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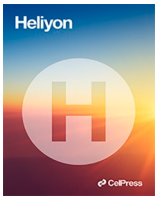

Research article

L.acidophilus HSCC LA042 and HKL suspension ameliorate DSS-induced ulcerative colitis in mice by improving the intestinal barrier inhibiting the NLRP3 inflammasome and pathogenic bacteria

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ABSTRACT

Ulcerative Colitis(UC) is a chronic intestinal inflammation affecting the intestines, yet its underlying causes remain unclear. In recent decades, the global prevalence of UC has been on the rise, leading to an increasing demand for therapeutic drugs with minimal side effects. Huan Kui Le (HKL), a traditional Chinese medicine compound, has demonstrated promising efficacy when combined with *Lactobacillus acidophilus (Lac.)* for UC intervention. However, the precise therapeutic mechanism of this combination remains unknown. The study focused on understanding the mechanisms of UC by examining the effects of Lac. and HKL (LH) treatment. The outcomes discovered that the disruption of gut microbiota, triggered by the activation of the NLRP3 inflammasome, plays a crucial role in UC development. This disruption exacerbates UC symptoms by causing disturbances in inflammatory cytokines and mucosal permeability. We investigated the dynamic changes following the application of this treatment using 16S rRNA sequencing, HE, WB, IHC, and ELISA. Compared with the UC group, LH treatment reduced colon pathological injury, improved colon length, and decreased IL-1 β serum levels. Furthermore, it restored the expression of TJs and preserved mucosal barrier integrity. LH treatment also mitigated colon injury by attenuating the expression of pyroptosis-related genes and proteins, such as NLRP3 and Caspase-1. Additionally, LH treatment altered the gut microbiota's microecology, characterized by a reduction in pathogenic bacteria abundance like Escherichia-shigella and an increase in beneficial bacteria abundance like Akkermansia and Erysipelatoclostridium. Overall, our findings indicate that LH therapy may be associated with intestinal barrier repair, inflammasome inhibition, and gut microbiota regulation, suggesting its potential as a UC treatment.

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1. Introduction

Ulcerative colitis (UC) is a chronic and non-specific inflammatory bowel disease (IBD) that causes inflammation in the colon for various reasons [1]. Histologically, UC is mainly characterized by extensive mucosal ulcers, gland disappearance, and neutrophil infiltration [2]. Patients with UC for many years face an increased risk of colon cancer [3], making UC a precancerous lesion. Hence, the World Health Organization considers UC to be one of the refractory diseases [4]. The global incidence rate of UC has been rising [5]. However, the pathogenesis remains incompletely understood.

Research indicates a close association between the occurrence and progression of UC and members of the NOD-like receptor (NLR) family, particularly NLRP3, which is extensively studied [6,7]. NLRP3 is widely distributed in epithelial cells, macrophages, and T and B lymphocytes and is closely linked to various diseases, including UC, obesity, Alzheimer's disease, and depression. Studies have shown that NLRP3 inflammasome is closely related to the UC [8]. Upon activation, NLRP3, along with activated caspase-1 and adapter protein ASC, generates NLRP3 inflammasomes, which in turn act on pro-IL-1β and pro-IL-18, influencing the maturation of IL-18 and IL-1β and thereby affecting the occurrence of inflammatory cascade reactions [8]. Studies have shown that inhibiting the increase of intestinal Tight Junctions(TJ) permeability caused by IL-1β can prevent DSS(Dextran Sulfate Sodium)-induced intestinal inflammation [9]. However, these two inflammatory factors also directly contribute to intestinal inflammation by damaging intestinal TJ proteins and elevating intestinal permeability through their impact on the immune system [9].

TJ protein plays, including Zonula Occludens (ZO), Occludin, and Claudins, are crucial for maintaining the permeability of the intestinal mucosal barrier [10]. Positioned at the apex of cell-to-cell junctions, they regulate microbial invasion and the spread of toxins. Claudins, in particular, govern the paracellular pathway, also known as the small molecule "pore pathway," expressed in both endothelial and epithelial cells, and it involves the TJ complex to regulate the outflow of molecules [11]. This pathway utilizes the TJ tight junction complex to control molecule outflow. Moreover, TJ proteins, such as ZO-1, directly bind to cytoskeletal myosin fibers [12,13]. Bacterial toxins and inflammatory factors destroy TJ protein, impacting tight cell connections and ZO-1 activity [14–16]. In UC, TJ protein is damaged due to the action of inflammatory factors, and the function of the intestinal mucosal barrier is reduced, resulting in bacterial translocation [17]. Additionally, alterations in intestinal flora can disrupt barrier function by affecting TJ proteins. Nevertheless, maintaining the bidirectional balance between intestinal flora and TJ protein the regulation of inflammasome is of great importance in treatment and molecular mechanism studies of UC.

At present, the therapeutic drugs for UC mainly rely on 5-aminosalicylates, corticosteroids, and immune-suppressants [18]. However, anti-inflammation may not suffice to repair the damage to the intestinal barrier or restore microflora balance. Changes in the intestinal flora could potentially exacerbate inflammation and metabolic imbalance [19]. Thus, a combined approach involving both anti-inflammatory treatment and microecological regulation might offer a safer and more effective strategy for treating UC, ultimately reducing its recurrence rate.

Presently, probiotics treatment for UC has become the new direction to regulate the disturbance of intestinal flora and restore the homeostasis between host and intestinal microbes. It is increasingly recognized as a safe, efficient, cost-effective, and innovative method with minimal side effects [20,21]. Research indicates that combining probiotics with traditional therapeutic drugs enhances the therapeutic efficacy of UC treatment [22,23].

Traditional Chinese medicine (TCM) is a kind of complementary therapy with multifaceted targets and applications. TCM is crucial in treating chronic diseases and has unique advantages in the prevention and treatment of UC in terms of improving efficacy and reducing inflammatory factors [24]. It is known for improving efficacy and reducing inflammation in patients with UC [25,26]. Our research team developed a traditional Chinese medicine HKL composed of eight components based on previous experiments. In the early stage, HKL alone was used to treat UC rats, and it was found that the HKL suspension had a certain effect on the improvement of UC [27]. Based on previous studies on UC pathogenesis, our research team employed a combination of HKL and Lactobacillus acidophilus HSCC LA042 to treat UC in mice, demonstrating significant therapeutic effects. However, the specific therapeutic mechanism remains unclear.

This study aims to analyze the interplay between TJ, NLRP3 inflammasome, and intestinal microflora in DSS-induced UC mice. Additionally, we aim to investigate the relationship between intestinal microflora composition and environmental factors using 16S rRNA technology to elucidate the potential molecular mechanism underlying combined UC treatment.

2. Materials and methods

2.1. Drugs and reagents

Lactobacillus acidophilus** HSCC LA042 **is a lyophilized bacterial powder produced through a freeze-drying process, containing a strain culture medium and a protective agent as excipients. The powder appears white to pale yellow without visible impurities. The enterprise identifies the strain as HSCC LA042, with a minimum viable bacteria count of at least 2.0×10^{11} CFU/g. *Escherichia coli* $\leq$ 10 CFU/g, Numbers of non-lactic acid bacteria$\leq$ 500 CFU/g, and water content$\leq$8.0 %. *This strain of Lactobacillus acidophilus* is an edible fungus approved as safe by the Ministry of Health. Production adheres to quality systems including, ISO/HACCP/GMP, and holds a food production license ISO/HACCP/GMP/Food production license. Product standard code: Q/WKHS 0003S.Production License Number: SC13137030600445.

HKL suspension: HKL suspension was optimized according to the original prescription [28]. The preparation process involves several steps: First, amber, Tabasheer, halloysitum rubrum, and sangusis draconis are finely crushed into powder. Next, Coptis chinensis, gallnut, pomegranate flower, and polygonum bistorta are subjected to reflux extraction with water three times, each lasting 1.5

h, filtered and combined with the filtrate, concentrated into a thick paste with a relative density of 1.30–1.40 (80 °C), mixed with the above fine powder, added auxiliary materials, granulated, dried, and pressed to obtain the drug.

2.2. Experimental animals

Male C57BL/6 N mice (20–24 g) were purchased from Vital River (Beijing, China) Laboratory Animal Technology Co., Ltd(Selling Unit License No: SCXK(JING)2021-0006. They were housed in a controlled environment with a temperature of 25 ± 3 °C, humidity of 55 ± 5 %, and a 12-h light/dark cycle, each in individual cages. The mice were provided with a standard diet and water ad libitum. Bedding-change and cage-washing were carried out frequently, and the preparation of recycled air was used to reduce the stress associated with the experiment. All the experiments were approved by the Ethics Committee of Xinjiang Medical University (Permit Number: IACUC-20230301-3).

2.3. Induction of UC in mice

Regarding previous relevant studies and pre-experiment results, C57BL/6 N mice were administered 2.5 % DSS(MPbio, USA) in

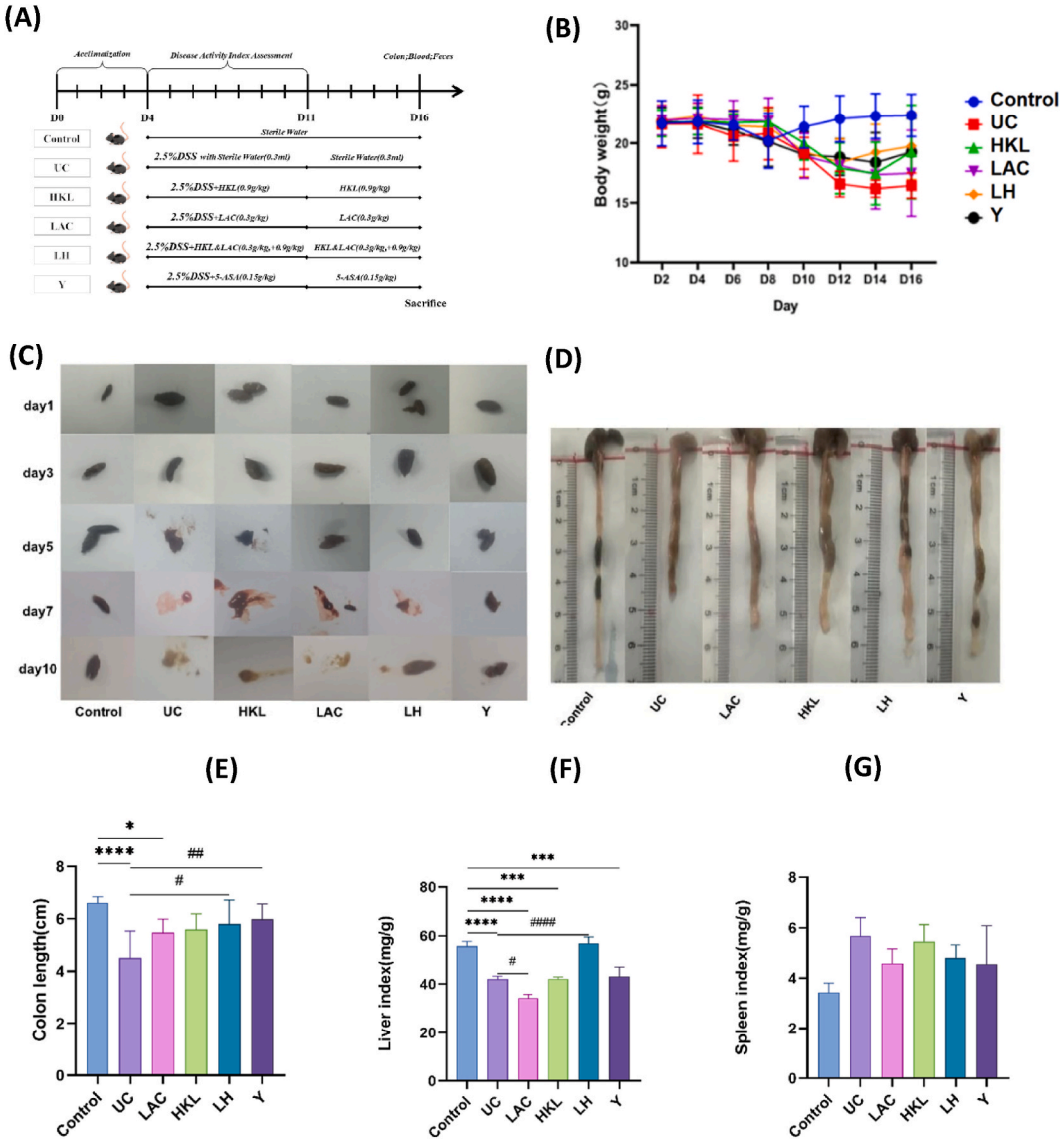

Fig. 1. LH treatment ameliorate DSS-induced damage. (A)Experimental design. (B) Body weight changes. (C) Fecal changes. (D)Colon length of mice (E)Colonic length comparison histogram of mice.(F)liver index comparison histogram.(G)spleen index comparison histogram.*$P < 0.05$, ****$P < 0.0001$ compared with control group. $^{\#}P < 0.05$,$^{\#\#}P < 0.01$, $^{\#\#\#\#\#}P < 0.0001$ compared with UC group.

sterile water to establish the UC model [29,30]. After adaptive feeding, the mice were randomly divided into six groups, including the Control group (n = 8), UC group (n = 12), HKL treated group (n = 12), *L. acidophilus HSCC LA042* (Lac) treated group (LAC group, n = 12), Lac and HKL treated group (LH group, n = 12) and 5-aminosalicylates treated group(Y group, n = 12). Briefly, except for the Control group, mice in each group received 2.5 % DSS in sterile drinking water for 7 days. Meanwhile, each group was intervened with different drugs. Subsequently, except for the Control and UC groups, the other groups received drug intervention for an additional 5 days. LAC group: *L.acidophilus HSCC LA042* 0.3 g/kg; HKL group: HKL suspension 2.6 g/kg; LH group: *L.acidophilus HSCC LA042* 0.3 g/kg+HKL 2.6 g/kg; Y group: 5-aminosalicylates 0.15 g/kg; Control group and UC group: sterilized water 0.3ml/mouse. After 12 days, all mice were sacrificed, and colon, liver, spleen, and serum were collected (Fig. 1A).

2.3.1. Hematoxylin and eosin staining

After euthanization, distal colon specimens were fixed in 4 % paraformaldehyde and embedded in paraffin. Sections were then cut and stained with hematoxylin and eosin (H&E) using a standard protocol (Biosharp, China). Subsequently, an experienced pathologist evaluated the pathological changes in the colon tissues.

2.3.2. Elisa

The concentrations of IL-1β and IL-18 in serum were determined using an ELISA kit according to the manufacturer's instructions (Reed Biotech, China). Following incubation, washing, and the addition of the terminating solution, the samples were determined at the absorbance of 450 nm and 630 nm. The serum concentration measured in each sample was expressed as pg/ml.

2.3.3. Immunohistochemical staining

Phosphate-buffered saline (PBS) was used to deparaffinize, hydrate, and wash colon tissue slices. Sections were blocked using 10 % goat serum, and then primary (incubated overnight at 4 °C) and secondary (incubated for 30 min at 37 °C) antibodies were applied (ZSGB-BIO, China). Then, hematoxylin (Biosharp, China) and DAB were used to counterstain. Under a microscope, the protein expression of Claudin1, Claudin2, Occludin, ZO-1, NLRP3, and caspase-1 in colon tissue was examined and documented. Additionally, the mean gray value (staining intensity) and the relative quantitative analysis of the positive area (staining area) of the positive signal were calculated using the Image J program to examine the data.

2.3.4. Western blotting analysis

Using RIPA lysis buffer, total proteins were extracted from the colon samples (Solarbio, China). The protein was quantified using the BCA test kit. Protein samples (30 μg) were subjected to 8 % or 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 70 V for 20 min and 150 V for 40 min. The resulting protein mixtures were subsequently transferred onto polyvinylidene fluoride membranes. 10 % non-fat milk was used to block the membranes for 2 h at room temperature. The following primary antibodies were then incubated on the membranes: anti-β-actin (1:11000, Affinity Biosciences), anti-claudin1 (1:2000, Affinity Biosciences), anti-claudin2 (1:2000, Affinity Biosciences), anti-Occludin (1:2200, Affinity Biosciences), anti-ZO-1 (1:1400, Affinity Biosciences), anti-NLRP3 (1:1400, Affinity Biosciences), and anti-caspase-1 (1:1000, Affinity Biosciences) were then incubated on the membranes at 4 °C for several hours. After three rounds of TBST (Solarbio, China) washing, secondary antibodies were added to the membranes and incubated. Following TBST washing, the protein bands were seen using an ECL kit. β-actin was used as an internal reference.

2.3.5. Microbiome analysis by 16S rRNA gene sequencing

We followed the manufacturer's protocol to extract total DNA from colon samples using the Fast DNA ®Spin Kit (MP Biomedicals, US). PCR was conducted using qualified DNA templates. The V3–V4 region of 16S rRNA was amplified for sequencing using the Illumina Miseq platform (PE 300; Major Bio-Pharm Technology Co. Ltd., Shanghai, China). The study evaluated the variations in alpha-diversity indices via the Welch T-test (Mothur, v1.30.1, http://www. mothur.org/wiki/Schloss_SOP# Alpha _diversity). The non-parametric Kruskal-Wallis H rank test evaluated variations in the microbiota composition, as determined by β-diversity measurements. R 3.2.2 software's RDA/CCA test was used to examine correlations between the gut microbiota and cytokines, TLRs, or metabolites (heatmap package). We conducted LEfSe (Linear discriminant effect size) analysis using default parameters based on the non-parametric factorial Kruskal–Wallis test. This analysis was performed from the phylum to the genus taxonomic level to identify microbial biomarkers in the gut microbiota of LH and UC groups. LEfSe used LDA (linear discriminant analysis) to assess the effect of each species' abundance on the observed effects. The threshold on the LDA score for discriminative biomarkers was 3.0. All statistical analyses were conducted using R 3.2.2.

2.3.6. Statistical analysis

Data were presented as means ± standard deviation (SD), while categorical variables were expressed as percentages. A one-way analysis of variance (ANOVA) was performed using GraphPad Prism 8.0 software to find differences between groups. Significant findings were determined at $p < 0.05$.

3. Results

3.1. LH treatment can alleviate DSS-induced colitis in mice

At the beginning of the DSS intervention, all mice experienced weight loss. Starting from the fourth day of modeling, mice in the UC group exhibited a significant decrease in weight, accompanied by the presence of blood in the stool around the anus and a decline in physical fitness. At the end of the intervention, the average body weight of mice in other intervention groups (LAC, HKL, LH) was higher compared to the UC group, particularly in the LH and Y groups (Fig. 1B and C). Observation of the colon and measurement of its length revealed that UC mice displayed a rough colonic appearance, edema, and significantly shortened colon length compared to the Control group. There was no significant change in the colon length of mice in the LH group (Fig. 1D and E). Furthermore, following the DSS intervention, the liver index of mice in the UC group decreased significantly. At the same time, there was no significant difference in the liver index of mice in the LH group. However, no changes in the spleen index were observed among the groups in this study (Fig. 1F and G).

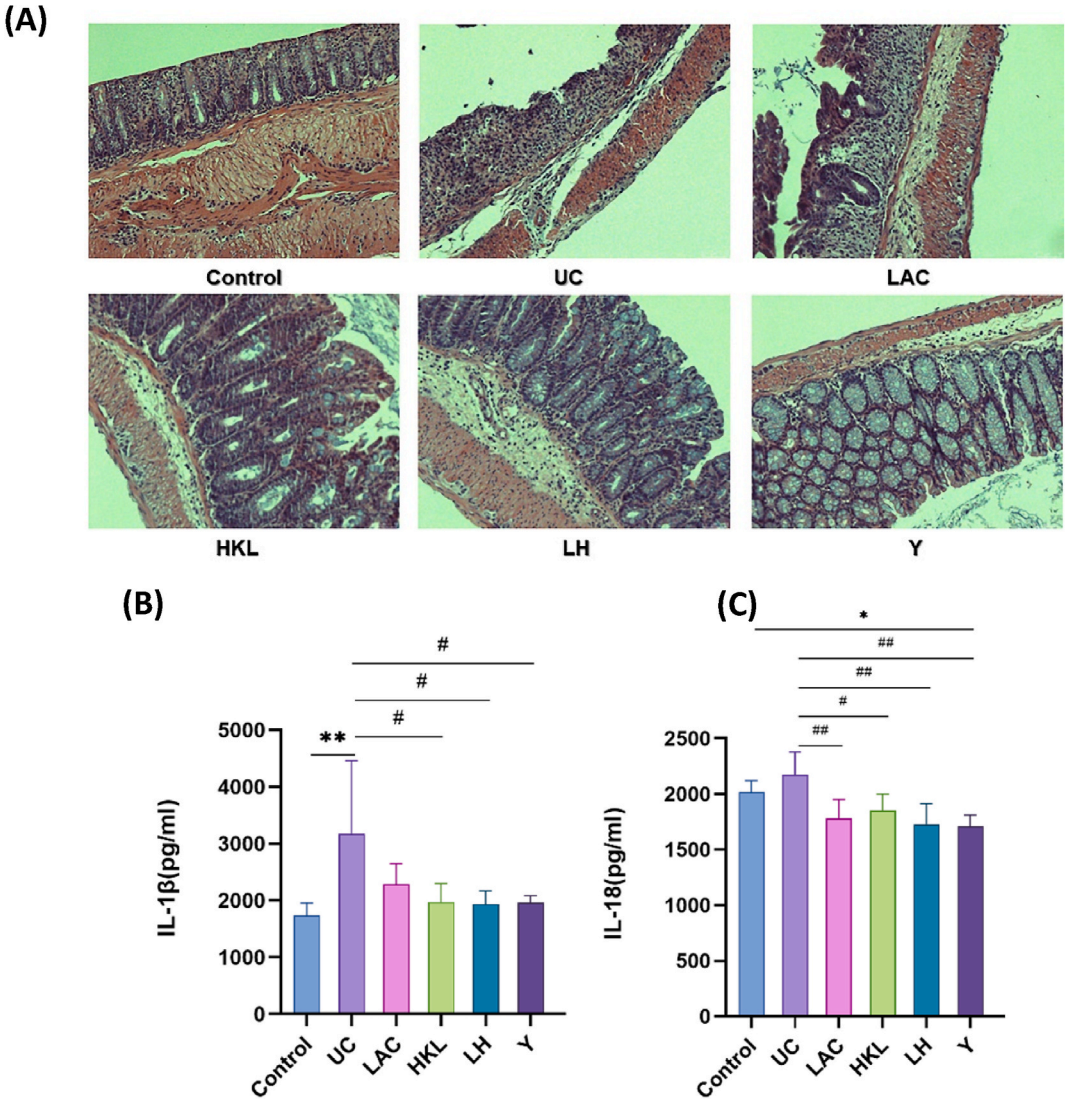

Fig. 2. Colonic histopathological changes and inflammatory factor levels in mice (n = 6). (A)Hematoxylin and eosin staining of colon tissue; (B)The expression level of IL-1β; (C)The expression level of IL-18. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the UC group.

3.2. LH treatment promoted the repair of colon mucosa in UC mice

H&E staining was used to observe the histopathological changes of colons in each group, and ELISA was used to detect the levels of inflammatory factors. As shown in Fig. 2A, mice in the UC group exhibited the disappearance of glandular structure in colon tissue, damaged crypts, visible ulcers, and extensive neutrophil infiltration, indicating severe damage to the intestinal barrier caused by DSS. However, the LH and Y groups showed improved mucosal barrier repair compared to the UC group. In these groups, the glandular arrangement was orderly, ulcers were mostly healed, and inflammatory cell infiltration was reduced. Additionally, the protein expression level of IL-1β was significantly increased in the UC group compared to the Control group ($P < 0.01$). Conversely, the intestinal protein expression levels of IL-1β and IL-18 were significantly decreased in all intervention groups compared to the UC group ($P < 0.05$ or $P < 0.01$) (Fig. 2B and C).

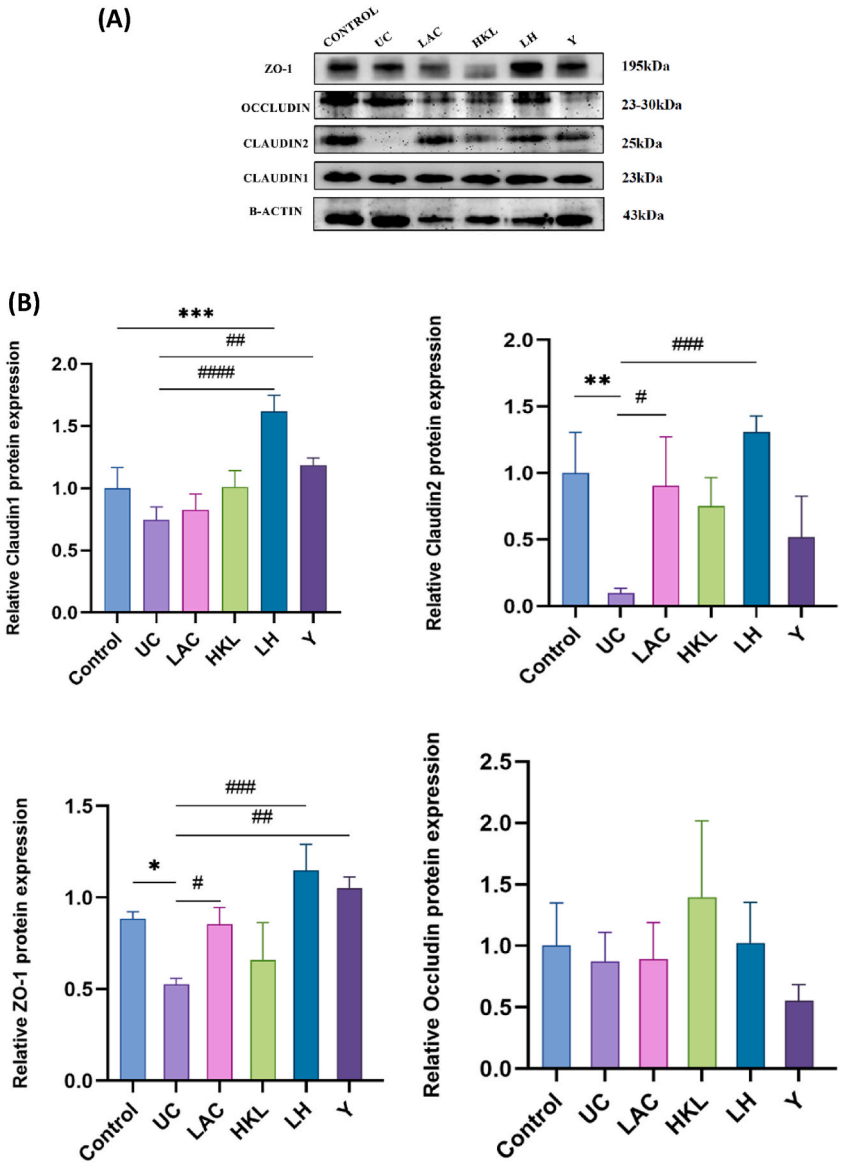

Fig. 3. Effect of LH treatment on the expression of TJ protein in UC.(A) Western blotting images of Claudin1, Claudin2, Occludin, ZO-1 and β-actin. (B) Compare the expression of Claudin1, Claudin2,ZO-1, Occludin in colon tissue. (C) Expression of TJ proteins was verified by IHC.(D)Comparative analysis of the positive results of TJ-related proteins intden/area). $^{*}P < 0.05$,$^{**}P < 0.01$, $^{***}P < 0.001$,$^{****}P < 0.0001$ compared with control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, $^{\#\#\#\#}P < 0.0001$ compared with UC group.

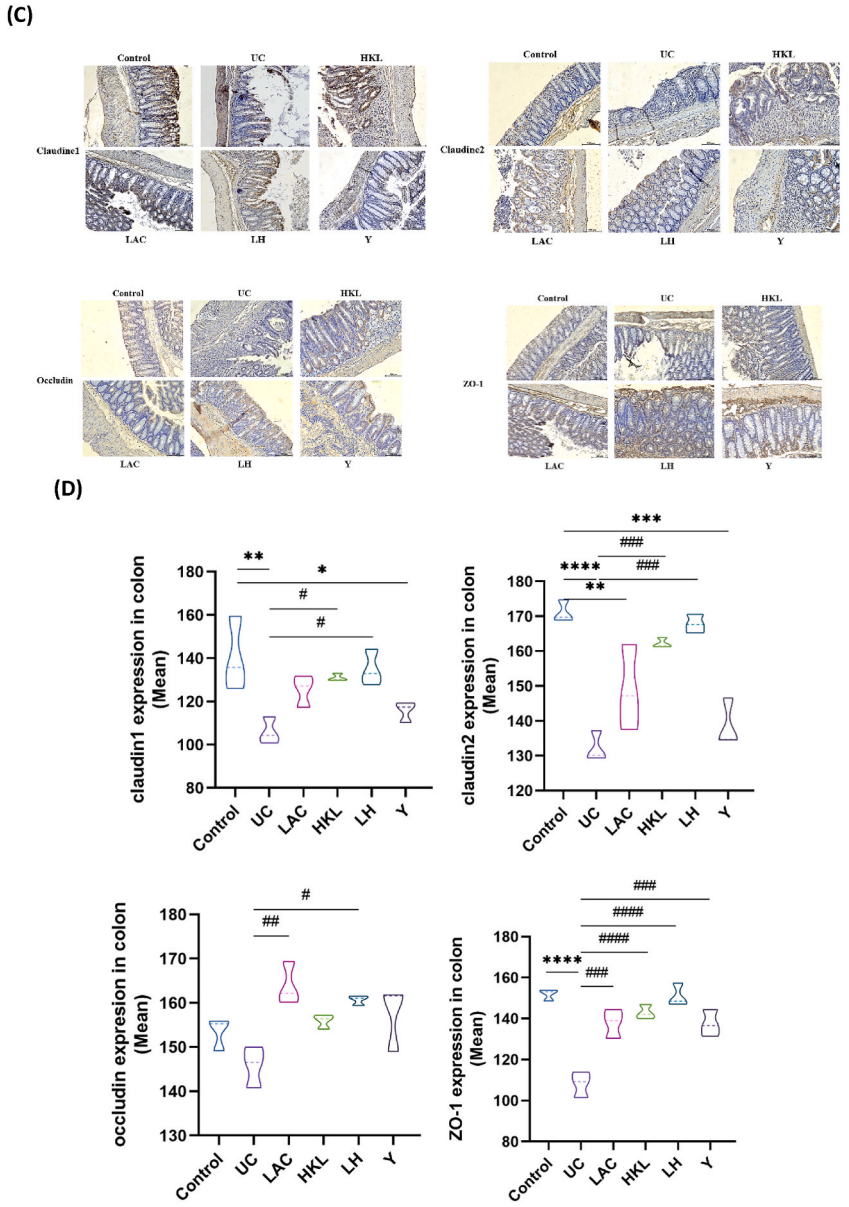

Fig. 3. (*continued*).

3.3. LH treatment affects the expression of colon TJ protein in UC mice

TJ is an important component of the intestinal mucosal barrier and is closely related to inflammation. Western blotting analysis in this study revealed significant decreases in the expression levels of Claudin-2 and ZO-1 in the UC group compared to the Control group ($P < 0.01$ or $P < 0.05$). Although statistical differences were not observed in Claudin-1 and Occludin, a decreasing trend (Fig. 3A,B). Conversely, the protein expression levels of Claudin-1, Claudin-2, and ZO-1 in the LH group were significantly increased compared to the UC group ($P < 0.001$) (Fig. 3A and B). However, none of the drugs appeared to have a significant improvement effect on Occludin.

TJ proteins are a mechanical barrier, safeguarding the intestinal mucosa against toxins and pathogens, and are essential for maintaining colon health. They are mainly expressed in the mucosal layer and some submucosal cell membranes. This research employed immunohistochemistry (IHC) to detect several crucial proteins within the TJ family. Regions exhibiting positive expression of these proteins after staining appeared as diffuse yellow particles, as shown in Fig. 3C. The results showed that Claudin1 and Claudin2 were less expressed in colon epithelial cells in the UC group than in the Control group($P < 0.01$ or $P < 0.0001$). Conversely, compared to the UC group, the expression of Claudin1 and Claudin2 in the LH group was significantly up-regulated ($P < 0.05$ or $P < 0.001$). Furthermore, the expression of ZO-1 was significantly increased in all intervention groups (LAC, HKL, LH, Y) compared to the UC group ($P < 0.0001$), and the expression of Occludin was also significantly up-regulated in the LH group ($P < 0.05$) compared to the

UC group(Fig. 3D).

3.4. LH treatment reduces the activation of inflammasome

The NLRP3 inflammasome has also been shown to play an important role in the UC [31].NLRP3 inflammasome is widely found in epithelial cells and immune cells, functioning by inducing downstream inflammatory factors. The expressions of NLRP3 and Caspase-1 were also detected by IHC in this research, as shown in Fig. 4A,B. Prominent cytoplasmic staining was observed in some cells of the mucous lamina propria, with additional staining observed around epithelial cells and goblet cells in the UC group. Quantitative analysis confirmed a significant increase in NLRP3 and Caspase-1 expression in the UC group compared to the Control group ($P <$

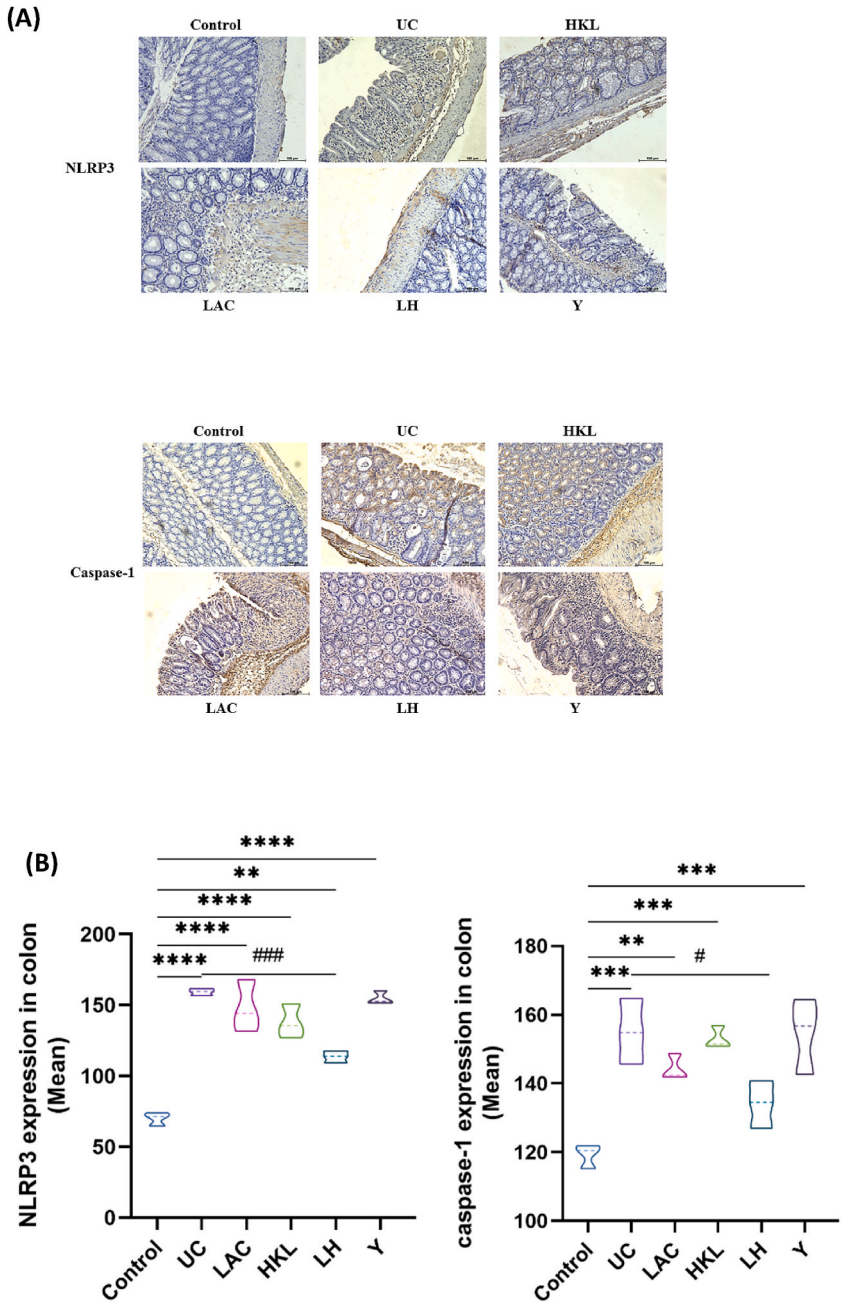

Fig. 4. LH treatment could attenuate the activation of inflammasome to some extent. (A)The expressions of NLRP3 and Caspase-1 were verified by IHC. (B)Comparative analysis of the positive results of NLRP3 and caspase1 in each group(intden/area). $^{*}P < 0.05$,$^{**}P < 0.01$, $^{***}P < 0.001$,$^{****}P < 0.0001$ compared with Control group, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ compared with UCgroup.

0.0001, $P < 0.001$). Conversely, compared to the UC group, there was a significant decrease in NLRP3 and Caspase-1 expression ($P < 0.001$, $P < 0.05$) in the LH group. Interestingly, LH treatment had little effect on NLRP3 and casapase1 protein expression.

3.5. Microbial diversity analysis of colon contents

To further elucidate the precise impact of LH treatment on UC will examine, we detected the composition and distribution of intestinal flora in the Control, UC, and LH groups using a 16S rRNA high-throughput sequencing. Changes in the richness and coverage of the gut microbiota were analyzed using Sobs, ACE, Chao, and Coverage indices of α-diversity analysis. The α-diversity analysis showed a significant decrease in bacterial diversity in the UC group, with bacterial diversity increasing after LH treatment (Fig. 5A–C). Community coverage significantly increased in the UC group and markedly decreased in the LH group (Fig. 5D). Rarefaction curves tested the data rationality of each group of the samples. A plateaued rarefaction curve of OTUs indicated that these sequencing depths covered all the species in the samples (Fig. 5E). Venn analysis showed that the number of unique species in the gut of UC mice (59 species) was less than in the Control group (259 species) and LH group (109 species), indicating a decline in the diversity of bacteria in the gut of UC mice (Fig. 5F).

3.6. LH treatment changed the intestinal flora of UC mice

To assess the impact of LH treatment on UC mice, the gut microbiota of each group was analyzed at the phylum and genus levels. As shown in Fig. 6A, Firmicutes were the most abundant phylum in the intestinal tracts of mice in all groups. However, the proportion of Proteobacteria and Campilobacterota in the intestinal tracts of mice in the UC group was significantly higher compared to the Control group. At the genus level, the bacteria with relatively high abundance in the gut included *Dubosiella*, *Lactobacillus*, *Escherichia-Shigella*, *norank-f-norank-o-Clostridia-UCG-014*, *Helicobacter, Turicibacter, norank-f-muribaculaceae, Bacteroides, Romboutsia*, and *Klebsiella* (Fig. 6B).

The dominant species composition and distribution proportions among three different groups were depicted using a Ternary phase diagram. In the diagram, the opportunistic pathogens Escherichia-Shigella and Klebsiella were predominantly concentrated in the UC group region. Conversely, *Clostridia_UCG-014, Dubosiella,* and *Turicibacter* were notably clustered in the LH group region (Fig. 6C). Further microbiota differences were analyzed at the genus level. Compared with the Control group, the abundance of *Lachnospiraceae_UCG-006, Eubacterium_brachy_group, unclassified_f_Oscillospiraceae, norank_f_Oscillospiraceae, norank_f_Eggerthellaceae* were significantly lower in the UC group (Fig. 6D). Additionally, compared to the UC group, the LH group had a significantly higher relative abundance of *Erysipelatoclostridium, Parvibacter*, and *unclassified c-Clostridia.* (Fig. 6E). This study observed considerable variation in the abundance of certain bacterial genera across different groups. Still, there were also substantial intra-group differences, leading to non-significant results in statistical analysis. As a consequence, using prior research findings, we compared microorganisms with higher and lower abundance in the UC and LH groups. We discovered that the abundance of *Klebsiella* and *Escherichia-Shigella* dropped in UC mice, whereas the amount of *Bifidobacterium* rose in the LH group(Fig. 6F–H). After LH intervention, previous studies have

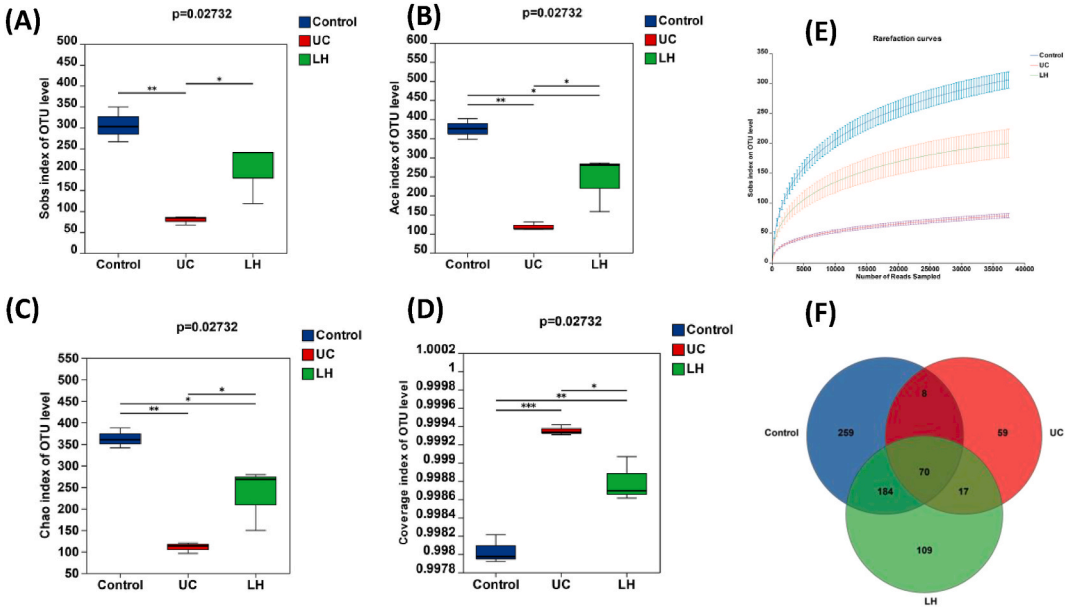

Fig. 5. Gut microbiota diversity analysis. (A) α-Diversity evaluated by Sobs index. (B) α-Diversity evaluated by Ace index. (C) α-Diversity evaluated by Chao index.(D) α-Diversity evaluated by Coverage index.(E) Sobs rarefaction curve of samples. (E) Veen analysis. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

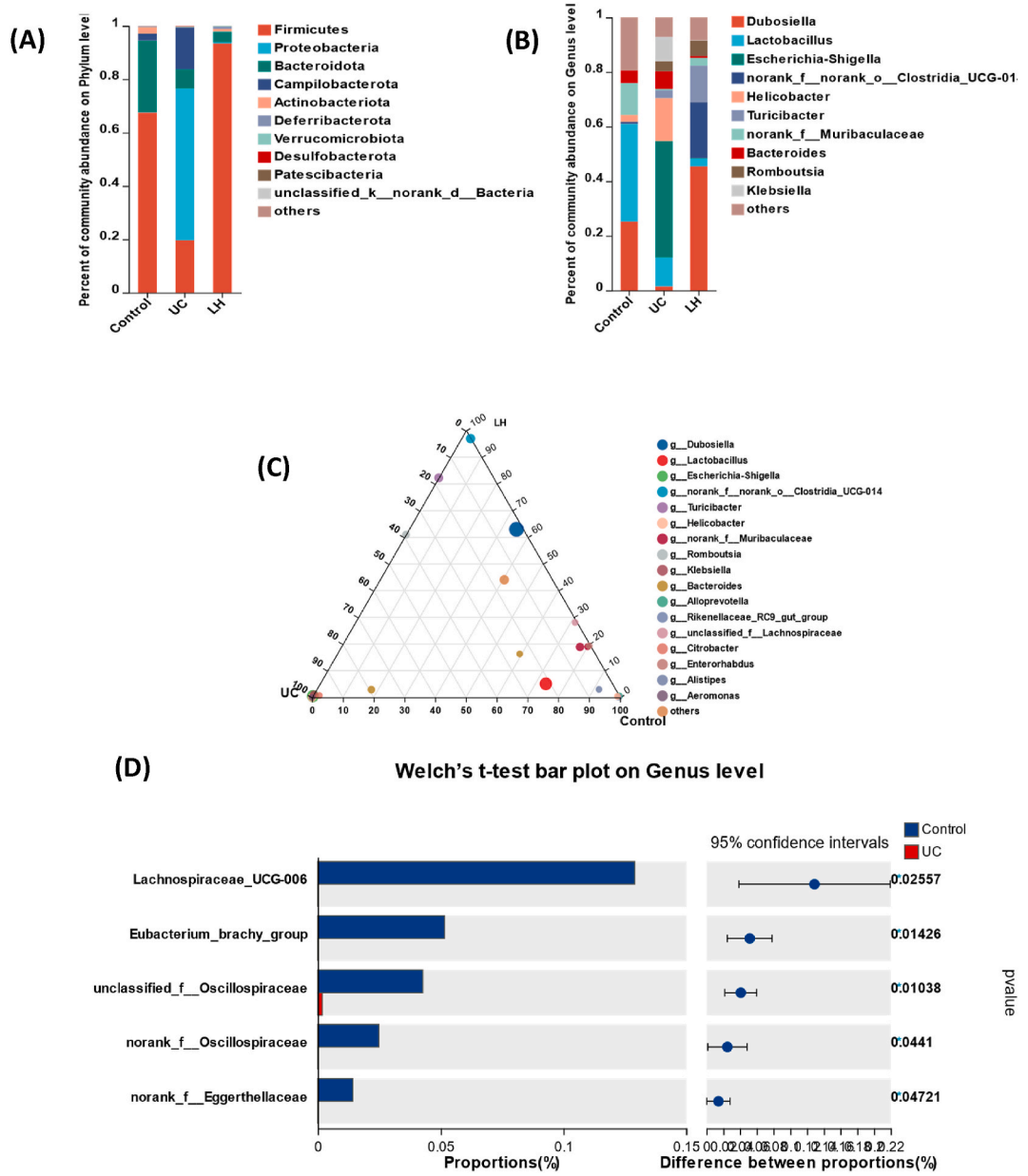

Fig. 6. Gut microbiota composition analysis; (A) Bar plot of bacterial richness distribution at the phylum level; (B) Bar plot of bacterial richness distribution at the genus level; (C)Ternary chart in three groups. (D)Differences of gut microbiota between Contro and UC groups at genus level; (E) Differences of gut microbiota between UC and LH groups at genus level; (F,G,H)Comparison the abundance of *Escherichia-shigella*, *Klebsiella, Bifidobacterium*.

shown a significant increase in the abundance of *Bifidobacterium* in the gut of UC rats. *Bifidobacterium* had a strong positive correlation with anti-inflammatory cytokines TGF-β and IL-13 [23]. These results suggest that the mechanism of LH treatment may be related to the immune response mediated by *Bifidobacterium*.

LEfSe analysis revealed significant differences in microbial taxa among the three groups with LDA scores >3. *Eacherichia-Shigella and Klebsiella* were over-represented in the UC group, whereas *Turicibacter* and *g_norank_f_norank_o_Clostridia_UCG-014* were more abundant in the LH group(Fig. 7A). Correlation analysis results showed a positive correlation between the expression levels of Claudin2, ZO-1, and the abundance of *Akkermansia*, *_f_Oscillospiraceae, Erysipelatoclostridium,* and *Bacteroides*. The abundance of *Bifidobacterium* is positively correlated with the abundance of Claudin1 and Claudin2. Meanwhile, the abundance of *Klebsiella and Escherichia-Shigella* was highly positively correlated with the NLRP3 inflammasome (Fig. 7B).

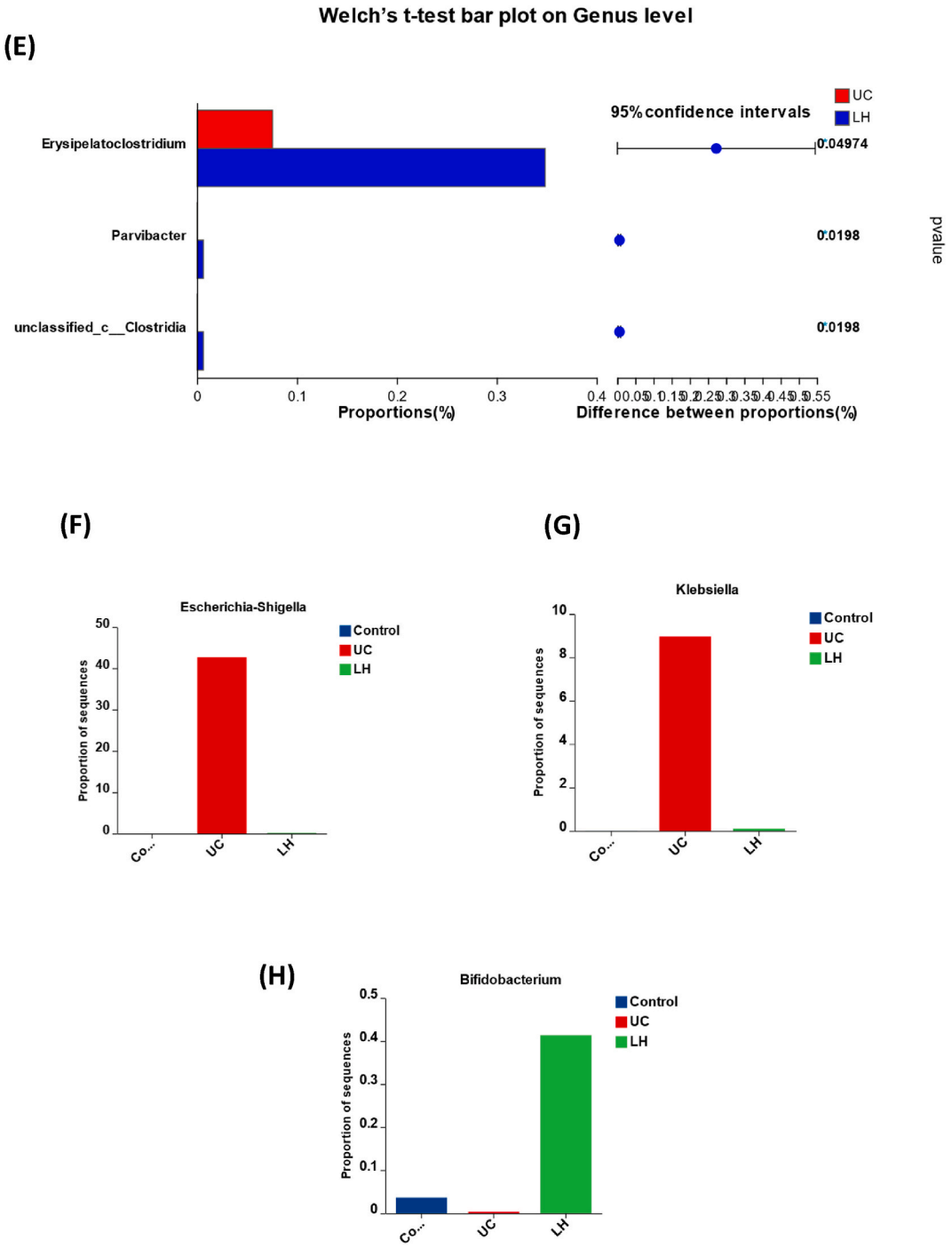

Fig. 6. (*continued*).

4. Discussion

The precise pathogenic mechanism of UC remains elusive, although the primary factors involved are the infiltration of inflammatory agents leading to loss of intestinal permeability and barrier function. Impairment of intestinal mucosal barrier function is an important indicator for assessing the degree of UC. This barrier function relies significantly on the integrity of the mucosal layer, which is solely determined by the expression and assembly of TJ proteins [32]. Changes in TJ protein levels within colon epithelial cells can enhance bacterial permeability. Additionally, the NLRP3 inflammasome, comprising NLRP3, Asc, and Pro-caspase-1, releases

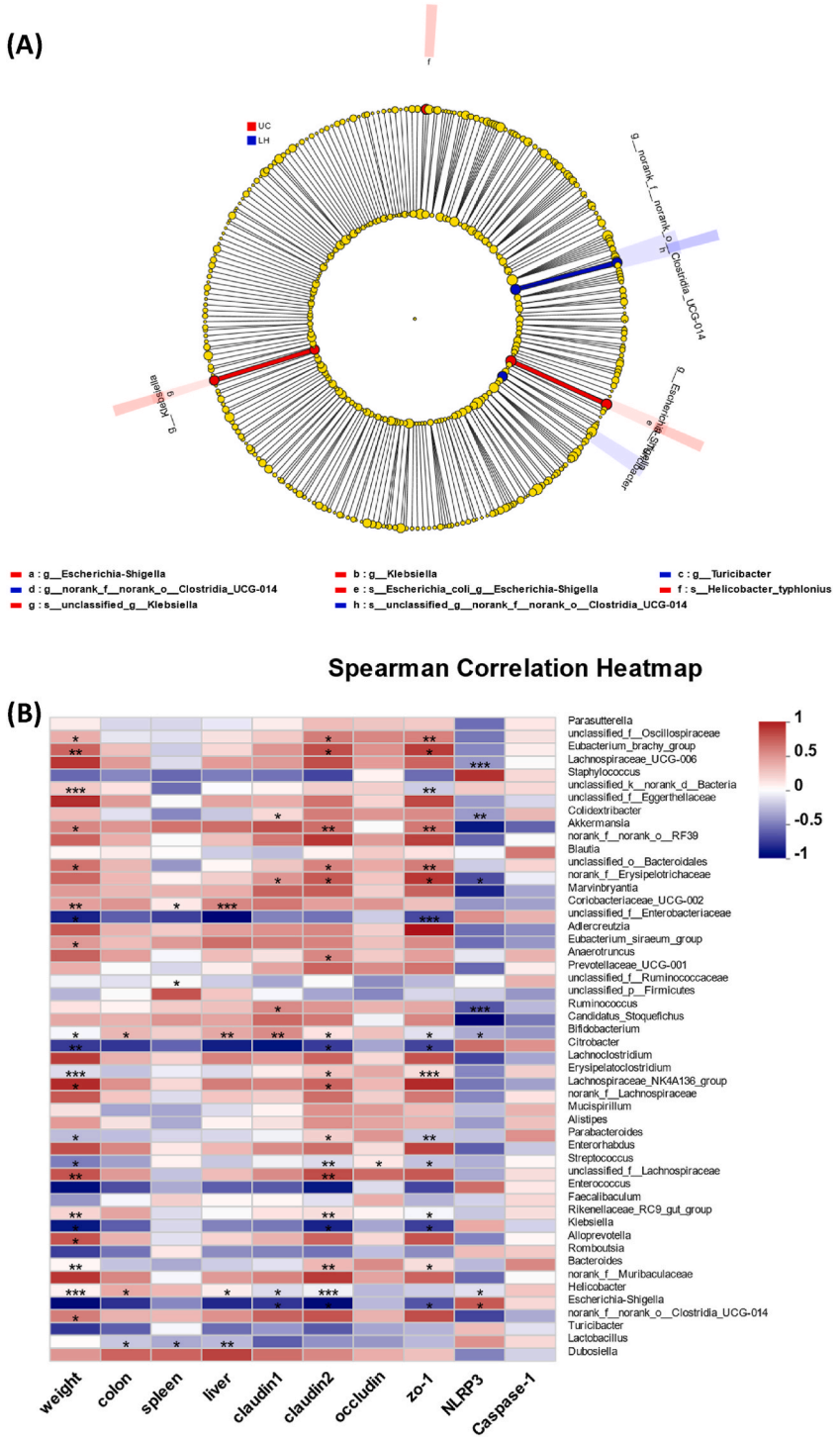

Fig. 7. Spearman Correlation Heatmap and LEfSe cladogram (LDA >3). (A)Cladogram based on LEfSe analysis. Circles represent taxonomic categories of organisms from the genus level (outermost circle) to the phylum level (innermost circle). Within each given taxon, each small circle represents its lower clade. Yellow nodes indicate no statistically significant differences in a given taxon between the samples of the three groups. (B) The correlation matrix shows the intensity of correlation between intestinal microbiota (genus level) and the weight, colon length, weight of spleen, weight of liver, expression level fo Claudin1,Claudin2,occludin,ZO-1,NLRP3 and Caspase-1. Spearman method was used to determine the statistical significance of all pairwise comparisons. Spearman r values range from −0.5(blue) to 0.5(red).$P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

inflammatory factors, which jointly aggravate colitis [33]. Nonetheless, strategies to enhance intestinal mucosal barrier function and suppress the spread of inflammation in UC patients remain subjects of ongoing research endeavors. TCM has been broadly applied to treat UC in China for a long time due to its safety and efficacy. In this study, *L.acidophilus HSCC LA042* and HKL were used to treat UC in mouse, and the mechanism of the LH treatment was explored from the perspectives of intestinal flora, intestinal mucosal barrier, and NLRP3 inflammasome. Previous research has indicated the effectiveness of HKL treatment in rat UC. It has been observed that the expressions of IL-4, IL-13, and TGF-β in the gut of UC rats significantly increase following HKL combined with Lactobacillus acidophilus treatment, while the expressions of cell membrane proteins TLR4 and TLR9 decrease [34]. Although HKL compounds have not been systematically studied, some studies have been reported on some components of HKL and their effects. Studies have revealed the anti-inflammatory properties of Bistortae Rhizoma and Quercus infectoria galls [35,36]. Moreover, Coptis Chinensis Franch and Halloysitum rubrum have been shown to maintain intestinal permeability and mucosal barrier integrity [37,38], significantly improving the prognosis of ulcerative colitis. The active components of Bistortae Rhizoma have anti-oxidation, promote autophagy protection, and can increase the content of IL-10 in serum and regulate the release of inflammatory cytokines [39]. Quercus infectoria galls contain main active components such as gallic acid (GA), methyl gallate (MG), and ethyl gallate (EG) etc. Among these, ethyl gallate (EG) directly targets phosphatidylethanolamine binding protein 1 (PEBP1), thus inhibiting the NF-κB inflammatory pathway [40]. By influencing the gut-brain axis, decreasing NF-κB, TNF-α, and IL-1β levels, and blocking astrocyte proliferation, Coptis chinensis Franch is thought to have anti-inflammatory effects on the brain [41]. Berberine is the main active ingredient in Coptis chinensis Franch [42]. Studies have demonstrated that berberine can improve the function of the intestinal mucosal barrier by upregulating the expression of Claudin-1, Occludin, and ZO-1 in the colon tissue of mice with ulcerative colitis (UC). Additionally, in the colon tissues of mice with DSS-induced UC, berberine has been shown to inhibit mRNA expression of TNF-α, NF-κB, and IFN-γ [43]. It is hypothesized that the combination of HKL and L. acidophilus may successfully treat ulcerative colony syndrome (UC) by healing damaged intestinal mucosal barriers, decreasing inflammation, and regulating intestinal flora, based on these findings and earlier investigations. A major element in the development of UC is the inflammatory response, controlled by a number of pro-inflammatory factors [44]. The intestinal barrier is disrupted when the NLRP3 inflammasome is abnormally activated because it emits a lot of pro-inflammatory cytokines such as IL-1β and IL-18 [45,46]. It was shown by Bauer C et al. [47] that mice that had the NLRP3 gene deleted were resistant to colitis brought on by DSS. Furthermore, it has been demonstrated by several studies that medications that prevent the NLRP3 inflammasome from activating may also be able to cure ulcerative colitis (UC) [48–51]. Moreover, the degradation of NLRP3 inflammasome can also regulate inflammation in the colon [47]. A 2.5 % DSS mouse model of UC was established in this investigation, and L. acidophilus and HKL were utilized both singly and in combination to intervene.The findings indicate that LH treatment significantly mitigates excessive fluctuations in body weight and colon length, while notably enhancing the integrity of the damaged intestinal mucosal structure and reducing inflammatory cell infiltration. IHC experiment results showed that the LH treatment had a certain inhibitory effect on the protein expression of NLRP3 and Caspase-1. Additionally, ELISA results reveal a reduction in the protein expression level of the inflammatory factor IL-1β in the gut of UC mice following LH intervention. Previous studies have suggested that by downregulating key regulatory factors of the NF-κBp65 transduction pathway, NF-κB activation is inhibited, thereby influencing the subsequent cascade release of downstream NLRP3 inflammasome and pro-inflammatory factors [52]. However, NLRP3 activation via TLR4/NF-κB leads to Caspase-1 cleavage, resulting in the fragmentation of Gasdermin (GSDMD). The N-terminal fragment is polymerized and inserted into the plasma membrane to form pores with an inner diameter of 10–20 nm and cause cell pyrosis [53]. Meanwhile, IL-1β and other inflammatory factors are released through pores, exacerbating inflammation and disrupting mucosal proteins. IL-1β and TNF-α reduce Occludin and claudin-1 expression, leading to intestinal inflammation and increased permeability [54].Previous research has shown that LH therapy reduces the expression of TLR4 protein in the stomach of UC rats [22], while HKL intervention alone reduces the expression of NF-KB protein [55]. The study demonstrated that LH administration dramatically increased the expression of TJ-related proteins ZO-1, claudin1, and claudin2, while down-regulating IL-1β, NLRP3, and Caspase-1. This suggests that LH treatment may safeguard the intestinal mucosa by inhibiting cell pyroptosis induced by excessive NLRP3 activation.

It is well known that UC is associated with dysregulation of the gut microflora [56]. In this study, it was found that the α diversity of gut microbiota decreased in the UC group, while the abundance of *Proteobacteria*, *Escherichia-shigella,* and *Klebsiella* increased, which aligns with the intestinal flora composition of rats with UC studied by our team [22].In this study, *Klebsiella* and *Escherichia-shigella*, highly abundant in the intestinal tract of mice in the UC group, have also been detected in patients with colitis and colorectal cancer [57,58], resulting in a disturbance in the intestinal microflora. Moreover, the abundance of *Escherichia-Shigella* was found to be negatively correlated with the expression of Occludin, as well as the body weight and colon length of mice. This suggests that the destruction of the intestinal barrier function of mice may be related to the abnormal increase of *Escherichia-shigella*. Studies have shown a significant increase in *Proteobacteria* and *Bacteroidetes*, while *Bacteroidetes* have decreased significantly in UC colon samples. The Study has revealed that changes in the number of *Bacteroidetes* are usually accompanied by changes in the expression of various amino acids, such as the increase of pyruvate metabolism and the decrease of citric acid expression [59]. Metabolites of short-chain fatty acids (SCFA), tryptophan, and bile acids, which provide nutrients and energy and protect the epithelium, are also reduced in UC [60,61]. We found a reduction in the number of butyrate-producing bacteria *Romboutsia* and *norank_f_Muribaculaceae* in the UC group, indicating a decrease in SCFA levels, leading to reduced nutrient and energy metabolism and diminished resilience of the colon mucosa against external threats. Correspondingly, the abundance of *Bacteroides* decreases as energy and nutrient metabolism declines [62]. After LH treatment, there was an increase in the abundance of SCFA-producing bacteria such as *Dubosiella* and *Romboutsia*. Studies suggest that SCFA can mitigate colitis by enhancing macrophage activity [63]. As a result, LH therapy may control the energy deficit of colon epithelial cells in UC mice by promoting the proliferation of SCFA-producing bacteria. In this work, LH therapy reversed the reduction of α diversity in UC mice and increased the relative abundance of *Akkermansia, Bifidobacterium, Erysipelatoclostridium,* and *Parvibacter*. Erysipelatoclostridium belongs to Firmicuts. Some have found that increased physical activity/fitness is associated with an increase in

butyrate-producing taxa including, Erysipelotrichaceae [64]. Chang et al. [65] found that the relative abundance of *Erysipelatoclostridium* was enriched in azoxymethane (AOM)/DSS mice treated with corylin. However, other studies have proposed the opposite, suggesting that this genus may harm fat metabolism and inflammation [66,67]. The results showed significant enrichment of *Erysipelatoclostridium* in the LH treatment group following LH intervention. We therefore suspect that *Erysipelatoclostridium* may be potentially beneficial bacteria in the gut, although further investigation is needed to elucidate its specific role. Other studies have demonstrated a significant reduction in protective microbiota, such as *Clostridium* and *Bifidobacterium* in UC [68]. Notably, we observed a notable increase in the abundance of the probiotic Bifidobacterium in the LH group. Related reports of the main component of HKL show that some intestinal flora can enhance the metabolic absorption of flavonoids of Bistortae Rhizoma in the body and reduce the toxicity of harmful substances. Moreover, flavonoids provide metabolic substrates for intestinal flora, promoting the growth of beneficial flora such as *Bifidobacterium*, thus optimizing the structure of intestinal flora and having positive significance for health. In addition, flavonoids, such as butyric acid and acetic acid, can also promote the fermentation of dietary fiber by bacteria, leading to SCFA production, . These compounds exert physiological effects [69] countering the impact of increased *Proteobacteria* on SCFA after DSS-induced UC. Therefore, this study tentatively proposes that Bistortae Rhizoma may play a significant role in modulating the intestinal flora during the LH treatment. Therefore, this study tentatively proposes that Bistortae Rhizoma may play a significant role in modulating the intestinal flora during LH treatment. Additionally, the local mucosal thickening during chronic colitis may affect *Akkermansia*, a bacterium implicated in mucus production and intestinal metabolism, which could impact its proliferation [70]. Previous research suggests that *Akkermansia* acts as a potential probiotic [71], primarily breaking down host mucin into various metabolites, including SCFA, to maintain intestinal barrier function [72]. This bacterium has been associated with various metabolic diseases, including diabetes. Interestingly, our findings revealed a reduction in *Akkermansia* abundance in the UC group, while the combined therapy restored its abundance.

5. Conclusions

Our study highlights the anti-inflammatory effects and mucosal barrier repair of LH treatment. The combination of HKL and *L. acidophilus* alleviated the expression of NLRP3 inflammasome, decreasing inflammatory cytokines and mitigating mucosal barrier damage. This is mainly reflected in increasing the expression of Claudins and ZO-1. LH treatment inhibited the growth of *Escherichia-shigella* and *Klebsiella* and promoted potential probiotics such as *Akkermansia*, *Erysipelatoclostridium, and Bifidobacterium*. LH treatment partially restored intestinal dysbiosis and mucosal barrier integrity. The combination of probiotics and Chinese medicine HKL may be a potential candidate for UC treatment.

Limitations of this research

The possible mechanism of LH treatment for UC has been revealed in our study, but some limitations also need to be addressed in our further research. Many prospective works focusing on the cellular level and clinical trials are needed to confirm the therapeutic and side effects of LH in humans. We believe the LH treatment has promising potential in treating UC.

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Ethics statement

The animal study was reviewed and approved by the Ethics Committee of Xinjiang Medical University (Permit Number: IACUC-20230301-3).

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Jiwei Zhu: Writing – original draft, Project administration, Formal analysis, Data curation. **Hanming Wang:** Writing – original draft, Project administration, Methodology. **Muaitaer Aisikaer:** Project administration, Formal analysis, Data curation. **Zainaipuguli Yisimayili:** Resources, Supervision. **Tongtong Yang:** Project administration. **Wenjun Zhou:** Project administration. **Jianfeng Zhao:** Project administration. **Kurexi Yunusi:** Conceptualization, Funding acquisition. **Kasimujiang Aximujiang:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33053.

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