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Lacidophilin tablets relieve irritable bowel syndrome in rats by regulating gut microbiota dysbiosis and intestinal inflammation

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Irritable bowel syndrome (IBS) is a common clinical functional gastrointestinal disease. It has a complex pathophysiological mechanism, in which the imbalance of gut microbiota might play an important role. Lacidophilin tablets (LH) can regulate gut microbiota, but their effect on IBS is unknown. In this study, the IBS model was established by acetic acid enema combined with the constrained stress method, and rats were fed LH for 2 weeks. LH significantly reduced visceral sensitivity and intestinal propulsion rate and improved IBS-induced anxiety and depressive behavior in IBS rats. LH elevated the expression levels of mucin 2, claudin1, and occludin, and ameliorated IBS-induced structural damage to colonic tissues. The gut microbiota analysis revealed that LH altered the structure and composition of the gut microbiota in IBS rats. In addition, LH reduced the expression levels of inflammatory factor-related genes. These results suggest that LH could significantly improve the visceral sensitivity and intestinal motility disorders of IBS rats, relieve anxiety and depression levels, and alleviate the symptoms of IBS rats by regulating gut microbiota and reducing intestinal inflammation.

Keywords Irritable bowel syndrome, Lacidophilin tablets, Gut microbiota, Inflammation

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder with about 4% prevalence worldwide[1]. IBS is typically characterized by recurrent abdominal pain, accompanied by abnormal bowel behavior and frequency[2]. In addition, IBS patients often have mental comorbidities[3], such as anxiety, irritability, depression, and other mental symptoms. Although IBS does not affect the long-term survival and general condition of patients, its recurrence seriously affects the quality of patients' lives and increases the incidence of mental comorbidity and economic burden on families and society[4]. IBS has a relatively complex pathogenesis, which has not yet been fully clarified[5]. Modern medical studies have shown that IBS may be related to high visceral sensitivity[6], abnormal gastrointestinal motility[7], mental tension[8], abnormal brain-intestinal axis regulation[2], dysbiosis of gut microbiota[9,10], etc. However, the specific causes of IBS require further investigation. Therefore, there is no specific therapy in clinical practice, and symptomatic treatment is often the main treatment, which cannot cure the disease.

An in-depth study of IBS demonstrated that the imbalance of gut microbiota might be related to the pathogenesis of IBS[11]. The gut microbiota is a complex microbial community, consisting of bacteria, fungi, viruses, and protozoa, that exist in a symbiotic environment to maintain the structural integrity of the intestinal mucosal barrier, immune regulation, and protection against pathogens[12]. In addition, gut microbiota is closely related to immune and metabolic functions by producing short-chain fatty acids, the common bacterial metabolites, as a mediator[13]. Studies have shown that the imbalance of gut microbiota can trigger host immune responses and impair intestinal movement and barrier function[14,15]. In addition, the composition of healthy gut microbiota was significantly different from those of the IBS patients[16]. Given its critical role in IBS, recent IBS treatment research has focused on treatments based on gut microbiota.

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Extensive research and clinical evidence have demonstrated that probiotics, prebiotics, and synbiotics, can improve IBS by regulating the gut microbiota[7,17,18]. However, with the widespread use of probiotics, safety concerns have surfaced in certain patient populations (immunodeficient, susceptible patients, etc.) regarding the use of live strains. The use of non-viable postbiotics is gaining popularity as a safer option, they can significantly reduce risk of microbial translocation and infection[19]. In 2021, the International Scientific Association of Probiotics and Prebiotics published a consensus statement on the definition and scope of postbiotics[20], which is preparations of inanimate microorganisms and/or their components that confers health benefits to the host. They include a variety of components, such as inactivated microbial cells, short-chain fatty acids, cell wall components, peptides, polyamines, functional proteins, bacteriocins, and other bioactive metabolites[21]. Postbiotics exert their beneficial effects through a variety of mechanisms, similar to probiotics and prebiotics. They can regulate the composition of the gut microbiota by inhibiting the growth of harmful bacteria and enhancing the function of beneficial bacteria, enhance gut barrier function, exhibit antioxidant and anti-inflammatory properties, and modulate immune responses[22,23].

Lacidophilin tablets (LH) are a postbiotic preparation made from defatted milk by *Lactobacillus acidophilus*, which contains L-lactic acid, D-lactic acid, and seven short-chain fatty acids (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid)[24,25]. It is commonly used to improve the intestinal environment and regulate intestinal function. It has the advantages of high safety[25], heat resistance, and easy storage. A previous study has shown that LH can reverse the dysbiosis of gut microbiota and increase its diversity[26]. As a postbiotic preparation rich in short-chain fatty acids, its effect and mechanism of action in improving irritable bowel syndrome are worth exploring. In this study, acetic acid enema in combination with stress restraint was used to establish IBS models, and the effects of LH on visceral sensitivity, anxiety and depression behavior, intestinal motility, intestinal mucosal barrier function, and gut microbiota in IBS rats were investigated.

Results

Effects of LH on visceral sensitivity and intestinal propulsion in IBS rats

In this study, at the AWR score of 3, the volume of normal saline injected into the colorectal of rats was used as the score standard for visceral sensitivity. As compared to the control group, at the AWR score of 3, the volume threshold of the IBS group was significantly lower ($P < 0.0001$). After LH administration, the volume threshold of the IBS + LH group was significantly higher than that of the IBS group ($P < 0.0001$) (Fig. 1A). The intestinal propulsion rate of the IBS group rats was significantly higher as compared with control group ($P = 0.0091$), whereas the intestinal propulsion rate of the IBS + LH group rats was significantly lower than that in the IBS group ($P = 0.0470$) (Fig. 1B). This showed that LH treatment could significantly improve visceral sensitivity and intestinal motility disorders.

Effects of LH on anxiety and depressive behavior in IBS rats

The open-field test results showed that the total moving distance and the number of span degree by the IBS group rats decreased significantly as compared to the control group ($P = 0.0017$, 0.0008), which increased significantly in the IBS + LH group as compared to the IBS group ($P = 0.0416$, 0.0280) (Fig. 2A, B). The experimental results of sugar water preference showed that the percentage of sugar water preference of the IBS group rats decreased

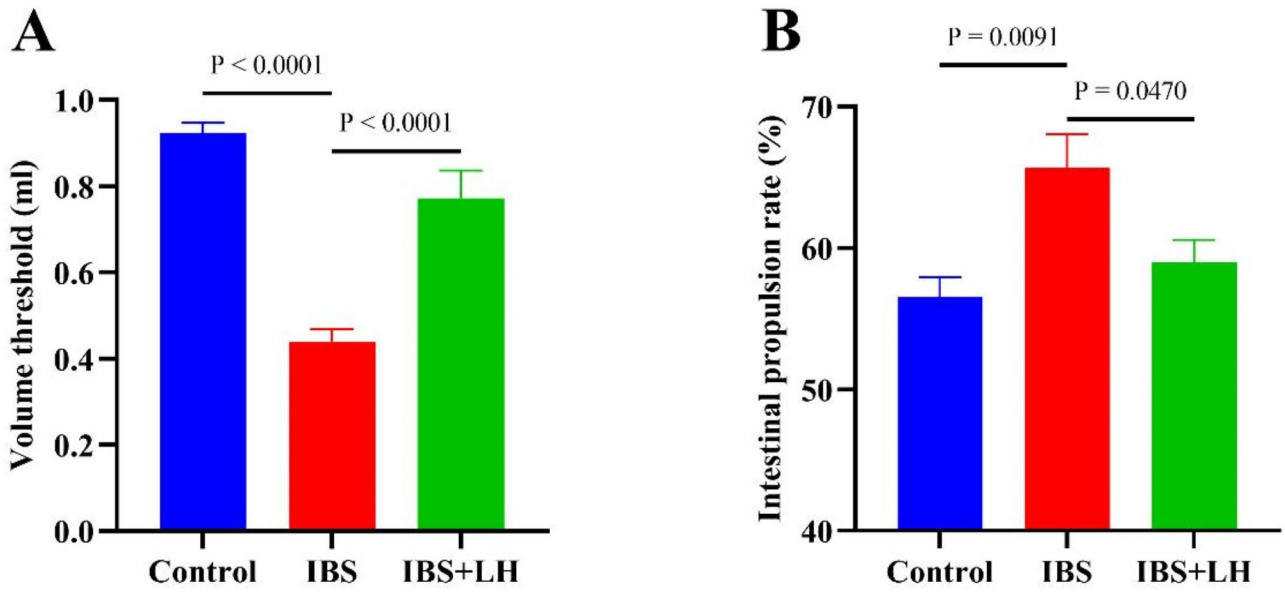

Fig. 1. Effects of LH on visceral sensitivity and intestinal propulsion in IBS rats. (**A**) Volume threshold at AWR score of 3. (**B**) Intestinal propulsion rate. Values are presented as the means ± SD ($n = 8$).

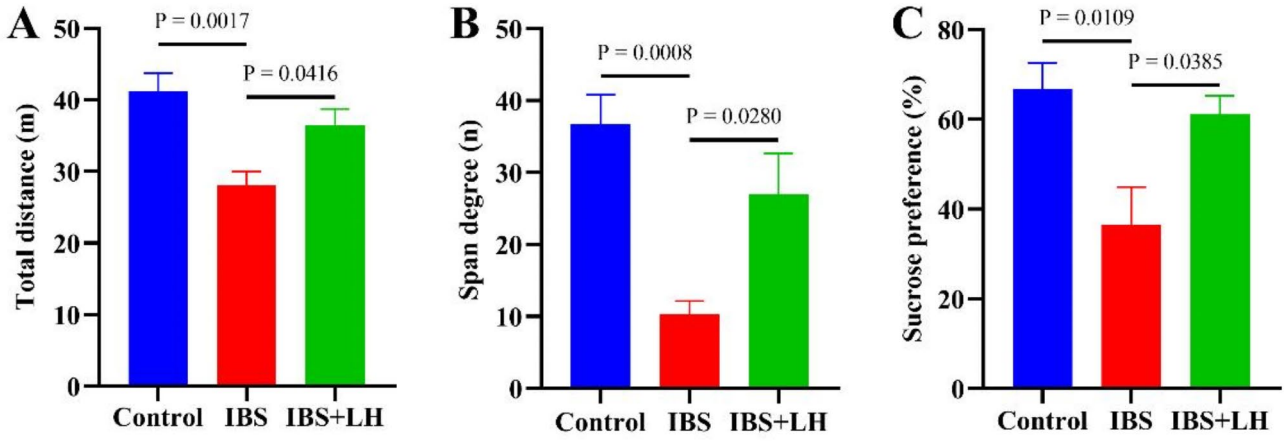

Fig. 2. Effects of LH on anxiety and depressive behavior in IBS rats. (**A**) Total movement distance of rats in open field. (**B**) Span degree of rats in open field. (**C**) The percentage of Sucrose preference. Values are presented as the means ± SD ($n = 8$).

as compared to the control group ($P = 0.0109$), which increased significantly in the IBS + LH group as compared to the IBS group ($P = 0.0385$) (Fig. 2C).

Effects of LH on pathological histology of colon tissue in IBS rats

The results of HE staining showed no significant pathological changes in the proximal colon tissues in all groups (Fig. 3A). The HE staining results of distal colonic tissue staining were shown in Fig. 3B. In the control group, the intestinal mucosal epithelium was relatively complete with regular cell arrangement, and the colon glands were not atrophied and arranged in an orderly manner with no intercellular edema or inflammatory cell infiltration. In the IBS group, some intestinal mucosal epithelial cells were necrotic and shed with slightly disorderly arranged glands, loose submucosal structure, edema and congestion, and a small number of inflammatory cell infiltration in the mucosa and submucosa. As compared to the IBS group, the colonic mucosal epithelium in the IBS + LH group tended to be more intact, more orderly arranged glands, reduced inflammatory cell infiltration, and no interstitial hyperemia and edema.

Effects of LH on the expression of MUC2, claudin1, and occludin in IBS rats

MUC2 is a major component of the colonic mucus layer, claudin1and occluding are tight junction proteins, which are associated with tight junctions between intestinal cells and can be used as markers for intestinal mucosal injury. The expression levels of MUC2 and claudin1 in distal colon tissue were detected using immunohistochemistry (Fig. 3C, D). As compared to the control group, the expression levels of MUC2 and claudin1 in the IBS group decreased, while the expression levels of MUC2 and claudin1 in the IBS + LH group increased as compared to the IBS group. And the Western blot results also showed that the expressions of claudin-1 and occludin were significantly elevated in IBS + LH group (Fig. 3E-G). These results suggested that the LH intervention could improve the IBS-induced colonic tissue damage.

Effects of LH on gut microbiota changes in IBS rats

Alpha-diversity analysis was performed to evaluate the effects of LH on the diversity of gut microbiota in rats. The α-diversity is expressed by the Chao and Shannon indices. Chao index is used to evaluate the richness of species in the sample, and the Shannon index determines the richness and evenness of species in the sample. Results as shown in Fig. 4A. As compared to the control group, the Shannon and Chao indices of the IBS group significantly decreased ($P < 0.01$, $P < 0.001$), indicating that IBS modeling significantly reduced the richness and evenness of gut microbiota in rats. As compared to the IBS group, the IBS + LH group showed no significant change in the Shannon index but exhibited a significant increase in the Chao index ($P < 0.05$), indicating that LH could restore the richness of gut microbiota in IBS rats to a certain extent. Principal coordinate analysis (PCoA) based on Bray Curtis distance was used to evaluate the structure and composition of rat's gut microbiota (β-diversity). As compared to the control group, the structure and composition of gut microbiota in the IBS group were significantly changed and were significantly restored by the LH treatment in IBS rats (Fig. 4B).

At the phylum level, Firmicutes, Verrucomicrobiota, and Actinobacteriota were the dominant microbiota in the three groups (Fig. 4C). The relative abundances of Bacteroidota and Desulfobacterota in the control group were higher than those in the IBS and IBS + LH groups. As compared to the control group, the relative abundance of Firmicutes in the IBS group significantly increased, while that of Bacteroidota significantly decreased, thereby increasing the Firmicutes/Bacteroidota (F/B) ratio. However, LH slightly increased the abundance of Bacteroidota and reduced the F/B ratio to a certain extent (Fig. 4D). Next, heatmap of relative abundance analysis and linear discriminant analysis effect size (LEfSe) analysis was used to test the significance of differences in the relative abundances of gut microbiota among the groups. Comparing the significant difference in the gut microbiota between the IBS and IBS + LH groups at the genus level (Fig. 4E, F) showed

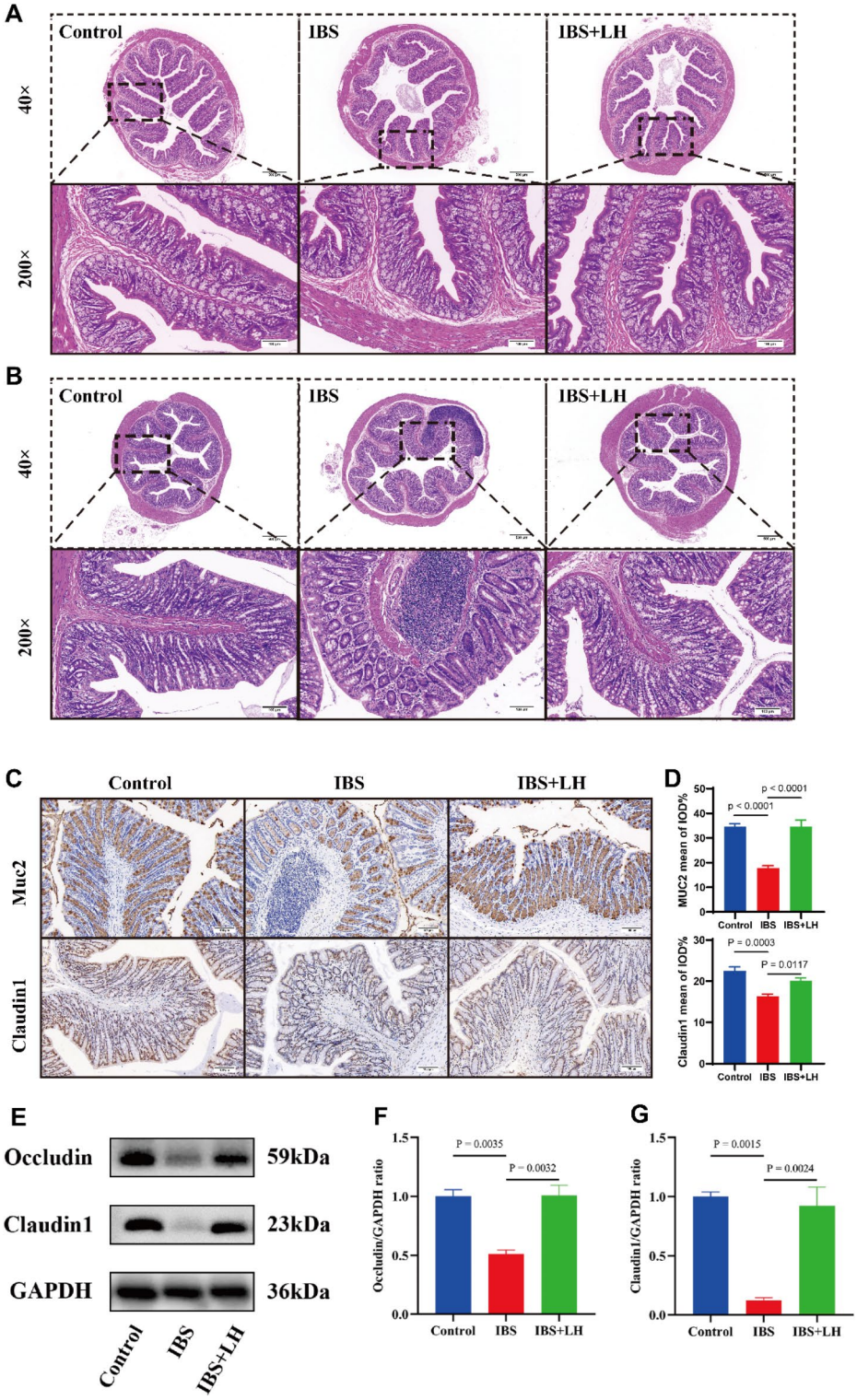

Fig. 3. Effects of LH on pathological histology of colon tissues and the expression of MUC2, claudin1, and occludin in IBS rats. (**A**) HE staining images of the proximal colon tissues. (**B**) HE staining images of the distal colon tissues. (**C**) Immunohistochemistry images of MUC2 and claudin1 in distal colon tissue. (**D**) The quantitative analysis of MUC2 and claudin1. (**E-G**) Claudin-1 and occludin expression in the colon tissues by the Western blot analysis. Values are presented as the means ± SD ($n = 3$).

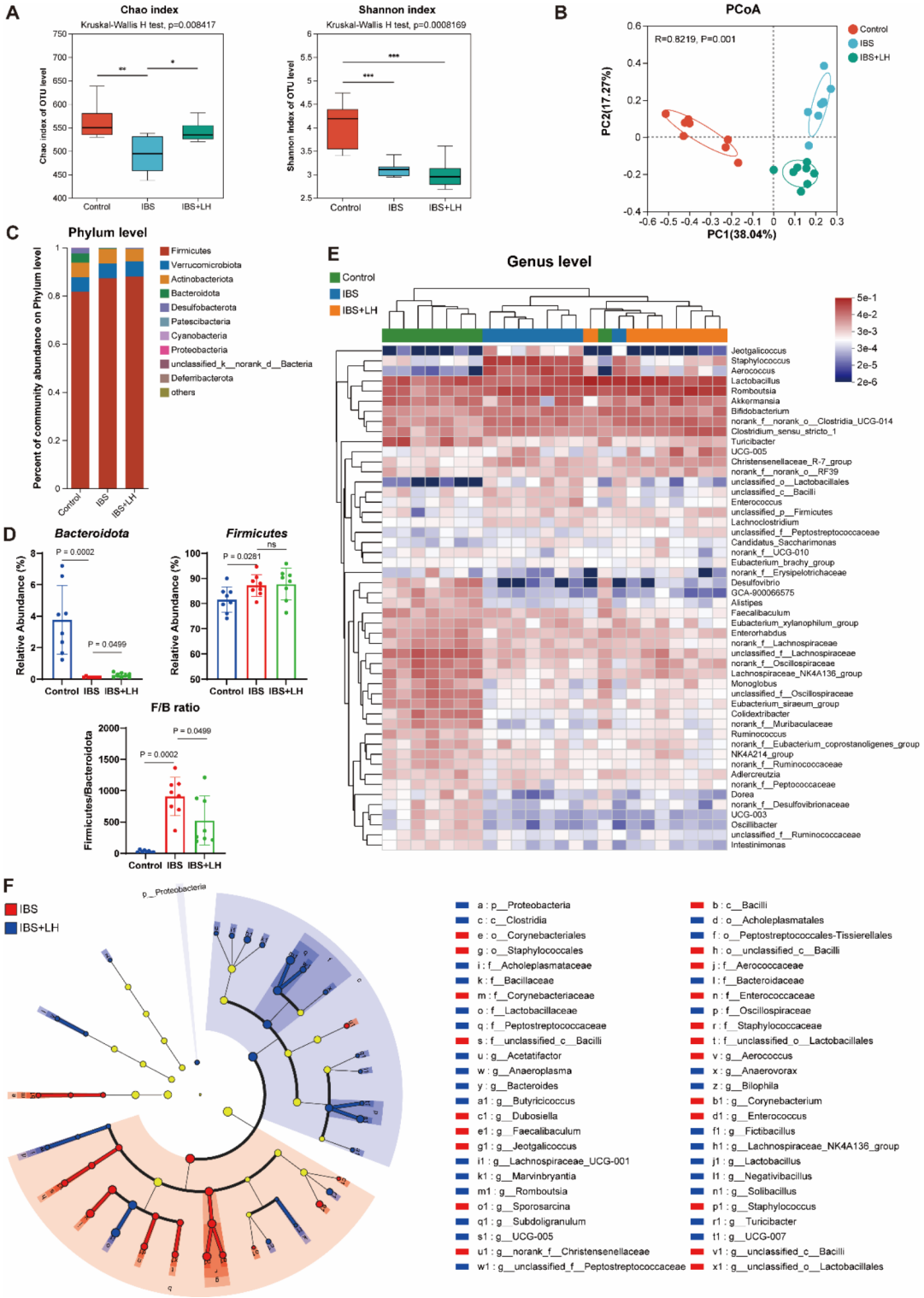

Fig. 4. 16 S rRNA sequencing analysis of the gut microbiota. (**A**) α-diversity (Chao and Shannon index) of the gut microbiota. (**B**) β-diversity (PCoA diagram) of the gut microbiota. (**C**) Histogram of relative abundance at the phylum level of the gut microbiota. (**D**) The relative abundance of *Firmicutes*, *Bacteroidota*, and F/B ratio. (**E**) Heatmap of relative abundance at the genus level of the gut microbiota. (**F**) Significant differences in gut microbiota abundance between groups by LEfSe anaylsis. Values are presented as the means ± SD ($n = 8$).

that LH could reverse the IBS modeling-induced changes in gut microbiota, including reduction in the relative abundances of *Lachnospiraceae_NK4A136_group* and *Turicibacter* and increasing that of *Aerococcus*, *Dubosiella*, *Enterococcus*, *Jeotgalicoccus*, *Sporosarcina*, and *Staphylococcus*. In addition, LH significantly increased the relative abundances of *Lactobacillaceae*, *unclassified_o_Lactobacillale*, *Lactobacillus*, *Bacteroides*, and *Negativibacillus* as compared to those in the IBS group.

Effects of LH on colon tissue gene expression in IBS rat

In order to study the effects of IBS modeling and LH treatment on gene expressions in the colon tissue of rats, a high-throughput RNA-seq analysis of the mRNA isolated from the colon tissue of rats in each group ($n=4$) was performed. In this study, the R software package "DESeq2" was used to screen differentially expressed genes (DEGs) according to $|\log2 \text{ (fold change)}| \geq 1$ and $P<0.05$. As compared to the control group, a total of 893 DEGs were identified in the IBS group, including 468 upregulated and 425 downregulated genes. Moreover, there were 121 DEGs between the IBS + LH and IBS group, including 45 were upregulated and 76 downregulated genes (Fig. 5A). Venn analysis showed 13,487 shared differential genes among the three groups, indicating that these genes may be related to the mechanism of LH treatment for IBS (Fig. 5B). Principal component analysis (PCA) of gene expression in each group showed that the samples in the IBS + LH and IBS groups could not be distinguished (Fig. 5C). As shown in the volcano plot and heatmap (Fig. 5D, E), the administration of LH reduced the expression levels of some inflammatory factor-related genes, including C-C motif chemokine ligand 4 (*Ccl4*), interleukin 17 C (*Il17c*), interleukin 12 receptor beta 2 (*Il12rb2*).

The Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis (Fig. 5F) indicated that the DEGs between the IBS and IBS + LH groups were significantly enriched in cytokine-cytokine receptor interaction, cortisol synthesis and secretion, peroxisome proliferator-activated receptor signaling pathway, and pantothenate and CoA biosynthesis and exhibited a certain relationship with renin angiotensin aldosterone system. Gene Ontology (GO) enrichment analysis of the DEGs between the IBS and IBS + LH groups (Fig. 5G) revealed that LH could significantly affect various immune-related functions, including activation or signaling pathway of B cell and leukocyte, regulation of various immune system processes, cell recognition and interaction, and some biological processes related to immunoglobulin. Notably, the significant enrichment of defense response to a bacterium, response to a bacterium, defense response to other organizations, and interactions between organizations and other pathways suggested that the LH-induced alteration of gut microbiota had a certain impact on regulating the gene expression and immunity of colon in IBS rats. In addition, the Gene-Set Enrichment Analysis (GSEA) of the gene expression levels in the IBS and IBS + LH group rats (Fig. 5H) showed significant upregulation in the pathways related to DNA replication and chromosome and tubulin binding in the IBS + LH group, suggesting that LH might affect the cell mitosis in the colon of IBS rats.

Discussion

IBS is a common gastrointestinal disorder characterized by abdominal discomfort, recurrent abdominal pain, and changes in bowel habits, which severely affect the quality of a patient's life[27]. Its pathogenesis is associated with visceral allergies, gastrointestinal motility disorders, intestinal permeability, and gut-brain axis; all these might be influenced by the gut microbiota[28]. Numerous studies have shown that probiotics can alleviate symptoms in patients with IBS by regulating gut microbiota, visceral allergies, gastrointestinal dyskinesia, and inflammatory responses[7,29–31]. A clinical study conducted by Lewis et al.[32] showed that both *Lactobacillus paracasei* HA-196 and *Bifidobacterium longum* R0175 could improve mental health and the quality of IBS patients' lives and reduce the severity of gastrointestinal symptoms. Andresen et al.[33] investigated heat-inactivated *Bifidobacterium*HI-MIMBb75 in IBS patients and showed that non-active probiotics could significantly relieve abdominal discomfort and bowel abnormalities in IBS patients. The mechanism of action of probiotics in IBS is diverse, heterogeneous, and strain-specific[7]. Studies have found that *Bifidobacteria*and lactic acid bacteria can specifically relieve IBS symptoms[34]. LH is a probiotic metabolite produced by lactic acid bacteria[35], which has a regulatory effect on the dysbiosis of gut microbiota. However, the effects and mechanism of LH in treating IBS are not clear. This study found that LH treatment could significantly reduce the visceral sensitivity and intestinal inflammatory response in IBS rats, improve damage to the intestinal mucosal barrier, relieve anxiety and depression, regulate gut microbiota, and promote the recovery of IBS rats.

Visceral hypersensitivity, the most important feature of IBS, is manifested by a decreased stress threshold of visceral tissues to external stimuli[36]. About 50–90% of IBS patients have visceral allergies, and the most significant and common clinical symptom is abdominal pain[37]. Clinical studies have shown reduced pain threshold of patients with IBS. Moreover, the chronic gastrointestinal stress response alters the hypothalamic-pituitary-adrenal axis, which promotes gastrointestinal movement and enhances visceral pain perception. The same degree of visceral sensitivity is an important mechanism, which causes pain response and participates in the peripheral mechanism of the intestinal response by altering intestinal secretion and movement in patients with IBS. AWR score is commonly used to evaluate the visceral sensitivity in rats. It had been reported that compared with the normal group, the visceral pain thresholds of IBS rats with were significantly reduced, indicating a significant increase in visceral sensitivity, which was consistent with the clinical characteristics of IBS. Baizhu shaoyao decoction and Linderae Radix water extract could effectively improve the visceral pain threshold and reduced the AWR score[38,39]. In this study, it was also found that the volume threshold of rats with IBS was significantly lower than that of the control group, indicating enhanced visceral sensitivity, which could be improved by LH.

An epidemiological study has shown that patients with IBS have psychiatric comorbidities (such as anxiety, depression, somatization, or neuroticism), and patients with psychiatric disorders have significantly higher incidence of IBS[3]. Gastrointestinal symptoms in patients with IBS undergoing psychiatric treatment are often overlooked. Psychological disorders are common in patients with IBS symptoms; therefore, IBS and

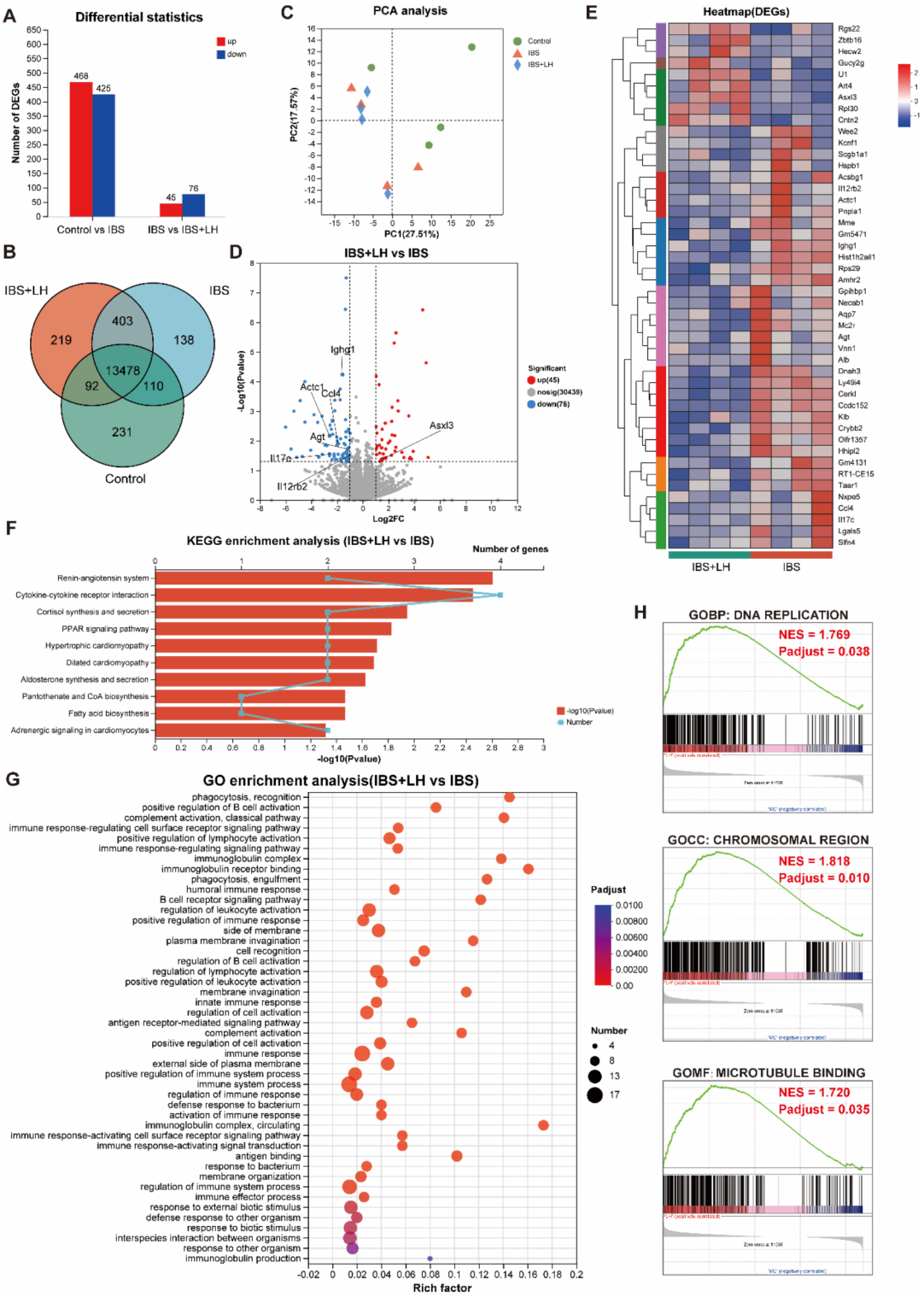

Fig. 5. RNA-seq analysis of mRNA in colon tissue. (**A**) Statistical table of differentially expressed genes. (**B**) Venn diagram of gene expression among different groups. (**C**) PCA of gene expression of samples in each group. (**D**) Volcano plot of differentially expressed genes between IBS and IBS + LH groups. (**E**) Heatmap of differentially expressed genes between IBS and IBS + LH groups. (**F**) GO enrichment analysis of DEGs between IBS and IBS + LH groups. (**G**) KEGG enrichment analysis of DEGs between IBS and IBS + LH groups. (**H**) GSEA of gene differential expression between IBS and IBS + LH groups ($n = 4$).

psychological disorders are considered comorbid and treated in combination[7]. In the current study, open-field and sugar-water preference experiments showed that LH could effectively improve the symptoms of anxiety and depression in IBS rats. In addition, LH could significantly reduce the intestinal propulsion rate in IBS rats and alleviate intestinal motility disorders. Psychological and social factors play an important role in the onset of IBS, mental stress, and external stress stimulation[40]. It had been reported in the literature that *Nigella sativa*seed oil exerted protective efficacy against stress-induced IBS mediated anxiety and depression-like symptoms[41]. A study found that IBS patients exhibited abnormal nerve central reflexes, which might be caused by mental stress stimulation[42]. Mental stress can change the intestinal migrating motor complexes[43], which might cause abnormal gastrointestinal motility in IBS patients. Stress response increases the secretion of certain hormones in the gastrointestinal tract, leading to abnormal gastrointestinal motility[44]. The neurohumoral regulation pattern might be one of the causes of gastrointestinal functional disorders in IBS patients. Therefore, this neurohumoral regulation pattern might also provide a basis for relieving the mental and psychological pressure of IBS patients as much as possible during the treatment of IBS patients, improving the treatment effect.

Transcriptomic analysis showed that LH had little effect on gene expression in the colon tissue of IBS rats. The gene expression and functional enrichment analysis showed that the effects of LH were mainly related to inflammation and immune response. Intestinal mucosal inflammation and immune changes play a significant role in the pathogenesis and progression of IBS. The IBS patients have a continuous increase in mucosal inflammation and recruitment of enteroendocrine cells in their gut as well as high levels of various proinflammatory factors. At the same time, they have abnormal immune activation in the local gut, which is mainly reflected in the increase in mast cell density and activity. These symptoms are closely related to the dysregulation of gut microbiota[45,46]. These immune and inflammatory changes lead to intestinal barrier dysfunction and induction of abdominal pain through the gut-brain-axis pain conduction nerve[47,48]. In the current study, LH reduced the expression of some proinflammatory factors and altered the immune state. The examination of pathophysiology also showed that LH could protect the intestinal barrier. Interestingly, LH affected DNA replication as well as chromosome and tubulin binding of colon tissue cells in IBS rats, which might be related to epigenetic modifications, such as chromatin remodeling, DNA methylation, and other factors in intestinal tissue[49]. However, the mechanistic role of LH requires further study.

GO enrichment analysis of the gene expressions in colon tissue also suggested that the effects of LH might be related to the regulation of gut microbiota. Gut microbiota is in direct contact with the intestinal epithelium of IBS patients and interacts with the intestinal barrier. Moreover, gut microbiota can regulate the immune system through metabolites and participate in the development of various human functions and diseases[50]. Currently, numerous clinical studies have shown that IBS is associated with the composition and dysfunction of gut microbiota[51,52]. Some bacteria have been proven to be associated with the therapeutic effects of IBS, and changes in their abundance might lead to some metabolic changes. This leads to the regulation of inflammation and immune system, intestinal motor dysfunction, and visceral hypersensitivity, thus promoting the development of IBS symptoms. Therefore, these bacteria are considered as potential therapeutic targets[53,54]. On the other hand, some bacteria can improve the host microecological balance and alleviate the IBS symptoms[33]. At present, these probiotics have become a key research direction of IBS treatment[55–57].

In this study, IBS modeling significantly altered the composition and structure of gut microbiota in rats, decreased the relative abundances of *Lachnospiraceae_NK4A136_group* and *Turicibacter*, and increased those of *Aerococcus*, *Dubosiella*, *Enterococcus*, *Jeotgalicoccus*, *Sporosaroina*, and *Staphylococcus*. LH reversed the IBS-induced change trend of some bacterial groups. Among them, *Lachnospiraceae_NK4A136_group*are butyrate-producing bacteria, and butyrate levels are negatively correlated with intestinal inflammation[58]. The abundance of *Turicibacter*is negatively correlated with 5-hydroxytryptamine (5-HT) levels in the peripheral system (serum and colon) and central nervous system[59]. 5-HT plays an important role in gastrointestinal motility and pain perception and is the main target in IBS intervention[60]. Among the bacteria with increased relative abundance after modeling, *Aerococcus*, *Enterococcus*, and *Staphylococcus*are human pathogens, and LH could significantly reduce their abundance[61–63]. IBS patients showed a significant increase in the number of α-diversity in gut microbiota was lower than that of the normal population[64]. In this study, IBS modeling significantly reduced the richness and evenness of gut microbiota in rats, while LH restored the richness to a certain extent. Similarly, consistent with the changes in gut microbiota in IBS-D patients in multiple clinical studies[64,65], the ratio of Firmicutes/Bacteroidete (F/B) in the IBS group increased significantly, which was reduced by LH to a certain extent. In addition, in this study, LH increased the relative abundances of *Lactobacillaceae*, *unclassified_O_Lactobacillus* and *Lactobacillus*are also similar to the results of LH dramatically increased the abundance of Lactobacillus in the past research[66]. In a clinical study, it was found that the relative abundance of *Lactobacillus*in IBS patients was significantly lower than that in healthy individuals[67]. In general, LH might play a role in the pathophysiology of IBS by altering gut microbiota and interfere with the imbalance of gut microbiota in IBS patients to some extent. The modulation of the gut microbiota has important therapeutic potential and requires further validation.

Conclusion

Collectively, LH exhibited a significant ameliorating effect on visceral sensitivity, intestinal motility disorders, and anxiety-depressive behavior in IBS rats. LH might improve IBS by reducing the relative abundances of *Lachnospiraceae_NK4A136_group* and *Turicibacter*, etc., regulating the structure and composition of the gut microbiota, and alleviating the intestinal inflammatory response.

Materials and methods

Materials and chemicals

The Lacidophilin tablets were obtained from Jiangzhong Pharmaceutical Co., Ltd. (Jiangxi, China), the acetic acid was purchased from Sichuan Jinshan Pharmaceutical Co., Ltd. (Sichuan, China). Anti-MUC2, anti-claudin 1, anti-occludin, goat anti-rabbit IgG, goat anti-mouse IgG, and the diaminobenzidine (DAB) chromogenic kit were purchased from Service-Bio Co., Ltd. (Wuhan, China).

Animals and experimental design

Seven-week-old male SD rats (160 ± 20 g) were purchased from Zhejiang Weitong Lihua Laboratory Animal Technology Co., Ltd. (Zhejiang, China). The rats were housed under the conditions of 12 h/12 h light/dark cycle, temperature of 22 ± 2 °C, and relative humidity of 50% ± 10%. Experimental protocols conformed to the requirements of the Experimental Animal Ethics Committee of Jiangzhong Pharmaceutical Co., Ltd. (20220415).

After adaptive feeding for one week, the rats were randomly divided into control group, IBS group, and IBS + LH group (n= 8 rats per group). The IBS model was established using the combination of acetic acid enema with restrain stress[6]. Except for the control group rats, all the rats were injected with 0.5 mL of 5% acetic acid solution into their colon every day for two weeks. Acetic acid enema rats were placed in a restraint device for 2 h twice a day (once in the morning and once in the afternoon) for 4 weeks. After the modeling, IBS + LH group rats were intragastrically administered 0.84 g/kg LH (Lacidophilin tablets pulverized into powder and dissolved in water) every morning for two weeks. The control and IBS groups rats were given an equal amount of pure water.

Visceral sensitivity assessment

Colorectal dilatation was performed on the 14th day of administration, and visceral sensitivity was assessed using the abdominal withdrawal reflex (AWR) score[68–70]. After the rats were fasted for 24 h, they were anesthetized with isoflurane. A double-cavity 6 F catheter was applied with paraffin oil and inserted into their anus, keeping the distance between the catheter balloon and the anus about 6 cm. The catheter was fixed at the tail of the rats using a medical tape. After the rats recovered from anesthesia, they were acclimated for 30 min, and normal saline was injected into the balloon to keep the balloon inflated for 20 s. The volume of normal saline injection that caused the rats to reach an AWR score of 3 was recorded. The measurement was repeated three times, and the average value was taken as the minimum AWR volume threshold of the rats. At 0 AWR score, the rats had no behavioral response to colorectal dilation; at 1 AWR score, the head movement of rats decreased but their body remained stationary; at 2 AWR score, the abdominal muscles of the rats contracted, and the abdomen did not move away from the desktop; at 3 AWR score, the abdominal muscles of the rats contracted, and the abdomen left the desktop; at 4 AWR score, the pelvis of the rat was raised, and their body was arched.

Open-field test

Open field text[71] was performed on the 14th day of administration. A 100 cm × 100 cm × 40 cm vertical column open-field test box with black inner and bottom sides was used. The rats were placed in the bottom center of the experimental box, and their movement within 5 min was tracked in real-time using VisuTrack animal behavior analysis software from Shanghai Xinsoft. The test was conducted in a dark room. After testing a rat, its urine and feces were cleaned with alcohol, and the box was dried before testing the next rat. The span times and total distance of movement were counted to evaluate the motor ability of rats.

Sucrose preference test

The sucrose preference test[72] was performed on the 10th day of administration. During the test, the rats were kept in a single cage, and each rat was given 2 bottles of 1% sucrose solution for 24 h. Then, the two bottles were replaced with one bottle of 1% sucrose solution and one bottle of water for 24 h. All the rats were then deprived of water for 23 h, and each rat was given a bottle of pre-weighed 1% sucrose solution and a bottle of pre-weighed water. After 1 h, the consumption of 1% sucrose solution and water was calculated by weighing the remaining bottles. Finally, the percentage of sucrose preference of rats was calculated using Eq. (1).

$$\text{Sucrose preference } (\%) = \text{Sucrose intake}/(\text{Sucrose intake} + \text{Water intake}) \times 100\% \quad (1)$$

Intestinal propulsion rate

The rats in each group were given 2 mL of Evans blue by instillation. After 20 min, the rats were euthanized. Their small intestines from the cardia to the end of the ileum were taken, and the length of Evans blue advancing in the intestine and the total length of the small intestine were measured[73]. The intestinal propulsion rate was calculated using Eq. (2).

$$\text{Intestinal propulsion rate } (\%) = \text{Evans Blue propulsion distance}/\text{total intestinal length} \times 100\% \quad (2)$$

Sample collection

The whole colons of rats were collected, and 2-cm long sections were cut from the proximal and distal colon. The colon sections were fixed in a 4% paraformaldehyde solution. The remaining colon and colonic feces were collected into sterile freezing tubes, frozen in liquid nitrogen, and stored at − 80 °C.

Hematoxylin and Eosin (HE) staining

The proximal and distal colon tissue sections were fixed in a 4% paraformaldehyde solution, dehydrated with gradient alcohol, and paraffinized. The paraffin blocks were fixed on the paraffin slicing machine, followed by

dewaxing, hydration, and HE staining. The changes in the mucosal morphology of proximal and distal colon sections were observed under an optical microscope.

Immunohistochemistry of mucin 2 (MUC2) and claudin1 expression in distal colon tissues

After dewaxing, the prepared slices were repaired using the heat-induced antigen retrieval method, followed by blocking with 3% bovine serum albumin for 30 min. The slides were then incubated with primary antibodies overnight at 4℃, followed by incubation with respective secondary antibodies at 37℃. After DAB color-rendering, the tissue sections were stained with hematoxylin solution, dehydrated with gradient alcohol, and made transparent with xylene. Finally, the slides were sealed with neutral resin and photographed under a Nikon positive fluorescence microscope.

Western blot analysis

The expressions and of claudin-1 and occludin in the intestine were determined by western blot analysis as described previously[74]. Take out the preserved colon tissues from the − 80℃ refrigerator, the BCA method was to determine the protein content of each sample, and then prepare a sodium dodecyl sulfate-polyacrylamide gel electrophoresis to load and separate the same amount of protein and transferred it to the PVDF membranes. The membranes were closed with rapid closure solution for 20 min at room temperature and incubated with primary antibody at 4 °C overnight, and then the secondary antibody was reacted at room temperature for 2 h. The protein bands were imaged through ultra-sensitive ECL and quantified with Image J software.

High-throughput sequencing of gut microbial 16 S rRNA gene

The total microbial DNA was extracted from frozen fecal samples using the DNeasy PowerSoil Kit (Qiagen). The V3-V4 regions of 16 S rRNA gene were amplified using the common primers (forward: 5′-ACTCCTACGG GAGGCAGCA-3′ and reverse: 5′-GGACTACHVGGGTWTCTAAT-3′) by the ABI GeneAmp® 9700 PCR thermocycler. The amplicons were sequenced on Illumina Miseq platform. The raw sequence reads were filtered for quality control and optimized. The gut microbiota was clustered using operational taxonomic unit (OTU) clustering with 97% similarity as threshold. The representative OTU sequences were obtained after removing the chimeric sequences in the clustering process. All the optimized sequences were mapped to representative OTU sequences, and those with more than 97% similarity were selected to generate OTU tables. The species were then classified based on the OTU table, and a phylogenetic tree was generated. The relative abundance of samples at each level was identified. The diversity of each library was analyzed using OTU tabular data.

Transcriptome sequencing

The colonic tissues were entrusted to Shanghai Majorbio Bio-pharm Technology Co., Ltd. for RNA-sequencing (RNA-Seq). Total RNA was extracted using Trizol Reagent (Thermo Fisher Scientific, USA). The library preparation and RNA-seq transcriptome sequencing were performed using Illumina Novaseq 6000 platform. RNA-Seq data were analyzed using the Majorbio Cloud platform (https://www.majorbio.com).

Statistical analysis

The data were expressed as mean ± standard deviation. Statistical analyses were performed using GraphPad Prism 9 software (GraphPad, La Jolla, USA) and SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The significance of differences between groups was analyzed using analysis of variance (ANOVA). *P*-value of less than 0.05 was considered statistically significant.

Data availability

All the data that were used to support the findings of this study are included within the article. The gut microbial 16 S rRNA data were submitted to SRA at https://dataview.ncbi.nlm.nih.gov/object/PRJNA1173965?reviewer=fbe2c4ib62mk51s3q9ol1coq5h (BioProject ID: PRJNA1173965). The RNA-seq data of colon were submitted to SRA at https://dataview.ncbi.nlm.nih.gov/object/PRJNA1174879?reviewer=kikfn438u6gg98263ph7dk33bv (BioProject ID: PRJNA1174879).

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References

1. Sperber, A. D. et al. Worldwide prevalence and burden of functional Gastrointestinal disorders, results of Rome foundation global study. *Gastroenterology* **160**, 99–114. https://doi.org/10.1053/j.gastro.2020.04.014 (2021).
2. Yu, S., Chun, E., Ji, Y., Lee, Y. J. & Jin, M. Effects of red ginseng on gut, microbiota, and brain in a mouse model of post-infectious irritable bowel syndrome. *J. Ginseng Res.* **45**, 706–716. https://doi.org/10.1016/j.jgr.2021.03.008 (2021).
3. Janssens, K. A., Zijlema, W. L., Joustra, M. L. & Rosmalen, J. G. Mood and anxiety disorders in chronic fatigue syndrome, fibromyalgia, and irritable bowel syndrome: results from the lifelines cohort study. *Psychosom. Med.* **77**, 449–457. https://doi.org/10.1097/PSY.0000000000000161 (2015).
4. Chang, L. et al. AGA clinical practice guideline on the Pharmacological management of irritable bowel syndrome with constipation. *Gastroenterology* **163**, 118–136. https://doi.org/10.1053/j.gastro.2022.04.016 (2022).
5. Niesler, B., Kuerten, S., Demir, I. E. & Schafer, K. H. Disorders of the enteric nervous system - a holistic view. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 393–410. https://doi.org/10.1038/s41575-020-00385-2 (2021).
6. Zhuang, Z., Huang, C., Zhang, Y. & Lv, B. Effects of Massa medicata fermentata on the intestinal pathogenic flagella bacteria and visceral hypersensitivity in rats with irritable bowel syndrome. *Front. Physiol.* **13**, 1039804. https://doi.org/10.3389/fphys.2022.1039804 (2022).
7. Simon, E., Calinoiu, L. F., Mitrea, L. & Vodnar, D. C. Probiotics, prebiotics, and synbiotics: implications and beneficial effects against irritable bowel syndrome. *Nutrients* **13**, 2112–2139. https://doi.org/10.3390/nu13062112 (2021).

8. Lee, C. et al. The increased level of depression and anxiety in irritable bowel syndrome patients compared with healthy controls: systematic review and meta-analysis. *J. Neurogastroenterol Motil.* **23**, 349–362. https://doi.org/10.5056/jnm16220 (2017).
9. Zhou, Y. et al. Bifico relieves irritable bowel syndrome by regulating gut microbiota dysbiosis and inflammatory cytokines. *Eur. J. Nutr.* **62**, 139–155. https://doi.org/10.1007/s00394-022-02958-0 (2023).
10. Thumann, T. A., Pferschy-Wenzig, E. M., Moissl-Eichinger, C. & Bauer, R. The role of gut microbiota for the activity of medicinal plants traditionally used in the European union for Gastrointestinal disorders. *J. Ethnopharmacol.* **245**, 112153. https://doi.org/10.1016/j.jep.2019.112153 (2019).
11. Shaikh, S. D., Sun, N., Canakis, A., Park, W. Y. & Weber, H. C. Irritable bowel syndrome and the gut microbiome: a comprehensive review. *J. Clin. Med.* **12**, 2558–2580. https://doi.org/10.3390/jcm12072558 (2023).
12. Jandhyala, S. M. et al. Role of the normal gut microbiota. *World J. Gastroenterol.* **21**, 8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787 (2015).
13. Levy, M., Blacher, E. & Elinav, E. Microbiome, metabolites and host immunity. *Curr. Opin. Microbiol.* **35**, 8–15. https://doi.org/10.1016/j.mib.2016.10.003 (2017).
14. Bhattarai, Y., Muniz Pedrogo, D. A. & Kashyap, P. C. Irritable bowel syndrome: a gut microbiota-related disorder? *Am. J. Physiol. Gastrointest. Liver Physiol.* **312**, 52–62. https://doi.org/10.1152/ajpgi.00338.2016 (2017).
15. Sciavilla, P. et al. Gut microbiota profiles and characterization of cultivable fungal isolates in IBS patients. *Appl. Microbiol. Biotechnol.* **105**, 3277–3288. https://doi.org/10.1007/s00253-021-11264-4 (2021).
16. Tap, J. et al. Identification of an intestinal microbiota signature associated with severity of irritable bowel syndrome. *Gastroenterology* **152**, 111–123. https://doi.org/10.1053/j.gastro.2016.09.049 (2017).
17. Yu, T. et al. Effects of prebiotics and synbiotics on functional constipation. *Am. J. Med. Sci.* **353**, 282–292. https://doi.org/10.1016/j.amjms.2016.09.014 (2017).
18. Choi, C. H. et al. Efficacy of combination therapy with probiotics and mosapride in patients with IBS without diarrhea: a randomized, double-blind, placebo-controlled, multicenter, phase II trial. *Neurogastroenterol Motil.* **27**, 705–716. https://doi.org/10.1111/nmo.12544 (2015).
19. Ma, L., Tu, H. & Chen, T. Postbiotics in human health: a narrative review. *Nutrients* **15**, 291–308. https://doi.org/10.3390/nu15020291 (2023).
20. Salminen, S. et al. The international scientific association of probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 649–667. https://doi.org/10.1038/s41575-021-00440-6 (2021).
21. Aguilar-Toalá, J. E. et al. Postbiotics: an evolving term within the functional foods field. *Trends Food Sci. Technol.* **75**, 105–114. https://doi.org/10.1016/j.tifs.2018.03.009 (2018).
22. Zolkiewicz, J., Marzec, A., Ruszczynski, M. & Feleszko, W. Postbiotics-a step beyond pre- and probiotics. *Nutrients* **12**, 2189–2206. https://doi.org/10.3390/nu12082189 (2020).
23. Ji, J., Jin, W., Liu, S. J., Jiao, Z. & Li, X. Probiotics, prebiotics, and postbiotics in health and disease. *MedComm (2020)*. **4**, 420–443. https://doi.org/10.1002/mco2.420 (2023).
24. Ma, X. J. et al. Analysis and study of organic acids in Lactobacillin material. *China Dairy. Ind.* **48**, 53–56. https://doi.org/10.19827/j.issn1001-2230.2020.08.013 (2020).
25. Zhan, Y. et al. Effects of lacidophilin in a mouse model of low-grade colitis and nonalcoholic fatty liver disease. *Food Bioscience*. **60** https://doi.org/10.1016/j.fbio.2024.104469 (2024).
26. Zhan, Y. et al. Effects of lactobacillus tablets on gut microbiota in antibiotic-associated diar-rhea model mice. *China Dairy. Ind.* **49**, 4–10. https://doi.org/10.19827/j.issn1001-2230.2021.08.001 (2021).
27. Xiao, L., Liu, Q., Luo, M. & Xiong, L. Gut microbiota-derived metabolites in irritable bowel syndrome. *Front. Cell. Infect. Microbiol.* **11**, 729346. https://doi.org/10.3389/fcimb.2021.729346 (2021).
28. Jeffery, I. B., O'Herlihy, E. & Shanahan, F. PW, O. T. Microbiome alterations in IBS. *Gut* **69**, 2263–2264. https://doi.org/10.1136/gutjnl-2020-320919 (2020).
29. Xu, H. et al. Adjunctive treatment with probiotics partially alleviates symptoms and reduces inflammation in patients with irritable bowel syndrome. *Eur. J. Nutr.* **60**, 2553–2565. https://doi.org/10.1007/s00394-020-02437-4 (2021).
30. Yang, B. et al. Lactobacillus plantarum CCFM1143 alleviates chronic diarrhea via inflammation regulation and gut microbiota modulation: a double-blind, randomized, placebo-controlled study. *Front. Immunol.* **12**, 746585. https://doi.org/10.3389/fimmu.2021.746585 (2021).
31. Nishida, K., Sawada, D., Kuwano, Y., Tanaka, H. & Rokutan, K. Health benefits of lactobacillus gasseri CP2305 tablets in young adults exposed to chronic stress: a randomized, double-blind, placebo-controlled study. *Nutrients* **11**, 1859–1873. https://doi.org/10.3390/nu11081859 (2019).
32. Lewis, E. D. et al. Efficacy of lactobacillus paracasei HA-196 and bifidobacterium longum R0175 in alleviating symptoms of irritable bowel syndrome (IBS): a randomized, placebo-controlled study. *Nutrients* **12**, 1159–1178. https://doi.org/10.3390/nu12041159 (2020).
33. Andresen, V., Gschossmann, J. & Layer, P. Heat-inactivated Bifidobacterium bifidum MIMBb75 (SYN-HI-001) in the treatment of irritable bowel syndrome: a multicentre, randomised, double-blind, placebo-controlled clinical trial. *Lancet Gastroenterol. Hepatol.* **5**, 658–666. https://doi.org/10.1016/S2468-1253(20)30056-X (2020).
34. Brenner, D. M., Moeller, M. J., Chey, W. D. & Schoenfeld, P. S. The utility of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Am. J. Gastroenterol.* **104**, 1033–1050. https://doi.org/10.1038/ajg.2009.25 (2009).
35. Chuah, L. O. et al. Postbiotic metabolites produced by Lactobacillus plantarum strains exert selective cytotoxicity effects on cancer cells. *BMC Complement. Altern. Med.* **19**, 114–126. https://doi.org/10.1186/s12906-019-2528-2 (2019).
36. Azpiroz, F. et al. Mechanisms of hypersensitivity in IBS and functional disorders. *Neurogastroenterol Motil.* **19**, 62–88. https://doi.org/10.1111/j.1365-2982.2006.00875.x (2007).
37. Brierley, S. M. & Linden, D. R. Neuroplasticity and dysfunction after Gastrointestinal inflammation. *Nat. Rev. Gastroenterol. Hepatol.* **11**, 611–627. https://doi.org/10.1038/nrgastro.2014.103 (2014).
38. Wei, Y. et al. Baizhu Shaoyao Decoction restores the intestinal barrier and brain–gut axis balance to alleviate diarrhea-predominant irritable bowel syndrome via FoxO1/FoxO3a. *Phytomedicine* **122** https://doi.org/10.1016/j.phymed.2023.155163 (2024).
39. Ouyang, L. et al. A multi-omics study reveals the therapeutic effect of Linderae Radix water extract on irritable bowel syndrome (IBS-D). *J. Ethnopharmacol.* **328** https://doi.org/10.1016/j.jep.2024.118050 (2024).
40. Qin, H. Y., Cheng, C. W., Tang, X. D. & Bian, Z. X. Impact of psychological stress on irritable bowel syndrome. *World J. Gastroenterol.* **20**, 14126–14131. https://doi.org/10.3748/wjg.v20.i39.14126 (2014).
41. Sharma, M. et al. Efficacy of Nigella sativa seed oil against psychophysical stress induced irritable bowel syndrome and anxiety-like symptoms in Wistar rats. *Psychopharmacology* **241**, 2609–2626. https://doi.org/10.1007/s00213-024-06713-7 (2024).
42. Schaper, S. J. & Stengel, A. Emotional stress responsivity of patients with IBS - a systematic review. *J. Psychosom. Res.* **153**, 110694. https://doi.org/10.1016/j.jpsychores.2021.110694 (2022).
43. Deloose, E., Janssen, P., Depoortere, I. & Tack, J. The migrating motor complex: control mechanisms and its role in health and disease. *Nat. Rev. Gastroenterol. Hepatol.* **9**, 271–285. https://doi.org/10.1038/nrgastro.2012.57 (2012).
44. Vincent, K. M., Sharp, J. W. & Raybould, H. E. Intestinal glucose-induced calcium-calmodulin kinase signaling in the gut-brain axis in awake rats. *Neurogastroenterol Motil.* **23**, 282–293. https://doi.org/10.1111/j.1365-2982.2011.01673.x (2011).

45. Ng, Q. X., Soh, A. Y. S., Loke, W., Lim, D. Y. & Yeo, W. S. The role of inflammation in irritable bowel syndrome (IBS). *J. Inflamm. Res.* **11**, 345–349. https://doi.org/10.2147/JIR.S174982 (2018).
46. Aguilera-Lizarraga, J., Hussein, H. & Boeckxstaens, G. E. Publisher correction: immune activation in irritable bowel syndrome: what is the evidence? *Nat. Rev. Immunol.* **22**, 332–333. https://doi.org/10.1038/s41577-022-00713-4 (2022).
47. Barbara, G. et al. Inflammatory and microbiota-related regulation of the intestinal epithelial barrier. *Front. Nutr.* **8**, 718356. https://doi.org/10.3389/fnut.2021.718356 (2021).
48. Ohman, L. & Simren, M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nat. Rev. Gastroenterol. Hepatol.* **7**, 163–173. https://doi.org/10.1038/nrgastro.2010.4 (2010).
49. Dothel, G. et al. New insights into irritable bowel syndrome pathophysiological mechanisms: contribution of epigenetics. *J. Gastroenterol.* **58**, 605–621. https://doi.org/10.1007/s00535-023-01997-6 (2023).
50. Camilleri, M. & Boeckxstaens, G. Irritable bowel syndrome: treatment based on pathophysiology and biomarkers. *Gut* **72**, 590–599. https://doi.org/10.1136/gutjnl-2022-328515 (2023).
51. Mars, R. A. T. et al. Longitudinal multi-omics reveals subset-specific mechanisms underlying irritable bowel syndrome. *Cell* **183**, 1137–1140. https://doi.org/10.1016/j.cell.2020.10.040 (2020).
52. Black, C. J. & Ford, A. C. Global burden of irritable bowel syndrome: trends, predictions and risk factors. *Nat. Rev. Gastroenterol. Hepatol.* **17**, 473–486. https://doi.org/10.1038/s41575-020-0286-8 (2020).
53. Zhai, L. et al. Ruminococcus gnavus plays a pathogenic role in diarrhea-predominant irritable bowel syndrome by increasing serotonin biosynthesis. *Cell. Host Microbe.* **31**, 33–44. https://doi.org/10.1016/j.chom.2022.11.006 (2023).
54. Gao, J., Xiong, T., Grabauskas, G. & Owyang, C. Mucosal serotonin reuptake transporter expression in irritable bowel syndrome is modulated by gut microbiota via mast cell-prostaglandin E2. *Gastroenterology* **162**, 1962–1974. https://doi.org/10.1053/j.gastro.2022.02.016 (2022).
55. Ford, A. C., Harris, L. A., Lacy, B. E., Quigley, E. M. M. & Moayyedi, P. Systematic review with meta-analysis: the efficacy of prebiotics, probiotics, synbiotics and antibiotics in irritable bowel syndrome. *Aliment. Pharmacol. Ther.* **48**, 1044–1060. https://doi.org/10.1111/apt.15001 (2018).
56. Konstantis, G. et al. Efficacy and safety of probiotics in the treatment of irritable bowel syndrome: a systematic review and meta-analysis of randomised clinical trials using ROME IV criteria. *Clin. Nutr.* **42**, 800–809. https://doi.org/10.1016/j.clnu.2023.03.019 (2023).
57. Goodoory, V. C. et al. Efficacy of probiotics in irritable bowel syndrome: systematic review and meta-analysis. *Gastroenterology* **165**, 1206–1218. https://doi.org/10.1053/j.gastro.2023.07.018 (2023).
58. Costantini, L., Molinari, R., Farinon, B. & Merendino, N. Impact of omega-3 fatty acids on the gut microbiota. *Int. J. Mol. Sci.* **18**, 2645–2663. https://doi.org/10.3390/ijms18122645 (2017).
59. Kong, Q. et al. Daily intake of Lactobacillus alleviates autistic-like behaviors by ameliorating the 5-hydroxytryptamine metabolic disorder in VPA-treated rats during weaning and sexual maturation. *Food Funct.* **12**, 2591–2604. https://doi.org/10.1039/d0fo02375b (2021).
60. Camilleri, M. & Boeckxstaens, G. Dietary and Pharmacological treatment of abdominal pain in IBS. *Gut* **66**, 966–974. https://doi.org/10.1136/gutjnl-2016-313425 (2017).
61. Rasmussen, M. Aerococcus: an increasingly acknowledged human pathogen. *Clin. Microbiol. Infect.* **22**, 22–27. https://doi.org/10.1016/j.cmi.2015.09.026 (2016).
62. Antonello, R. M., Riccardi, N., Saderi, L. & Sotgiu, G. Synergistic properties of linezolid against Enterococcus spp. Isolates: a systematic review from in vitro studies. *Eur. J. Clin. Microbiol. Infect. Dis.* **23**, 10096. https://doi.org/10.1007/s10096-023-04704-8 (2023).
63. Kumar, G. & Engle, K. Natural products acting against S. aureus through membrane and cell wall disruption. *Nat. Prod. Rep.* **40**, 1608–1646. https://doi.org/10.1039/d2np00084a (2023).
64. Duan, R., Zhu, S., Wang, B. & Duan, L. Alterations of gut microbiota in patients with irritable bowel syndrome based on 16S rRNA-targeted sequencing: a systematic review. *Clin. Transl Gastroenterol.* **10**, e00012. https://doi.org/10.14309/ctg.0000000000000012 (2019).
65. Jalanka-Tuovinen, J. et al. Faecal microbiota composition and host-microbe cross-talk following gastroenteritis and in postinfectious irritable bowel syndrome. *Gut* **63**, 1737–1745. https://doi.org/10.1136/gutjnl-2013-305994 (2014).
66. Yang, N. et al. Effects of lacidophilin tablets, yogurt, and bifid triple viable capsules on the gut microbiota of mice with antibiotic-associated diarrhea. *Can. J. Infect. Dis. Med. Microbiol.* **2022** (6521793). https://doi.org/10.1155/2022/6521793 (2022).
67. Napolitano, M. et al. Gut dysbiosis in irritable bowel syndrome: a narrative review on correlation with disease subtypes and novel therapeutic implications. *Microorganisms* **11**, 2369–2400. https://doi.org/10.3390/microorganisms11102369 (2023).
68. Shaidullov, I. F. et al. Short chain fatty acids and colon motility in a mouse model of irritable bowel syndrome. *BMC Gastroenterol.* **21**, 37–49. https://doi.org/10.1186/s12876-021-01613-y (2021).
69. Han, X. Y. Electroacupuncture protects the intestinal mucosal barrier in diarrhea-predominant irritable bowel syndrome rats by regulating the MCs/Tryptase/PAR-2/MLCK pathway. *Am. J. Translational Res.* **16**, 781–793. https://doi.org/10.62347/vzjl1218 (2024).
70. Song, S. et al. Effects of PAR2 gene knockout on visceral sensitivity, stress behaviors, and colonic electrical activities in irritable bowel syndrome. *Discov. Med.* **35** https://doi.org/10.24976/Discov.Med.202335178.75 (2023).
71. Zhou, D. et al. Protective effects of Liupao tea against high-fat diet/cold exposure-induced irritable bowel syndrome in rats. *Heliyon* **9**, e16613. https://doi.org/10.1016/j.heliyon.2023.e16613 (2023).
72. Xia, J. et al. The comparison of sex differences in depression-like behaviors and neuroinflammatory changes in a rat model of depression induced by chronic stress. *Front. Behav. Neurosci.* **16**, 1059594. https://doi.org/10.3389/fnbeh.2022.1059594 (2022).
73. Wang, L. et al. Transdermal administration of volatile oil from citrus aurantium-rhizoma atractylodis macrocephalae alleviates constipation in rats by altering host metabolome and intestinal microbiota composition. *Oxid Med Cell Longev* 9965334, (2022). https://doi.org/10.1155/2022/9965334 (2022).
74. Xiong, T. et al. Ganluyin ameliorates DSS-induced ulcerative colitis by inhibiting the enteric-origin LPS/TLR4/NF-κB pathway. *J. Ethnopharmacol.* **289** https://doi.org/10.1016/j.jep.2022.115001 (2022).

Author contributions

Huiqun Fan and Yang Zhan: drafted the manuscript, made data analysis, and participated in the design of the study. Huiqun Fan and Xiaoying Cheng: performed the experiments and collected the data. Mintao Tan: participated in analysis of intestinal microbiota data. Yang Zhan, Yingmeng Li, and Yanxia Xiong: supervised the scientific work and revised the manuscript. Qiong Li and Wenjun Liu: conceived and designed this study and supervised the research team. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethical declarations
All animal experiments in this study were approved by the experimental animal ethics committee of Jiangzhong Pharmaceutical Co., Ltd. (No. 20220415). All procedures were carried out according to Guidelines for the Care and Use of Laboratory Animals and the Chinese Legislation on Laboratory Animals. The reporting in this study follows the recommendations in the ARRIVE guidelines.

Additional information
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