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Effect of Jinhuahecha—A Fermented Green Tea on DSS-Induced Ulcerative Colitis in Mice and Its Preliminary Pharmacological Mechanism

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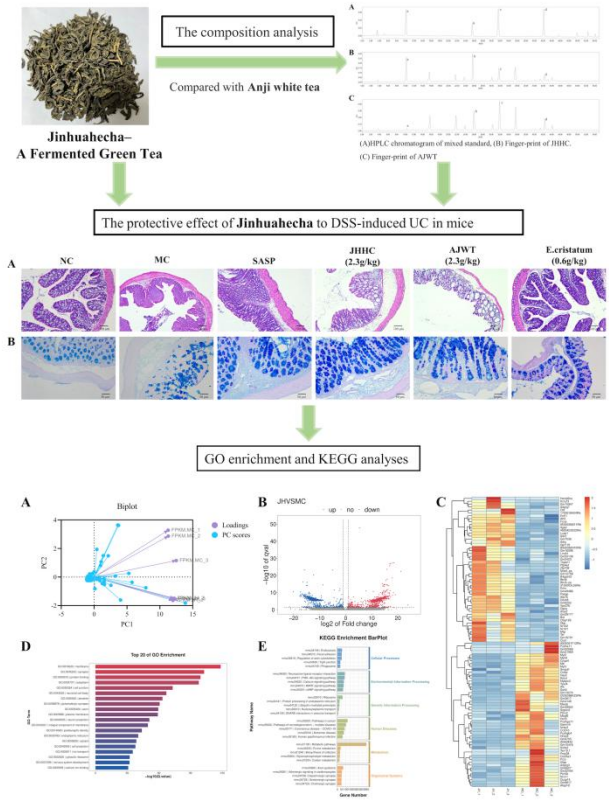
Abstract

Background: Ulcerative colitis (UC) is an inflammatory bowel disease. Jinhuahecha (JHHC)-a fermented green tea is obtained by micro-fermentation of Anji white tea (AJWT) inoculated with high-purity "*E. cristatum*". **Purpose**: This study is aimed to unravel the protective effect of JHHC to UC in mice. **Methods:** High performance liquid chromatography (HPLC) was applied to determine the effective components and their contents in JHHC and AJWT. UC mouse model was constructed by ingestion of 2.5% dextran sulfate sodium (DSS) aqueous solution for 9 days. Disease activity index (DAI) was evaluated with the daily weight, fecal characters, and bloody stool condition, the length of colon and the spleen coefficients in mice was measured. Hematoxylin and eosin and Alcian blue-periodic acid Schiff staining was performed to observe the histopathological changes of the colon tissue. The positive expression of MyD88 and TNF- α were observed by immunofluorescence and immunohistochemistry. The possible mechanism was speculated by via transcriptomics. **Results:** HPLC results indicated that compared with AJWT, in JHHC, the content of gallic acid was significantly increased, the caffeine content was slightly decreased, and the polyphenolic components were notably declined. Compared to normal control group, in model control group, DAI score and spleen coefficient were elevated, the length of colon was shortened, and the colon tissue was severely damaged. In contrast to model control group, the above indexes were improved in treatment group, especially in JHHC group, which was possibly associated with regulation of inflammatory response. **Conclusions:** JHHC, as a natural plant product, has anti-inflammatory effects that can alleviate UC in mice.

Graphic Abstract



1 Introduction

Ulcerative colitis (UC) is a chronic, non-specific and lifelong inflammatory bowel disease (IBD) [1], mainly involving the colon and extending from rectum, which affects the colon to varying degrees [2]. The clinical manifestations include severe abdominal pain, persistent or intermittent diarrhea, bloody purulent stool, etc. The etiology of UC is primarily related to genetic factors and environmental changes [3,4], but there is a lack of knowledge about pathogenesis of UC and most scholars consider UC as an autoimmune disease [5]. Since the 21st century, the incidence rate of IBDs, such as UC, has increased year by year, which greatly affects the normal life of patients owing to repeated condition and high recurrence rates after anti-inflammatory treatment [6]. Currently, the drug treatment of UC mainly depends on salicylic acid preparations (e.g., sulfasalazine), hormones (e.g., methylprednisolone), immunosuppressants (e.g., methotrexate) and biological agents (e.g., infliximab) that can effectively suppress intestinal inflammatory reaction; however, these drugs incur adverse reactions including gastrointestinal reactions, abnormal liver function, and decreased immune function, and may even increase the risk of malignant tumor [7,8].

Tea, a natural product, is one of the most popular drinks secondary to water because of its unique taste and rich nutrients [9,10]. According to the processing method and fermentation degree, tea can be divided into three types: unfermented green or white tea, semi-fermented oolong tea, and fully fermented black tea [9]. Anji white tea (AJWT) is a kind of special green tea [11] that is mainly produced in Anji County, Zhejiang province, China, and is a national geographic indication product [12], characterized by more amino acids and fewer tea polyphenols.

"Jinhua" bacteria, also known as *Eurotium cristatum* (*E. cristatum*), is a kind of fungus belonging to *Eurotiales*, *Trichocomaceae*, and *Eurotium*, and constituted by ascocarp and mycelium [13], which was first found in Fuzhuan brick tea [14] and is an enzymatic bacteria that is beneficial to human body. Inoculation of *E. cristatum* to tea can cause the covering of tea with golden yellow granular "*E. cristatum*" during the fermentation process, thereby forming a unique taste and flavor, and various physiological functions [15,16]. Jinhuahecha (JHHC) — a fermented green tea used in this study was obtained by microbial transformation of AJWT as raw material using high-purity *E. cristatum*. AJWT contains various active ingredients such as theanine, tea polyphenols (mainly Epicatechin, Epigallocatechin, Epigallocatechin gallate, Epicatechin gallate), gallic acid, caffeine, tea polysaccharides, etc. [17,18]. These active ingredients have been confirmed to have functions of anti-oxidant, anti-inflammation, anti-bacteria, reducing blood glucose, improving cardiovascular and cerebrovascular health, enhancing immunity, and maintaining digestive system health

[18-20]. Based on the above findings, this study was aimed to further observe the changes in the composition of AJWT after intervention with *E. cristatum* and the protective effect of *E. cristatum* on dextran sulfate sodium (DSS)-induced UC in mice, to promote the development and utilization of AJWT.

2.2 Materials and methods

2.1 Reagents and drugs

Anji white tea, 'Jinhuahecha' and *Eurotium cristatum* conidial powder were provided by Anji Hecha Biotechnology Co., Ltd (Anji, China). Gallic acid (GA, A0110), Caffeine (Caf, A0470), (-)-Epigallocatechin Gallate (EGCG, A0159), and (-)-Epicatechin gallate (ECG, A0160) were procured from Chengdu Must Bio-technology Co., Ltd (Chengdu, China). Dextran sulfate sodium (DSS, MW 36000-50000) was purchased from MP Biomedicals (Southern California, USA). Sulfasalazine (SASP) enteric coated tablets were obtained from Shanghai Sine Tianping Pharmaceutical Co., Ltd (Shanghai, China). Methanol and acetonitrile were of chromatographic grade, and other reagents were of analytical grade.

2.2 Sample preparation

100 g of Anji white tea and 'Jinhuahecha' was soaked in 2000 mL of pure water for 30 min, after which the filtrate was collected by reflux extraction for 1 h. After adding another 1800 mL of pure water for 45 min reflux extraction, the two filtrates were collected and concentrated with a rotary evaporator. 3 g of *E. cristatum* conidial powder was added to 50 mL of water and sonicated to make a suspension.

2.3 Analysis of characteristic components of AJWT and JHHC

High performance liquid chromatograph (HPLC) with a 2489 UV-Vis Detector (Waters Technology, New York, NY, USA) was applied to analyze effective components. The main effective components of AJWT were tea polyphenols, caffeine, theanine, tea polysaccharides, and vitamins. This study mainly observed the changes in the contents of gallic acid, caffeine, epigallocatechin gallate, and epicatechin gallate in AJWT and its JHHC fermented by *E. cristatum*. To acquire test solution, an appropriate amount of 2.5 mg/mL AJWT, JHHC dry powder were added to methanol solution and weighed, followed by ultrasonic extraction for 30 min. The extract was placed at room temperature and weighed, and the lost weight was made up and shaken evenly. After filtration through a 0.45 μm filter membrane, the filtrate was collected for later use. To prepare control solution, an appropriate amount of the four control substances mentioned above was added with methanol to prepare the corresponding concentration of control solution mother liquor. 0.2 mL of each standard stock liquor was added with methanol to a constant volume of 1 mL, and shaken evenly to obtain a mixed control solution (including 0.0120 mg/mL of GA, 0.0200 mg/mL of Caf, 0.0208 mg/mL of EGCG, and 0.0248 mg/mL of ECG). The content of GA, Caf, EGCG, and ECG was measured with a UV spectrophotometer detector (VWD) at the wavelength of 220 nm. The column (XB-C18, 4.6 × 250 nm, 5 μm) temperature was maintained at 30 ˚C. The mobile phase consisted of acetonitrile-0.2% phosphoric acid aqueous solution, the flow rate was 1.0 ml/min, and the elution procedure was as follows: 0-5 min, 1 %-5 % A; 5-15 min, 5 %-10 % A; 15-30 min, 10 %-15 % A; 40-45 min, 20 %-23 % A; 45-50 min, 23 %-28 % A; 50-55 min, 28 %-38 % B.

2.4 Animals

Specific pathogen-free (SPF) C57BL/6 male mice (6-8 weeks old) were purchased from Hangzhou Qizhen experimental animal Technology Co., Ltd (Hangzhou, China) with license number: SCXK(Hangzhou)2022-0005SCXK. All animal experiments were approved by the Medical Ethics Committee of Zhejiang Chinese Medical University

(IACUC-20230828-14). Mice were housed in the animal laboratory center under the conditions of 20-25 °C, 50-60% humidity, 12 h light-dark cycle, and free access to food.

2.5 UC mouse modeling and drug administration

After one week of habituation, 18 mice were randomly assigned into 6 groups (n = 3) as normal control (NC) group, model control (MC) group, SASP group, JHHC group, AJWT group, and *E. cristatum* conidial powder (*E. cristatum*) group. Except for the NC group, mice in the other groups were given 2.5% DSS aqueous solution instead of drinking water for 9 days to establish a UC model [21,22], with the DSS aqueous solution refreshed daily. Meanwhile, 0.45 g/kg SASP, 2.3 g/kg JHHC water extract, 2.3 g/kg AJWT water extract, and 0.6 g/kg *E. cristatum* conidial powder suspension were administered by gavage. The remaining water weight was measured daily to calculate the amount of water drinked. Isoflurane (Veterinary Medicine Standard 031217015, RWD, Shenzhen, China) was installed on the RWD small animal inhalation anesthesia gas anesthesia machine (R510-11, RWD) for euthanasia of mice on the 10th day.

2.6 Disease activity index (DAI) score

During the experiments, the general physical sign, body weight, fecal viscosity, and fecal occult blood of mice were observed and recorded to assess DAI score (Table 1) [23].

Table 1 DAI score standard.

Decline of weight	Fecal morphology	Fecal occult blood/gross blood stool	Score
0	Normal	Normal	0
1~5%	Incompact	Occult blood positive	1
5~10%			2
10~15%	Loose	Gross blood stool	3
>15%			4

2.7 Measurement of the length of colon and the coefficients of spleen and liver

At the end of experiment, the mice were fasted but had water *ad libitum* for 12 h, and then the weight of the mice was measured. After euthanasia, the spleen, liver, and complete colon tissues were harvested at low temperature. The length of the colon was measured, and the wet weight of the spleen and liver was detected to calculate the organ coefficient [organ wet weight (mg)/fasting body mass (g)].

2.8 Histopathological examination

The colon tissue was washed with ice 0.9% sodium chloride solution, and the same section was selected to be fixed in 10% formalin for 48 h. The samples were dehydrated with gradient ethanol solution, and then embedded in paraffin. Paraffin-embedded tissues were sliced into 4 μm sections, and stained with hematoxylin and eosin (H&E), after which histological changes of the colon were observed under a biological microscope (MshOt, Guangzhou, China).

2.9 Alcian blue-periodic acid Schiff (AB-PAS) staining

The colon mucus secretion capacity of mice in each group was analyzed by AB-PAS (G1285, Solarbio, Beijing, China) staining. After dewaxing and hydration, the slices were soaked in Schiff reagent, periodic acid solution, and Alcian blue dye successively. Followed dehydration with gradient ethanol for 5 min, the slices were transparentized by xylene for 5 min, and then sealed with neutral gum. The changes of mucin secretion by goblet cells were observed under an

optical microscope (C13210-01, HAMAMATSU, Japan).

2.10 Immunohistochemistry (IHC)

Colon tissue samples were fixed in 10% formaldehyde solution, followed by embedding in paraffin and sectioning. Subsequently, routine immunohistochemistry (IHC) was conducted to assess the expression of TNF alpha Rabbit pAb (TNF-α, 346654, Zenbio, Chengdu, China). Each slide was monitored with a light microscope, and images were captured at random fields of vision.

2.11 Immunofluorescent staining (IF)

Paraffin sections of colon tissue were dewaxed, rehydrated, and than used EDTA for antigen repair. The slides were blocked with QuickBlock Immunol Staining Blocking Buffer dilution (P0260, Beyotime, Shanghai, China) for 10 min. Then sections were incubated overnight with MyD88 Rabbit Polyclonal Antibody (MyD88, AF7524, Beyotime) at 4 ℃. Then incubated with FITC-labeled Goat Anti-Rabbit IgG (H+L) (A0562, Beyotime). Finally, the slices were stained with DAPI and subsequently sealed, observed under a Zeiss positive fluorescence microscope (AXIO SCOPE.A1, Carl Zeiss AG, Jena, Germany).

2.12 Transcriptome analysis of colon tissue

Transcriptome sequencing of colon tissues from each group was performed using a 2 × 150 bp paired-end sequencing (PE150) on an Illumina Novaseq™ 6000 (LC-Bio Technology CO., Ltd., Hangzhou, China) as per the recommended protocol. The sequencing data were analyzed on the Omicsmart platform (www.omicsmart.com).

2.13 Statistical analysis

SPSS 25.0 statistical software (International Business Machines Corporation, New York, USA) was adopted to conduct statistical analyses, and GraphPad Prism 9.0 software (San Diego, CA, USA) was used for drawing diagrams. All quantitative results were expressed as mean ± standard deviations (SD), and statistical differences were evaluated through a two-tailed Student's *t*-test or one-way analysis of variance (ANOVA). *p*-value less than 0.05 was defined to be statistically significant.

3 Results

3.1 *E. cristatum* fermentation changed the contents of active components

In line with the chromatographic conditions in 2.3, the four active components in the HPLC spectra of JHHC and AJWT (Figure 1) achieved baseline separation from adjacent peaks ($R \geq 1.5$) and met the requirements for quantitative analysis ($S/N \geq 10$). The changes in the contents of components from JHHC that were obtained by microbial fermenting AJWT with *E. cristatum* were displayed in Table 2. It can be noticed that gallic acid level was signally augmented, caffeine level was slightly diminished, and the levels of epigallocatechin gallate and epicatechin gallate were greatly dwindled.

3.2 JHHC improved the symptoms of DSS-induced UC mice

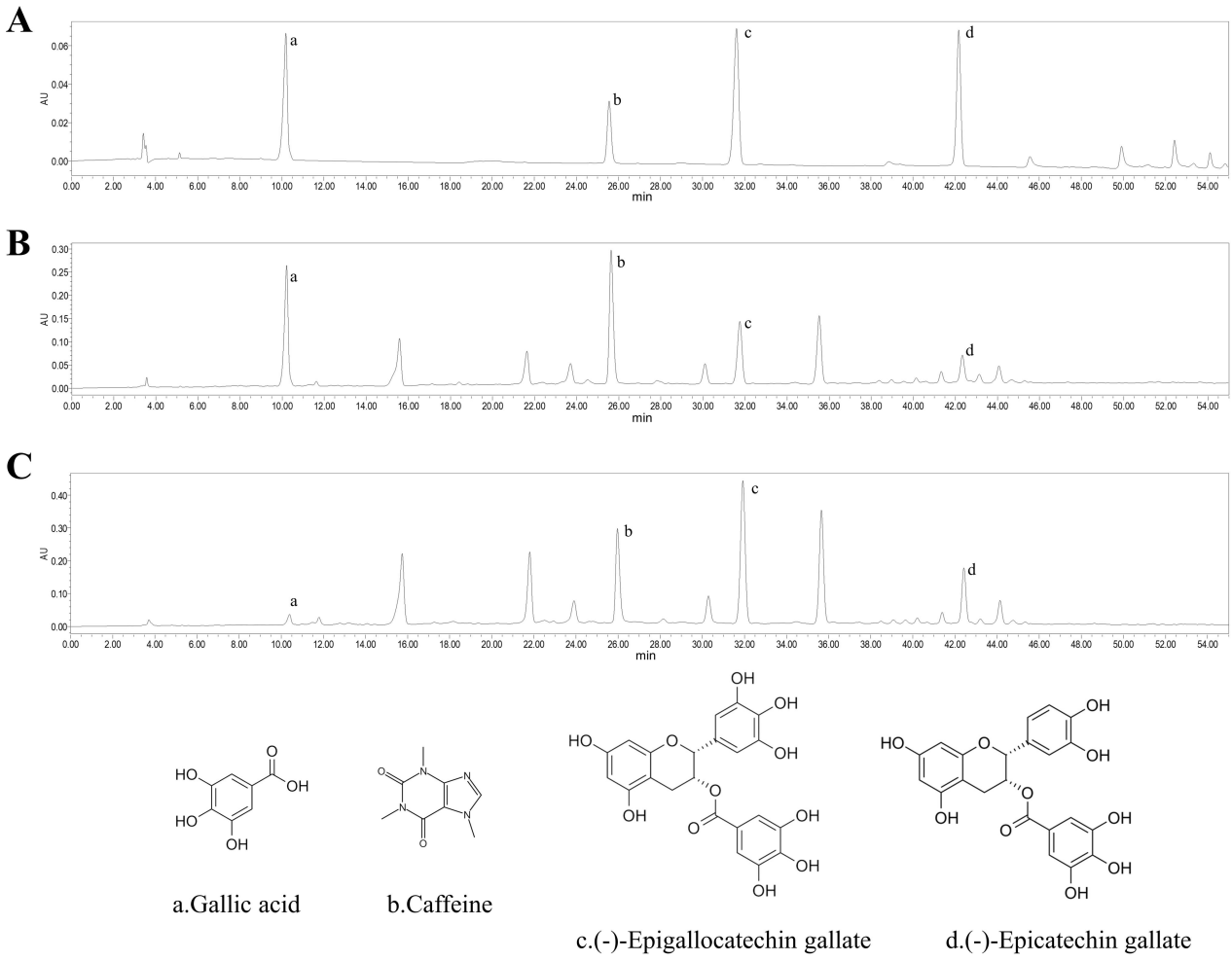
The body weight, daily weight loss percentage, fecal morphology, and occult blood of each group of mice were observed (Figure 2A,B). By contrast, drinking 2.5% DSS aqueous solution for 9 days resulted in significant weight loss, increased DAI score, severe hematochezia, and even anal prolapse in model mice. Administered with AJWT group, JHHC group, and *E. cristatum* group effectively improved weight loss in UC mice (Figure 2A), reduced DAI score and alleviated fecal occult blood (Figure 2B).

The colon tissue of the normal mice was intact and horseshoe-shaped, with obvious folds. However, in UC model mice, the colon length was markedly shortened ($p < 0.01$) (Figure 2C), the intestinal wall became thinner, the congestion and edema of the colon

mucosa were visible, and black bloody stools were present in the intestines (Figure 2D). Compared with the MC group, in the JHHC group and *E. cristatum* group, the colon length was remarkably increased ($p < 0.05$), and the thickness of the intestinal wall as well as the congestion and edema of the colon mucosa were improved, while in AJWT group, we noted an increase in colon length and a recovery in colon atrophy (Figure 2C,D). The fecal alterations in mice were observed before and after the induction of the UC model. Before modeling, the feces of all mice were oval-shaped, slightly brownish black, and slightly dry. After the success of UC model, fecal formless mice in MC group had severe hematochezia, while fecal characteristics and hematochezia of mice in JHHC group, AJWT group and *E. cristatum* group were improved (Figure 2E). Among the tested substances, *E. cristatum* conidial powder was more effective than JHHC and AJWT in improving the symptoms of DSS-induced UC in mice. JHHC water extract effectively reversed the shortened colon length induced by DSS in UC mice.

Table 2 The contents of effective components in JHHC and AJWT.

Group	Effective components			
	GA mg/g	Caf mg/g	EGCG mg/g	ECG mg/g
JHHC	17.79	70.46	13.98	8.8
AJWT	2.29	73.56	43.99	23.61



a.Gallic acid b.Caffeine c.(-)-Epigallocatechin gallate d.(-)-Epicatechin gallate

Figure 1 HPLC spectra of characteristic components in standard and tea samples. (A) Spectra of characteristic components blended with standard samples (a. Gallic acid; b. Caffeine; c. (-)-Epigallocatechin gallate; d. (-)-Epicatechin gallate). (B) Finger-print of JHHC. (C) Finger-print of AJWT.

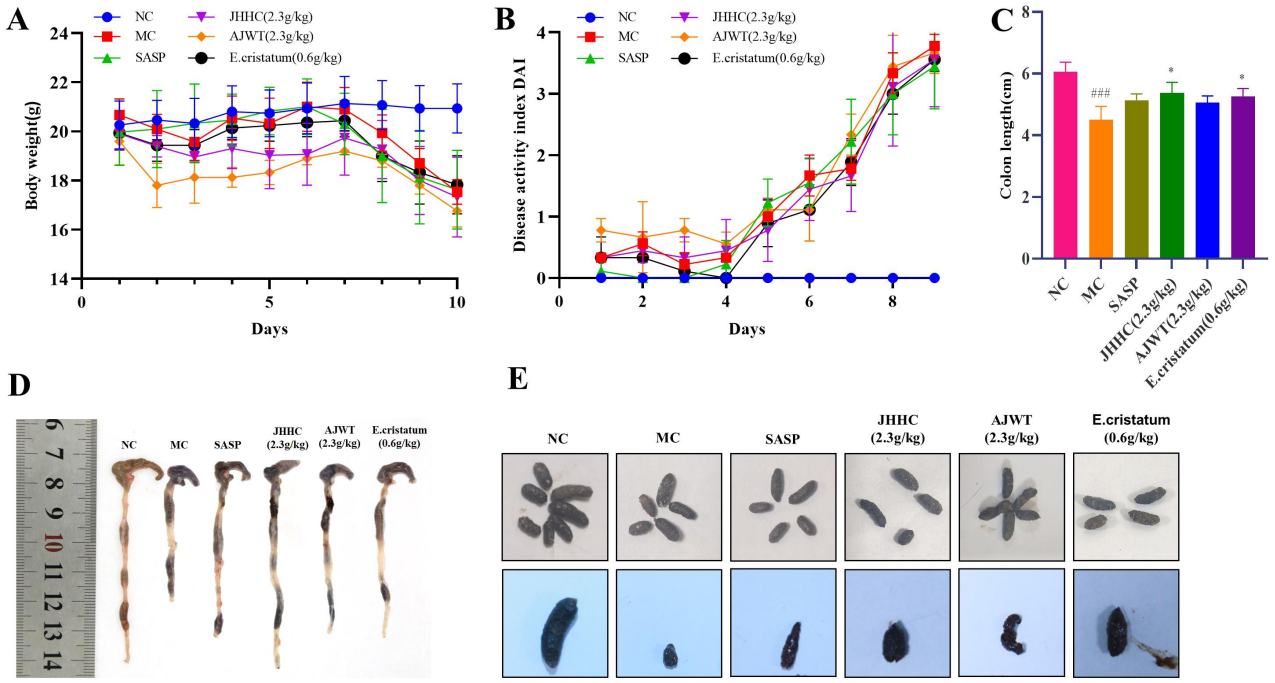


Figure 2 JHHC improved the symptoms of DSS-induced UC mice. (A) The weight of mice. (B) DAI score of mice. (C) The colon length of mice. (D) Representative colonic morphology of mice in each group. (E) Fecal morphology of mice before and after administration. (n = 3), compared with NC group, $^{###}p < 0.01$; compared with with MC group, $^{*}p < 0.05$.

3.3 JHHC improved the histopathological changes of DSS-induced UC mice

According to the H&E staining results of mouse colon tissues (Figure 3A), NC group of mice had intact intestinal mucosal tissue, a large number of intestinal villi, orderly arrangement of intestinal glands, and no inflammatory cell infiltration. Compared with NC group, in the MC group, mice presented varying degrees of damage to the upper colonic mucosa, crypt structures, and intestinal villi, with a wide range of inflammatory cell infiltration and prominent damage to the intestinal epithelium. As compared with mice in MC group, treatment of AJWT, JHHC and *E. cristatum* conidial powder can relatively restore crypt structures in colon and relieve the intestinal injury degree, among which the therapeutic efficacy was superior in *E. cristatum* group and JHHC group.

Mucin is an important component of the intestinal mucosal layer, mainly existing in the mucus secreted by the epithelial cells and glandular cells of the colon mucosa, which has an intimate association with intestinal mucosal inflammation and ulcer formation [24]. Colon AB-PAS staining results (Figure 3B) revealed heaps of goblet cells, good shape and orderly arrangement in NC group. However, MC group presented decreases in goblet cells of the colon tissue and mucus, and a disorderly arrangement. Intriguingly, AJWT, JHHC, and *E. cristatum* conidial powder increased goblet cells in mouse colon tissue and promote the recovery of mucosal layer.

3.4 JHHC improved the spleen and the liver index

The spleen participates in regulating the response of immune system and is a critical immune organ [25]. After modeling, mice of MC group showed a significant increase in spleen weight and spleen coefficient ($p < 0.01$). However, the spleen coefficient was pronouncedly lessened by JHHC ($p < 0.05$), while being reduced to varying degrees by AJWT and *E.*

cristatum conidial powder (Figure 4A). The liver coefficient had no obvious changes in all drug treatment groups (Figure 4B).

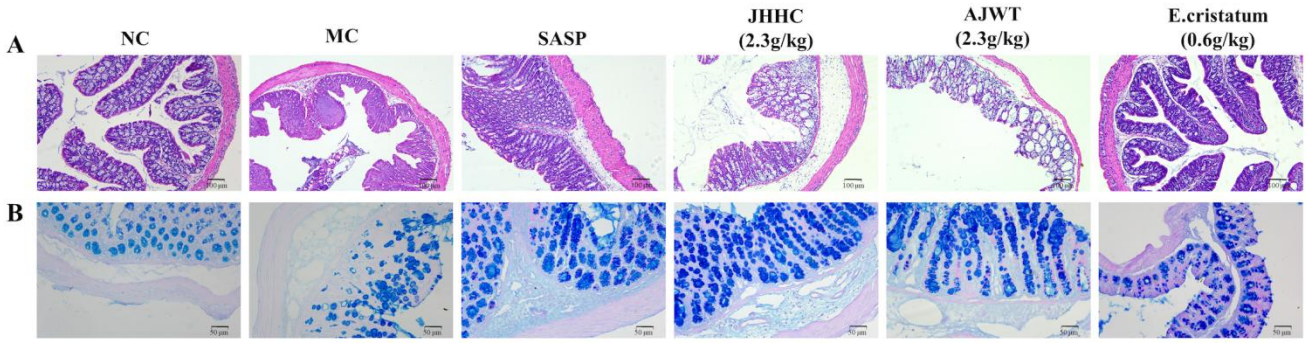


Figure 3 Histopathological changes in mouse colon tissue. (A) Histopathologic image of mouse colon (H&E staining, 100×). (B) Mucin staining image of goblet cells in mouse colon tissue (AB-PAS staining, 200×).

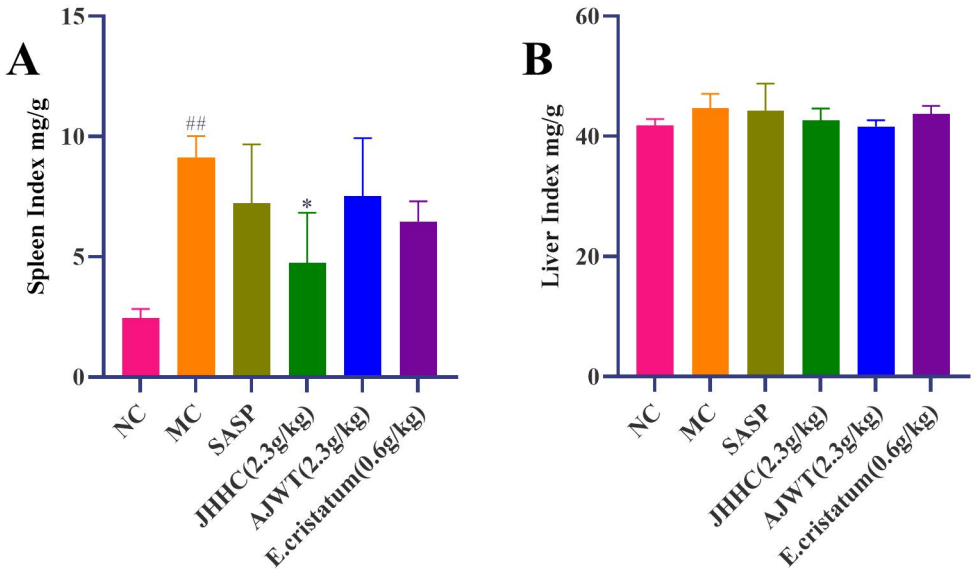


Figure 4 Spleen coefficient (A) and liver coefficient (B) of mice in each group. compared to NC group, $^{\#\#}p < 0.01$; compared to MC group, $^{*}p < 0.05$.

3.5 Effect of JHHC on gene expression in colon tissue

To fathom out the preliminary pharmacological mechanisms of JHHC improving UC in mice, we analyzed the transcriptome sequencing data of the colon tissues. In transcriptome sequencing data analysis, NC, MC and JH represent NC group, MC group and JHHC group, respectively. The results of principal component analysis (PCA) indicated a strong correlation in JH group, and there are differences in gene samples between JH group and MC group (Figure 5A). In light of the volcano plot as well as the heatmap of the top 100 differentially expressed genes of JH and MC group (Figure 5B,C), it can be seen that compared with the MC group, some genes in the JH group showed a downward trend, but there was no significant difference in the number of up- or down-regulated genes. The GO enrichment analysis of the top 20 differential genes (Figure 5D) revealed that the functions of the differential genes mainly included "synapse", "cell junction", "integral component of membrane", and "calculus ion binding". Based on this, further screening of the KEGG pathway was conducted

(Figure 5E). A therapeutic effect on UC was found in JH group, which may be closely related to the MAPK signaling pathway and tight junction signal. The FPKM data analysis on the key gene in these two pathways (Figure 5F) showed compared with NC group, MAPK signaling pathway-related *MAPK8IP2* ($p < 0.004$), *MAPK10* ($p < 0.005$), *MAPK8IP1* ($p < 0.008$) and *MYD88* expressions in the colon of UC mice were significantly increased, while tight junction-related *OCLN*, *CLDN1*, *CLDN7*, and *PARD3* expressions were decreased to varying degrees, with *OCLN* expression showing the largest decrease ($p < 0.003$). The changes of the above gene expressions can be reversed by JHHC water extract to different extent, hinting that the improvement of DSS-induced UC by JHHC water extract may be achieved via modulating MAPK signaling pathway to inhibit inflammatory response and mediating tight junction protein expression to protect the intestinal barrier.

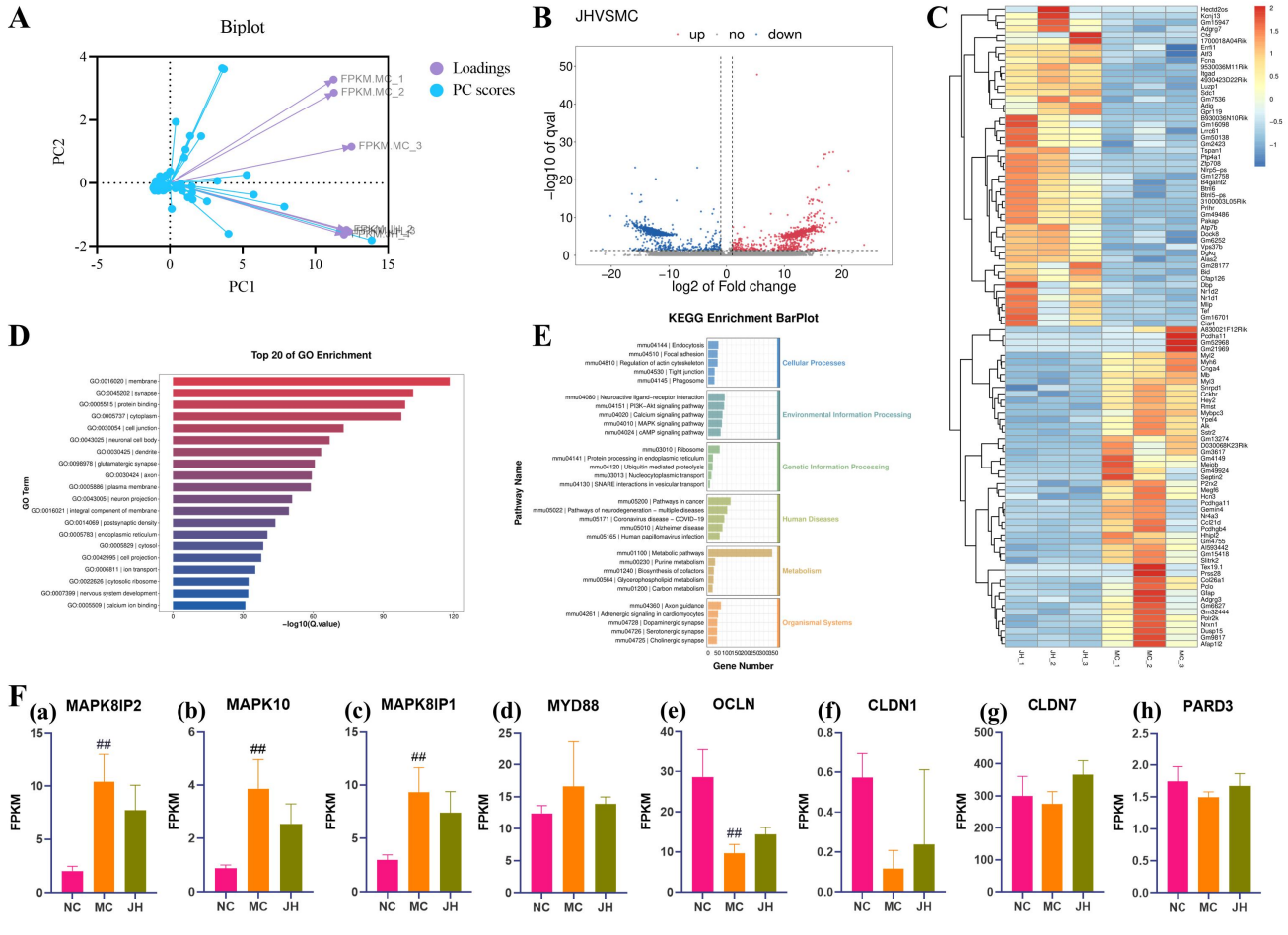


Figure 5 Effects of JHHC on differential genes and analysis of transcriptome data in DSS-induced UC mice (n = 3). (A) Principal component analysis. (B) Volcano plot of differential gene. (C) Differential gene clustering heatmap of top 100 genes. (D) Top 20 of GO enrichment. (E) KEGG enrichment BarPlot. (F) Expression level of MAPK signaling pathway and Tight junction related genes. Compared to NC group, $^{\#\#}p < 0.05$, $^{\#}p < 0.01$. NC: NC group; MC: MC group; JH: JHHC group.

3.6 JHHC reduced the expression of MyD88 and TNF-α in colon tissue

MyD88 is a protein that mediates inflammation, and mainly acts as an important medium of interleukin-1 receptor (IL-1R) signaling and participates in the activation of the MAPK signaling pathway [26,27]. To explore the mechanism by which JHHC improves DSS-induced inflammation in UC mice,

immunofluorescence staining and immunohistochemical staining were used to observe the expression changes of MyD88 and TNF-α in colon tissues, respectively. The results showed that compared with NC group, the MyD88, TNF-α positive cells were significantly increased in MC group. Compared with MC group, MyD88, TNF-α positive cells were significantly decreased in JHHC, AJWT, and *E. cristatum* group, indicated that JHHC, AJWT and *E. cristatum* could inhibit the inflammatory response in UC (Figure 6).

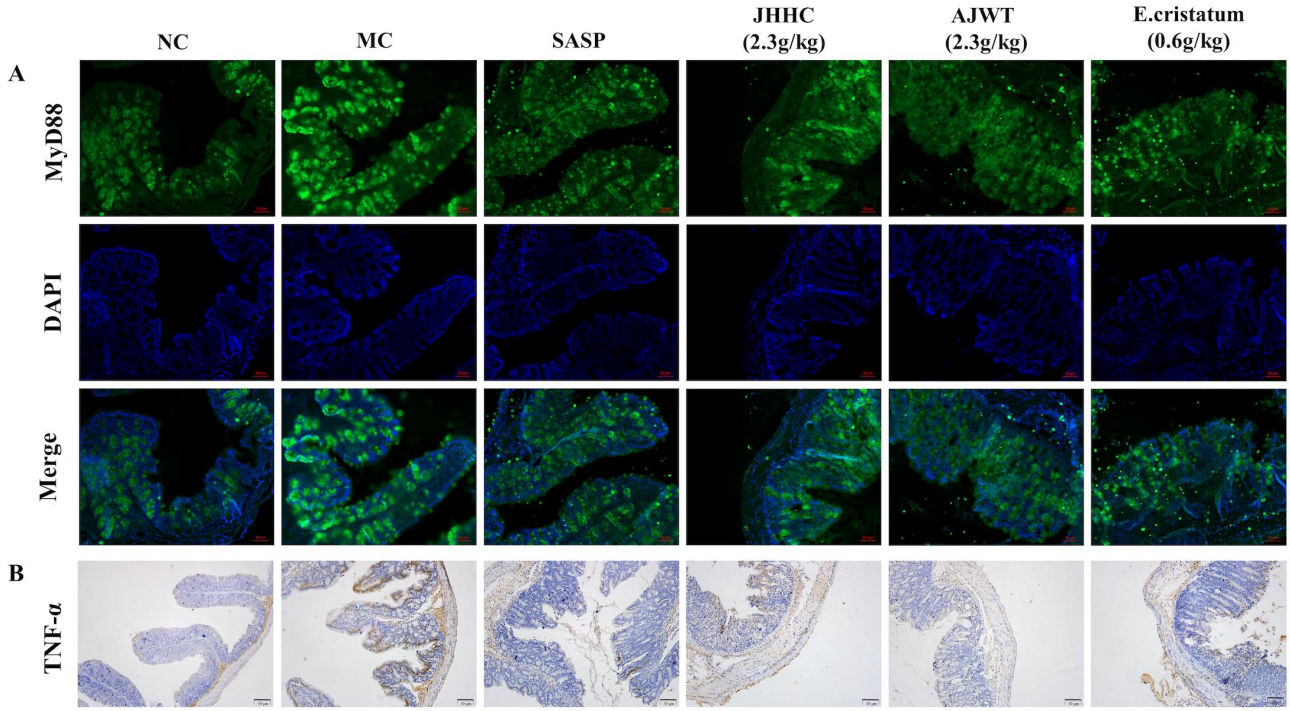


Figure 6 Effects of JHHC on protein expression of MyD88 and TNF-α. (A) Representative IF images of MyD88 (IF, 200×). (B) Representative IHC images of TNF-α (IHC, 200×).

4 Discussion

UC is characterized by long course of disease and relapse, and it cannot be cured at present [28]. To prevent recurrence and avoid complications, patients need long-term medication treatment to alleviate inflammation, which brings enormous economic burden to individuals and society. There are studies revealing that the pathogenesis of UC is primarily associated with intestinal mucosal barrier integrity and T lymphocyte immunity [29,30]. Anti-inflammation and immune regulation are the two important means for the treatment of UC. It has been documented that AJWT contains abundant tea polyphenols, as well as nutrients such as amino acids and gallic acid, which has anti-bacterial, anti-inflammatory, and immune enhancement effects [31,32]. The AJWT after fermentation using *E. cristatum* can modulate gut microbiota, improve intestinal barrier function, and subsequently regulate gastrointestinal function [18]. This experiment for the first time analyzed the changes in the composition of AJWT after fermentation, namely JHHC, and whether the kind of tea can improve DSS-induced UC in mice. The results of component analysis indicated that after microbial fermentation, the content of gallic acid was significantly increased, but that of caffeine was slightly decreased in JHHC, signifying a transformation of tea polyphenol components during fermentation [33], and the contents of EGCG and ECG were diminished. Gallic acid has been confirmed to exert anti-inflammatory, anti-bacterial, antiviral and anti-oxidant effects to assist in treating inflammatory diseases [34,35].

Caffeine plays an anti-oxidant and anti-inflammatory role, moderate intake of which contributes to physical health [36]. EGCG and ECG, as tea polyphenols, generate anti-oxidant, antiviral, and immunomodulatory effects [37,38], and can achieve anti-inflammatory effects via stimulating the MAPK pathway [39].

In this study, 2.5% DSS aqueous solution that replaces drinking water was applied to successfully establish UC mouse model [40]. Through analysis of experimental data, we proved that the fermented JHHC had decreased tea polyphenols and increased gallic acid, analogous to existing experimental reference data [9]. JHHC can also effectively improve the clinical symptoms of DSS-induced UC in mice, such as weight loss, hematochezia, rectal prolapse, shortened colon length caused by colon atrophy, pathological changes in colon tissue, and loss of intestinal barrier. Based on experimental results and transcriptome sequencing data, it was conjectured that the mechanism of JHHC improving UC in mice may be related to its rich nutrients that help strengthen immunity [41], or components such as gallic acid that inhibit inflammatory mediator production and release, reduce tissue damage caused by inflammation, and thus maintain the intestinal epithelial barrier [42,43].

MAPK signaling pathway is involved in the pathogenesis of inflammation and inflammatory response [44]. According to the transcriptome sequencing and staining results, the DSS-induced UC model may be related to IL-1R activating MAPK pathway through MyD88, leading to the release of inflammatory factors (such as interleukin-1β, tumor necrosis factor- α , etc.), and then triggering inflammatory response [45,46]. JHHC can reduce the expression of MyD88 and TNF-α in a certain extent, and regulate the level of inflammation. Nevertheless, further mechanisms and potential targets remain to be investigated.

In recent years, in the context of the growing public awareness of the health, people trust more in the efficacy of natural products and their active components [47]. Products for relieving UC made from natural ingredients such as TCM both as medicine and food have a certain market [48,49]. The JHHC and its active components transformed by microorganisms are expected to be developed into new products for the prevention and treatment of UC.

5 Conclusion

The composition of JHHC changed after fermentation. JHHC can improve DSS-induced UC by reducing intestinal inflammatory response and protecting colonic epithelial barrier function. It is a potential candidate for UC treatment.

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Conflicts of Interest

All authors declare no conflict of interest.

Author Contributions

K.L., Y.W., W.L., and X.Q.: Carried out the experiment; K.L.: Formal analysis and Writing-original manuscript; X.Z. and G.Z.: Conceptualization; Z.L. and J.G.: Experimental design and manuscript revising.

Ethics Approval and Consent to Participate

All animal experiments were approved by the Medical Ethics Committee of Zhejiang Chinese Medical University (IACUC-20230828-14).

Funding

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Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Supplementary Materials

Not applicable.

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