Ononin combined with *Lactobacillus paracasei* alleviates DSS-induced ulcerative colitis by ferroptosis via inhibiting JAK2/STAT3 signaling pathway

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SUMMARY

Purpose This study aimed to assess the efficacy of Ononin combined with *Lactobacillus paracasei* in treating dextran-sulfate sodium (DSS)-induced UC, and to investigate some of its underlying mechanisms. **Methods** BALB/c male mice were given 3% (w/v) DSS to induce ulcerative colitis. Assessments included changes in body weight, disease activity index (DAI), and colon length. The effects of Ononin combined with *Lactobacillus paracasei* on UC were evaluated by Hematoxylin and eosin (H&E) staining, ELISA, and Western blot. **Results** The UC models were established successfully by drinking DSS water. Ononin combined with *Lactobacillus paracase*i showed a significant effect against UC by the increased body weight and colon length, decreased DAI and alleviated histopathological changes. Meanwhile, compared with the DSS group, the inflammation indexes TNF-α, IL-1β and MPO, MDA levels were reduced, while the SOD and GSH levels were increased dramatically . The protein level of ACSL4 and JAK2/STAT3 were obviously downregulated, but the GPX4, Occludin and Claudin-1 expression were upregulated. **Conclusion** Ononin combined with *Lactobacillus paracase*i inhibits DSS-induced inflammation, enhances intestinal barrier function, and effectively ameliorates inflammatory injury in UC. These data revealed that suppressing ferroptosis and oxidative stress could effectively ameliorate DSS-induced UC involved in blocking JAK2/STAT3 signaling pathway.

Keywords: Ulcerative colitis; Ononin; *Lactobacillus paracasei*; Ferroptosis; JAK2/STAT3 pathway

1. Introduction

Ulcerative colitis (UC) is a chronic and frequently recurring nonspecific inflammatory disease of the intestinal tract. Its typical symptoms include periodic diarrhea, abdominal pain, and

the presence of mucus, pus, and blood in the stools[1]. In severe cases, complications may arise, such as intestinal perforation, hemorrhage, and carcinoma. At present, the exact etiology and pathogenesis of UC have not been fully elucidated. It is generally accepted that dysbiosis of the intestinal microbiota, compromised intestinal mucosal barrier function, and abnormal immune regulation significantly contribute to the pathogenesis of UC[2]. Among these factors, the impairment of the intestinal mucosal barrier is regarded as one of the key mechanisms underlying the development of UC[3]. In clinical practice, there are several treatment methods for UC, but most of these methods primarily focus on alleviating symptoms. Currently, drug therapy is the predominant treatment approach, utilizing medications such as aminosalicylates, corticosteroids, and immunosuppressants[4]. However, traditional drug therapies often come with significant side effects that can severely impact patients' quality of life. Therefore, it is essential to explore novel therapeutic strategies that are safe, effective, and associated with minimal side effects for the treatment of UC.

Ononin, a flavonoid compound derived from widely used traditional Chinese medicine(TCM) such as *Astragalus membranaceus* and *Pueraria lobata*, has been demonstrated by modern pharmacology to possess antioxidant, anti-tumor, and anti-inflammatory properties[5]. Some studies have demonstrated that Ononin can inhibit the expression of inflammatory factors, reduce intestinal inflammatory responses, regulate the structure of intestinal microbial communities, promote the growth of beneficial bacteria, and exhibit potential therapeutic effects on intestinal diseases such as UC[6,7]. *Lactobacillus paracasei*, a probiotic belonging to the Lactobacillus genus, is commonly found in both traditional fermented dairy products and the human gastrointestinal tract[8]. It exhibits remarkable biological activities, including antioxidant and anti-inflammatory properties. *Lactobacillus paracasei* has the capacity to enhance the intestinal environment, restore the balance of the intestinal microbiota, repair the intestinal mucosal barrier, and reduce intestinal inflammation[9]. Furthermore, it shows significant promise in providing therapeutic effects against a variety of intestinal diseases, with UC being one of the most notable[10].

Recent extensive studies have demonstrated that ferroptosis is closely associated with the onset and progression of UC. Ferroptosis is a novel form of programmed cell death that differs

from pyroptosis, apoptosis, necrosis, and autophagy. It is characterized by iron-induced lipid peroxidation and the accumulation of reactive oxygen species (ROS), which ultimately leads to oxidative cell death[11].

Although both Ononin and *Lactobacillus paracasei* demonstrate certain beneficial effects on UC, when used individually, it remains uncertain whether their combined application could produce enhanced therapeutic results. Furthermore, no studies have confirmed whether these agents exert their effects on UC through the regulation of ferroptosis. Therefore, this study utilized a dextran sulfate sodium (DSS)-induced murine model of UC to investigate the combined therapeutic effects of Ononin and *Lactobacillus paracasei* in relation to ferroptosis. By analyzing their synergistic intervention, we aim to elucidate the underlying mechanisms and provide novel insights for the clinical treatment of UC.

2. Materials and methods

2.1. Animals

Fifty-four male specific pathogen-free (SPF) BALB/c mice, aged 6 to 8 weeks and Body mass (20 - 22) g, were provided by Liaoning Changsheng Biotechnology Co., Ltd. All mice were housed in a standard SPF environment with a temperature of 22 ± 2 °C, humidity of 55% ± 5%, and a 12-hour light/dark cycle. They had ad libitum access to food and sterilized tap water, as well as a standard commercial diet. The animal study protocol was approved by the Animal Research Welfare Council of Henan University of Traditional Chinese Medicine Affiliated Zhengzhou Anorectal Hospital (Ethics No. ZG20250302A). All animal experiments in this study were conducted in compliance with the ARRIVE guidelines and in accordance with the U.K. Animals (Scientific Procedures) Act 1986, the EU Directive 2010/63/EU, and the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Materials

DSS (MW: 36,000–50,000) was purchased from Yea Sen Biotechnology (Shanghai). Ononin was obtained from Yuan Ye Bio-Technology (Shanghai, China). SOD, GSH, MDA, MPO, TNF-α and IL-1β ELISA kits were acquired from Jiangsu Enzyme Exemption Industry Co., Ltd. *Lactobacillus paracasei* was sourced from BNCC. Mesalazine sustained-release granules were

obtained from Shanghai Ethypharm Pharmaceuticals Co., Ltd. The following antibodies were purchased from BioDragon Technology Co., Ltd.: Occludin (10Z10) Rabbit Monoclonal Antibody, Claudin-1 Rabbit Polyclonal Antibody, JAK2 Rabbit Polyclonal Antibody, JAK2 (Phospho Y1007 + Y1008) (17F11) Rabbit Monoclonal Antibody, STAT3 Rabbit Monoclonal Antibody, phosphorylated (p)-STAT3 Rabbit Monoclonal Antibody, HRP-labeled Goat Anti-Rabbit IgG (H+L), HRP-labeled Goat Anti-Mouse IgG (H+L), and β-actin Mouse Monoclonal Antibody.

2.3. Methods

2.3.1. Preparation of bacterial suspension

Lactobacillus paracasei strains were inoculated into conical flasks containing MRS liquid medium and incubated in a constant temperature shaker at 37 ℃ for 18 h. Remove, centrifuge and collect and adjust the concentration of the bacterial solution to 3.33×10^9 CFU/mL for spare.

2.3.2. Model construction and drug intervention

After acclimatizing mice in the experimental environment for one week, they were divided into six groups: a control group (CON), a DSS-induced colitis group (DSS)[12], a mesalazine group (5-ASA, 200 mg/kg)[13], an Ononin group (Ononin, 20 mg/kg)[7], a *Lactobacillus paracasei* group (LP, 3.33×10^9 CFU/mL)[14] and a combination treatment group(Ononin +LP). Following the 7-day acclimatization period, the CON group was given free access to double-distilled water, while the other five groups were provided with free access to a 3% DSS solution daily for 7 days to establish a model of UC. After successful modeling, intragastric administration of the appropriate medication (10 mL/kg) commenced. The CON and DSS groups were gavaged with 10 mL/kg of normal saline for 7 consecutive days. A schematic representation of the experimental study is provided in Figure 1A. Prior to sacrifice by cervical dislocation, blood samples were collected via eyeball exsanguination.

2.3.3. Disease Activity Index (DAI) Score

After the DSS-induced model was established, all mice were weighed and recorded at the same time each day. The mental state, coat condition, food and water intake, body weight changes, stool characteristics, and instances of hematochezia were observed in each group. And their DAI was assessed according to the scoring criteria outlined in Table 1. DAI score = (weight loss percentage score + fecal traits score + fecal bleeding score) / 3. Evaluation was performed as

describe[15].

2.3.4. Determination of colon length

The colon was cut out, the morphology of the colon was observed and recorded, and the length of the colon was measured.

2.3.5. Measurement of spleen index

The spleens of the mice were isolated and their weights were recorded. Calculate the spleen indices of the mice.

2.3.6. Hematoxylin-Eosin Staining

A part of colon was cut, fixed with 4% paraformaldehyde, embedded in paraffin, cut into 5 μm sections, stained with HE, and observed the histopathological changes of colon under microscope.

2.3.7. Detection of inflammatory factors TNF-α, L-1β content

Serum from mice was taken and the levels of TNF-α and IL-1β in mouse serum were detected according to the instructions of the ELISA kits.

2.3.8. Measurement of oxidative stress markers

The serum of mice was taken and the levels of MPO, MDA, SOD and GSH were measured according to the instructions of the ELISA kits.

2.3.9. Detection of protein expression level

Appropriate amounts of mouse colon tissues were collected, and protein samples were extracted according to the instructions provided in the tissue protein extraction kit. Protein quantification was performed using the BCA method, followed by heat denaturation at a high temperature for 10 minutes. Subsequent procedures included electrophoresis, membrane transfer, blocking, and overnight incubation with primary antibodies against GPX4, ACSL4, JAK2, p-JAK2, STAT3, p-STAT3, Occludin, and Claudin-1. After washing with TBST, membranes were incubated with secondary antibodies (1:10,000 dilution) at room temperature for 1 hour, followed by exposure and development. The gray value of protein bands was detected using Image J software, and the gray value of the internal reference protein β-actin was used as a control for calculation. The expression level of the target protein was determined using the formula: expression level = gray value of the target protein band / gray value of the reference protein band. All experiments were conducted in triplicate.

2.3.10. Statistical analysis

All experimental results were statistically analyzed using GraphPad Prism 8.0.2 statistical software, and the data were presented as the mean ± standard deviation (SD). All experiments were performed at least in triplicate using independent assays. The statistical significance of data comparisons was determined using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test or student's t-test. Values of $p< 0.05$ were considered to be statistically significant.

3. Results

3.1. Ononin combined with *Lactobacillus paracasei* ameliorated the symptoms of DSS-induced colitis mice

The characteristics of DSS-induced colitis are marked body weight loss, diarrhea, and severe bloody stools. In this study the DAI score of the CON group was stable at 0 with time, and the growth status was good. Mice in the DSS group showed significant body weight loss unlike mice in the normal group ($p<0.05$, Figure 1B). Following drug intervention, the weight loss symptoms in each group improved, and the DAI score significantly decreased ($p<0.01$, Figure 1C). The increase in body weight and the decrease in DAI score were more pronounced in the combined group ($p<0.001$). These results suggest that Ononin combined with *Lactobacillus paracasei* could alleviate colitis symptoms in UC mice.

3.2. Comparison of colon length in mice in each group

Compared to the CON group, DSS administration resulted in noticeable colon shortness as an indication of colon inflammation ($p<0.001$, Figure 1D). Compared with the DSS group, colon shortening was significantly suppressed after drug treatment ($p<0.001$), in which the improvement of colon shortening symptoms in mice in the Ononin+LP group was extremely significant ($p<0.001$, Figure 1D, 1E)). The difference between the colon lengths of the Ononin group, the *Lactobacillus paracasei* group, and the 5-ASA group was not statistically significant ($p>0.05$).

3.3. Comparison of spleen index of mice in each group

The spleen is the most important lymphoid organ and is enlarged in response to infection or inflammation in the body. In this study, compared with the CON group, the splenic index of mice in the DSS group was significantly higher ($p<0.001$, Figure 1F). It demonstrate that UC mice not only exhibit intestinal damage but also cause spleen damage. Compared with the DSS group, the

splenic index of mice after drug intervention was significantly decreased (p<0.01), among which the decrease in the splenic index of mice in the Ononin+LP group was particularly significant (p<0.001, Figure 1F), indicating that Ononin combined with *Lactobacillus paracasei* can improve the inflammatory response and spleen injury induced by DSS in UC mice.

3.4. Comparison of histopathology of the colon of mice in each group

As shown in Figure 2, the intestinal mucosa and glands of mice in the CON group were morphologically and structurally intact and neatly arranged, no inflammatory cell infiltration or ulcer formation was observed, and no obvious edema was seen in the submucosa. Compared with the CON group, the intestinal wall integrity of mice in the DSS group was severely damaged, with obvious thickening of the intestinal wall, significant reduction in the number of glands, damage to the crypt structure, accompanied by extensive inflammatory cell infiltration and extensive ulcer coverage, and a more severe degree of pathological damage to the colonic tissues. After the administration of drug intervention treatment, the colonic tissues of mice in each intervention group were significantly improved, the area of inflammatory cell infiltration was reduced, the glands and crypt structures were rearranged in a neat and orderly manner, and the submucosal edema was alleviated. Among them, the Ononin+LP group of mice showed the most significant pathological improvement, with repair of damage to the epithelial cell layer of the colonic mucosa, no obvious edema or thickening of the intestinal wall, and a significant reduction in inflammatory cell infiltration.

3.5. Comparison of serum levels of inflammatory factors TNF-α and IL-1β in mice in each group

Compared with the CON group, the levels of TNF-α and IL-1β in the serum of mice in the DSS group increased significantly (p<0.01, Figure 3A, 3B), indicating increased colon inflammation. the levels of TNF-α and IL-1β in the serum of mice in the 5-ASA group, the Ononin group, the *Lactobacillus paracasei* group, and the Ononin+LP group decreased significantly compared with those in the DSS group (p<0.01, p<0.001); the Ononin+LP group and 5-ASA group showed the most significant decrease (p<0.001, Figure 3A, 3B). These data suggest that the protective effect of Ononin combined with *Lactobacillus paracasei* in UC is related to its

modulation of inflammation.

3.6. Comparison of serum levels of MPO, MDA, SOD and GSH in mice in each group

Compared with the CON group, the levels of MPO and MDA in the serum of mice in the DSS group were significantly increased (p<0.01, Figure 3C, 3D), and the levels of SOD and GSH were significantly decreased (p<0.01, Figure 3E, 3F), revealing a decrease in free radical scavenging and an increase in oxidative stress. Compared with the DSS group, the levels of MPO and MDA in the serum of mice in the 5-ASA group, the Ononin group, the *Lactobacillus paracasei* group and the Ononin+LP group were significantly decreased (p<0.01, p<0.001, Figure 3C, 3D); the levels of GSH and SOD increased significantly (p<0.05, p<0.01, Figure 3E, 3F). Among them, the most significant decrease was observed in the Ononin+LP group (p<0.001), suggesting recovery of the ability to eliminate free radicals. This indicates that Ononin combined with *Lactobacillus paracasei* can effectively enhance the antioxidant level in the body to alleviate UC.

3.7. Comparison of the relative expression levels of Occludin and Claudin-1 proteins in the colonic tissues of mice in each group

Compared with the CON group, the expression of Occludin and Claudin-1 proteins in the colonic tissues of mice in the DSS group was significantly higher (p<0.001, Figure 4A, 4B, 4C). Compared with the DSS group, Occludin and Claudin-1 protein expression was significantly elevated in all treatment groups (p<0.01), with extremely significant elevations in the Ononin+LP and 5-ASA groups (p<0.001, Figure 4A, 4B, 4C). Together, these results indicated that Ononin combined with *Lactobacillus paracasei* had a beneficial effect on DSS-induced mucosa disruption.

3.8. Comparison of differences in the protein expression levels of GPX4 and ACSL4 in the colonic tissues of mice in each group

Compared with the CON group, the ACSL4 protein expression level was significantly higher (p<0.01, Figure 5 A, 5B), and the GPX4 protein expression level in the colonic tissues of mice in the DSS group was significantly lower (p<0.01, Figure 5 A, 5C); compared with the DSS group, the ACSL4 protein expression level of the protein expression level was significantly lower (p<0.01, Figure 5A, 5B), and the GPX4 protein expression level of the intervention groups was significantly higher (p<0.01, Figure 5A, 5C). Among them, the change in protein expression level

of Ononin+LP group was significantly higher than that of other groups (p<0.001). These data indicated that Ononin combined with *Lactobacillus paracasei* could effectively improve the phenomenon of colon ferroptosis.

3.9. Comparison of the differences in the expression levels of p-JAK2 and p-STAT3 proteins in the colonic tissues of mice in each group

Compared with the CON group, the expression levels of p-JAK2 and p-STAT3 proteins in the colonic tissues of mice in the DSS group were significantly increased (p<0.01, Figure 6A, 6B, 6C), and compared with the DSS group, the expression levels of the proteins in the intervention groups were significantly decreased (p<0.01, Figure 6A, 6B, 6C); the decrease in the expression of p-JAK2/p-STAT3 proteins in the Ononin+LP group was significantly higher than that in the other groups (p< 0.001). These results demonstrated that the combination of Ononin combined with *Lactobacillus paracasei* suppressed JAK2/STAT3 phosphorylation, concurrently attenuating inflammation and ferroptosis, which contributed to anti-inflammatory and cytoprotective outcomes.

4. Discussion

According to its clinical manifestations, UC is classified in TCM under categories such as "diarrhea," "dysentery," "intestinal wind," "chronic dysentery," "intestinal pi," and "hematochezia." In recent years, TCM has shown distinct advantages in the treatment of UC. Numerous studies indicate that TCM achieves significant clinical efficacy through multi-target, multi-molecular, and multi-mechanism interventions[16]. Astragalus (Huang Qi), a widely utilized traditional Chinese herb, is particularly effective in enhancing immune function and reducing inflammation[17]. As a key bioactive component, Ononin can reduce the overexpression of IL-1β and TNF-α while inhibiting the abnormal activation of the MAPK and NF-κB pathways. This action suppresses IL-1β-induced pro-inflammatory responses in chondrocytes and prevents extracellular matrix degradation, ultimately improving the inflammatory state of chondrocytes[6]. These findings further underscore the therapeutic potential of Ononin in treating inflammatory diseases. Additionally, evidence indicates that Ononin alleviates DSS-induced colitis by promoting mitophagy and inhibiting the NLRP3 inflammasome[7]. In the field of gut microbiota research, *Lactobacillus paracasei* has been shown to alleviate intestinal inflammation and promote gut health, effectively preventing or treating diseases associated with gut dysbiosis[18]. Further

research has demonstrated that *Lactobacillus paracasei* NTU 101 alleviates DSS-induced UC by boosting antioxidant enzymes (GR/GSH/CAT/SOD), lowering MDA and IFN-γ levels[9]. Previous studies have confirmed the therapeutic effects of both Ononin and *Lactobacillus paracasei* in DSS-induced UC. Our current study provides further evidence that the combination of Ononin and *Lactobacillus paracasei* as an adjunctive therapy demonstrates superior efficacy compared to Ononin alone in the treatment of UC.

Intestinal inflammation is the primary pathogenic mechanism underlying the activity of UC, characterized by the persistent infiltration of inflammatory cells in the mucosa, which serves as the hallmark pathological feature throughout the entire course of the disease[19]. TNF-α and IL-1β are crucial pro-inflammatory factors primarily produced by monocytes and macrophages, with their levels significantly increasing during the active phase of UC[20]. As a key signaling molecule, TNF-α is instrumental in immune regulation and the initiation of inflammatory response[21]. IL-1β is produced by activated mononuclear macrophages and plays a crucial role in both acute and chronic inflammation, as well as in autoimmune processes. Its concentration can serve as an indicator of the severity of UC[22]. In this study, compared to the CON group, the levels of TNF-α and IL-1β in the serum of DSS-induced UC mouse models were significantly elevated. Following drug treatment, the levels of TNF-α and IL-1β in the serum of mice were both reduced when compared to the DSS group. These results suggest that the combined medication may alleviate the immune inflammatory response in the intestine and promote the repair of the intestinal mucosa by more effectively down-regulating the levels of TNF-α and IL-1β in the serum of UC mice.

Oxidative stress refers to a pathological process characterized by an imbalance between reactive oxygen species (ROS) and antioxidants in vivo, leading to cellular and tissue damage[23]. Peroxidation is a significant factor contributing to UC. Oxidative stress can impair the function of the intestinal mucosal barrier, trigger inflammatory responses, cause dysfunction in the intestinal mucosal immune system, and hinder the repair of the intestinal mucosa[24]. Abnormal oxidative stress in UC results in the release of myeloperoxidase (MPO), which leads to the over-activation of intestinal immune cells and exacerbates the inflammatory response in the intestines[25]. Superoxide dismutase (SOD), an essential antioxidant enzyme, plays a vital role in neutralizing

excess free radicals and reducing oxidative damage to cells[26]. Malondialdehyde (MDA), the final product of ROS-induced lipid peroxidation, serves as a significant biomarker for assessing cellular damage, with its concentration directly indicating the degree of oxidative injury[27]. As a vital non-enzymatic antioxidant within cells, glutathione (GSH) plays a crucial role in protecting against oxidative stress, regulating cell proliferation, apoptosis, and immune functions[28]. It has been employed as a biomarker for inflammation and oxidative stress, reflecting the extent of colonic mucosal damage[29]. Consequently, by measuring the levels of MPO, MDA, SOD, and GSH in the serum of mice with UC, researchers can assess the degree of lipid peroxidation, thereby enabling the evaluation of intestinal mucosal damage and oxidative stress levels associated with the condition. The results of this study demonstrate that Ononin, when combined with *Lactobacillus paracasei*, can enhance SOD and GSH activity in a dose-dependent manner, reduce MPO and MDA levels, and inhibit oxidative stress responses.

Studies have confirmed that dysfunction of the intestinal epithelium is a critical factor in the onset and recurrence of UC, with tight junctions serving as the key determinants of normal intestinal epithelial barrier function[30]. Tight junctions consist of structural proteins such as Occludin, Claudin-1, Zonula occludens proteins (ZO), and junctional adhesion molecules (JAM)[31]. Both Occludin and Claudin-1 serve as biomarkers for intestinal epithelial barrier integrity. Typically, these proteins form elongated, chain-like structures that bridge the gaps between adjacent epithelial cells, thereby regulating epithelial permeability[32]. Therefore, modulating tight junctions (TJs) to maintain epithelial barrier integrity represents a critical therapeutic target in UC. The results of this study indicated that, compared to the CON group, mice in the DSS group exhibited significantly down-regulated expression of Occludin and Claudin-1 proteins in the colonic tissues. After treatment with Ononin and *Lactobacillus paracasei*, the inflammatory state significantly improved, the expression of Occludin and Claudin-1 proteins was significantly up-regulated, suggesting that the pathogenesis of UC may be closely related to the abnormal expression of proteins that are integral to the intestinal mechanical barriers.

Ferroptosis is a novel form of programmed cell death characterized by iron-dependent lipid peroxidation and the accumulation of ROS[33]. Emerging evidence suggests that the pathogenesis of

UC is closely linked to intestinal iron overload and the dysregulation of iron metabolism[11]. Notably, GPX4 and ACSL4 have been identified as crucial regulators of ferroptosis. GPX4 functions as the key enzyme responsible for catalyzing the reduction of lipid peroxides in cells, thereby preventing cytotoxic effects and subsequent ferroptosis[34]. Conversely, ACSL4 serves as a critical biomarker of ferroptosis; its upregulation not only enhances lipid peroxide production but also significantly suppresses GPX4 expression, ultimately leading to impaired ROS clearance, oxidative stress, and the induction of ferroptosis[11,35]. In the present study, we observed that the DSS-induced UC model group exhibited decreased expression of GPX4 and increased expression of ACSL4 in intestinal mucosal tissues, indicating the occurrence of ferroptosis in UC mice. These findings collectively demonstrate that the therapeutic benefits of Ononin and *Lactobacillus paracasei* in alleviating intestinal mucosal damage in UC mice are at least partially mediated through the inhibition of ferroptosis pathways.

The JAK2/STAT3 signaling pathway is a critical pathway involved in oxidative stress and inflammatory activation[36]. Within this pathway, the phosphorylation of JAK2 initiates a cascade of reactions: activated JAK2 subsequently induces the phosphorylation and dimerization of downstream STAT3. The phosphorylated STAT3 dimer then translocates into the nucleus as a component of the transcriptional regulator complex, where it promotes the transcription of specific target genes involved in various physiological processes, including stem cell homeostasis, cell cycle progression, and apoptosis[37]. To further elucidate the role of the JAK2/STAT3 signaling pathway in the combination of Ononin and *Lactobacillus paracasei* in inhibiting ferroptosis and alleviating intestinal mucosal damage in mice with UC, this study administered Ononin in conjunction with *Lactobacillus paracasei* through intragastric administration to mice treated with DSS and observed its effects on intestinal mucosal injury in UC mice. The results demonstrated that Ononin, in combination with *Lactobacillus paracasei*, could inhibit the phosphorylation of the JAK2/STAT3 signaling pathway, disrupt signal transduction, and reduce the production of inflammatory factors as well as the activation of immune cells. This mechanism induces ferroptosis in intestinal mucosal epithelial cells, counteracting the protective effect of DSS on intestinal mucosal damage in mice with UC and ultimately ameliorating intestinal mucosal injury in these mice.

In summary, the combination of Ononin and *Lactobacillus paracasei* may slow the pathological progression of UC and inhibit ferroptosis. The underlying mechanism is speculated to involve the inhibition of the JAK2/STAT3 signaling pathway, downregulation of TNF-α and IL-1β, and effective regulation of oxidative stress markers such as (MPO, SOD, MDA, GSH, as well as key proteins including Occludin, Claudin-1, p-JAK2, p-STAT3, ACSL4, and GPX4.

The limitation of this study may include the following aspects. Firstly, the sample of the study is relatively small. Secondly, experimental model in this study is acute UC model and Ononin combined with *Lactobacillus paracasei* treatment in chronic UC remains unclear. Thirdly, the mechanism of the interaction of oxidative stress, ferroptosis, and JAK2/STAT3 with Ononin combined with *Lactobacillus paracasei* needs further elucidate. Thus, a study with large samples and chronic UC model is warranted in the future to further explore the mechanism. In addition, the combination of Ononin treatment with other probiotic would be investigated.

Collectively, our findings explored the mechanism of Ononin in conjunction with *Lactobacillus paracasei* treating UC and could be helpful in the development of new treatment methods in UC.

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Table 1 Quantitative table of DAI score

Weight loss (%)	Feces consistency	Hemafecia	Score
0	Normal	N/A	0

1-5	Mild soft	Slight bleeding	1
5-10	Soft and wet	Moderate bleeding	2
10-20	Half loose stool	Gross bleeding	3
＞20	loose stool	Blood clot around anus	4

Figure Legends

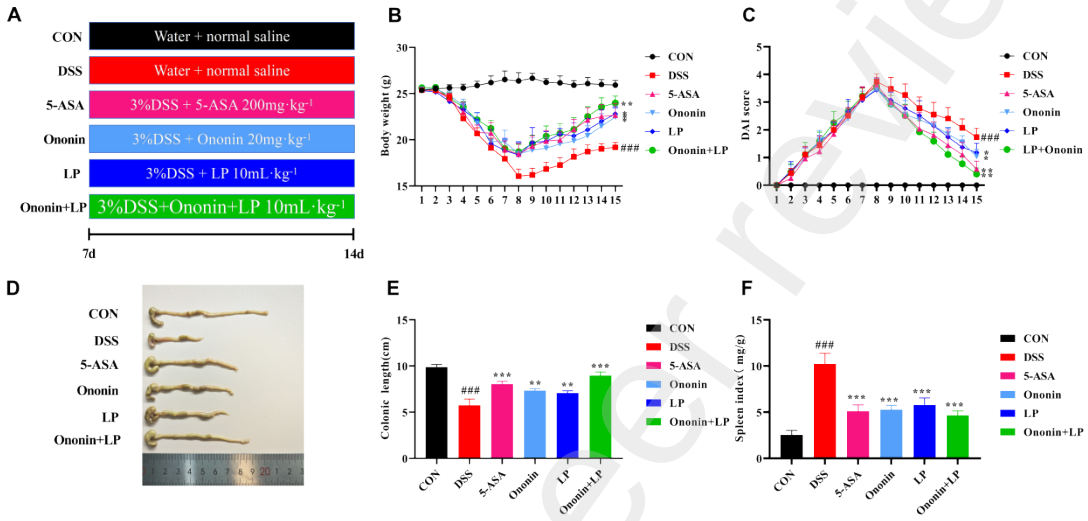

Figure 1. Effects of Ononin combined with *Lactobacillus paracasei* on ameliorating the general symptoms of murine colitis. (A) Schematic representation of the DSS-induced colitis model in BALA/c mice and the experimental design. (B) Changes in body weight of mice following the induction of colitis. (C) Daily disease activity index (DAI) of mice after colitis induction. (D) Representative image of colon lengths. (E) Colon length. (F) Spleen index. All data are presented as mean ± standard deviation. $^{###}p<0.001$ vs. CON group, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. DSS group.

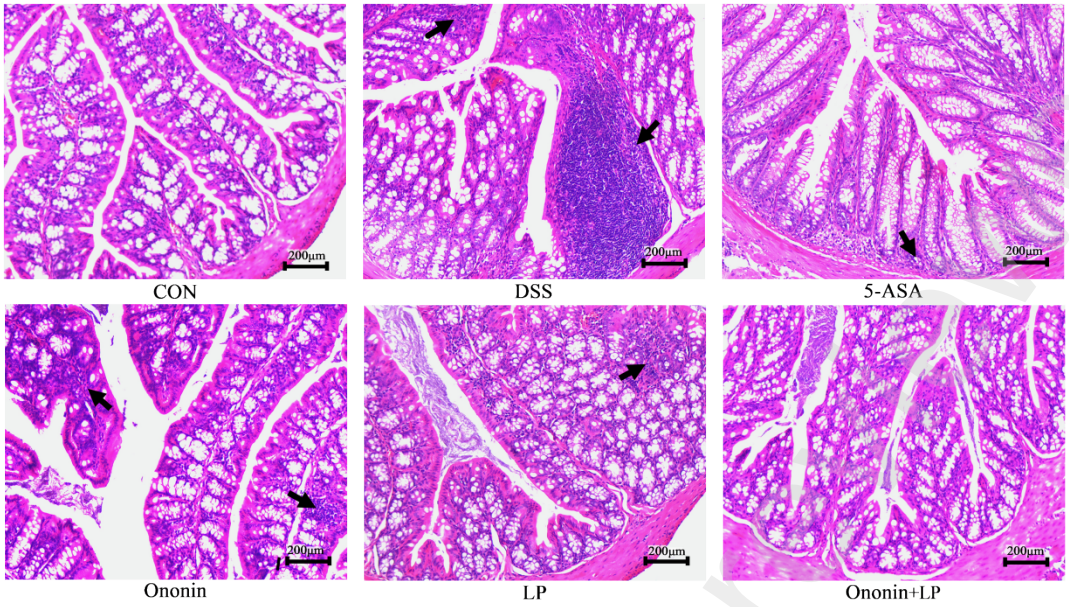

Figure 2. Histopathological analyses of H&E-stained colon tissue sections in a DSS-induced UC model in mice. Arrows indicated the inflammatory infiltration, mucosal erosion, and damage of crypts. Scale bar = 200 μm.

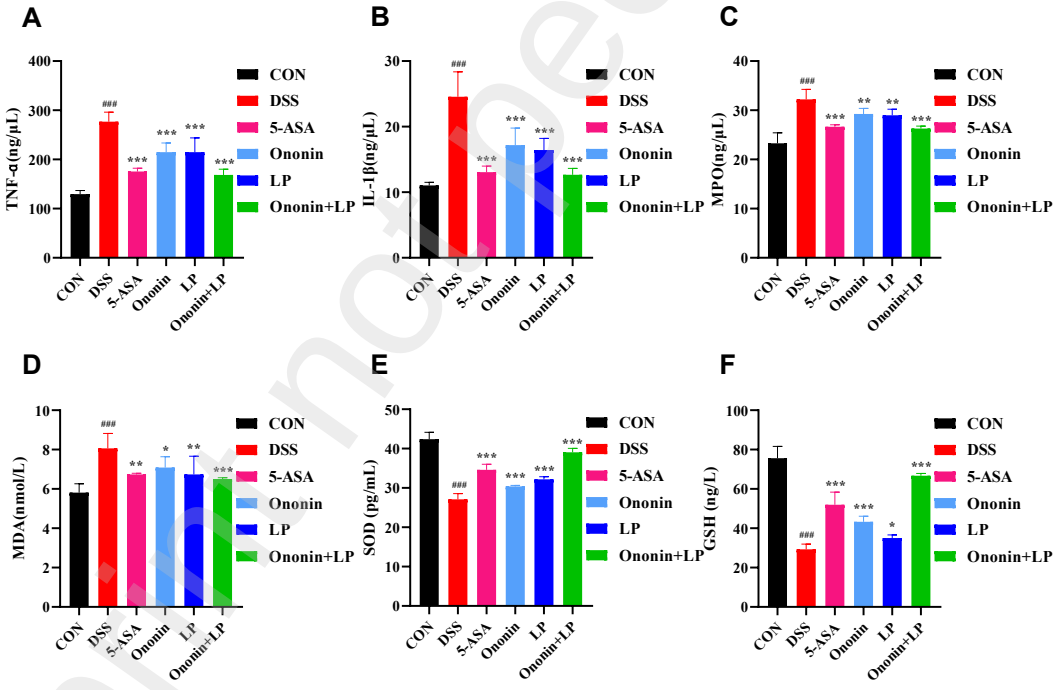

Figure 3. Effects of Ononin combined with *Lactobacillus paracasei* on pro-inflammatory factors and oxidative stress levels in mice. (A) Serum TNF-*α* levels. (B) Serum IL-1β levels. (C) Serum MPO levels. (D) Serum MDA levels. (E) Serum SOD levels. (F) Serum GSH levels. All data are presented as mean ± standard deviation. $^{###}p<0.001$ vs. CON group; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. DSS group.

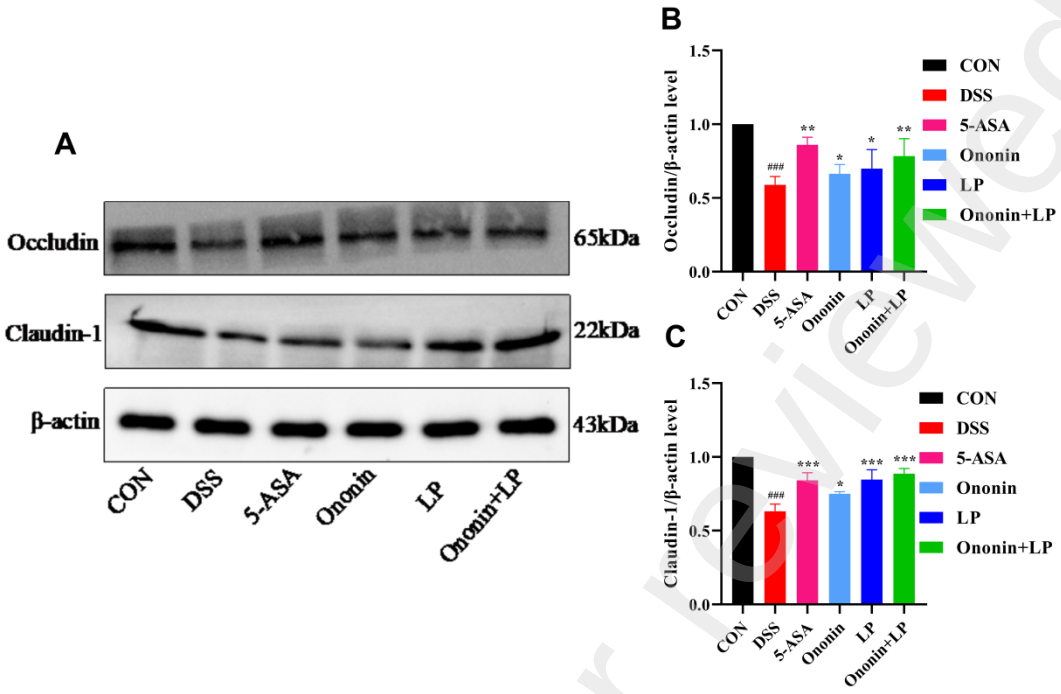

Figure 4. Effect of Ononin combined with *Lactobacillus paracasei* on the colon tissue protein levels of Occludin and Claudin-1 were examined by western blot, (A) Representative western blotting images of Occludin and Claudin-1 in each group.(B) Occludin. (C) Claudin-1. Relative levels of proteins were qualified by ImageJ software and was normalized by corresponding total protein content. All data were presented as mean ± standard deviation. $^{###}p<0.001$, vs CON group; $^{*}p<0.05$,$^{**}p<0.01$,$^{***}p<0.001$, vs DSS group.

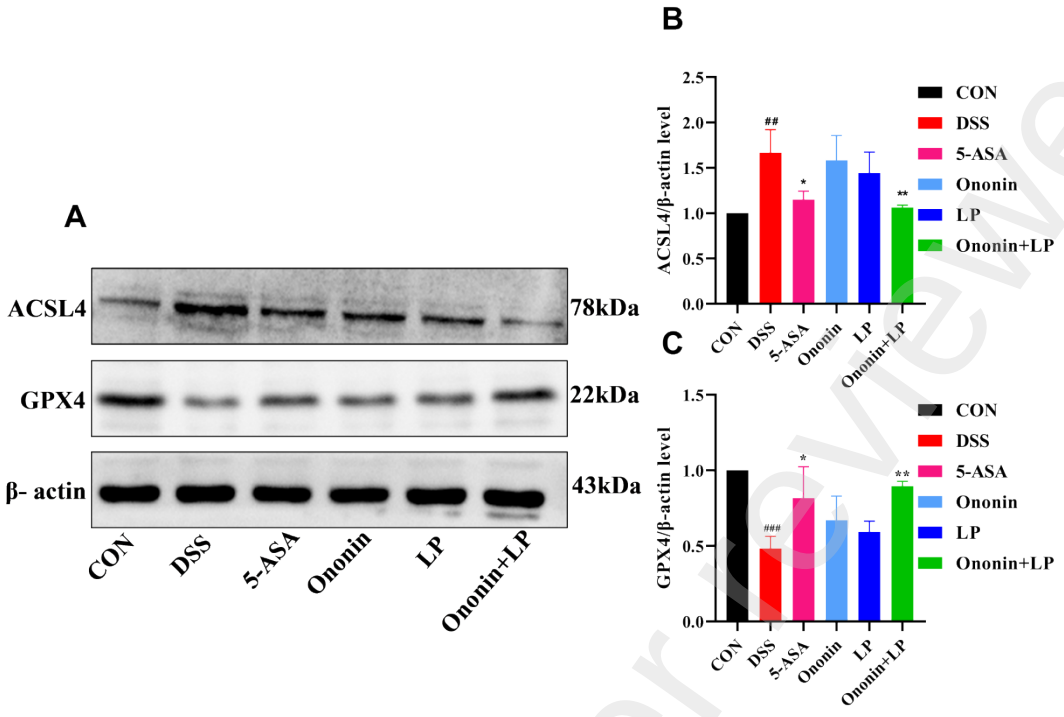

Figure 5. Effect of Ononin combined with *Lactobacillus paracasei* on the colon tissue protein levels of GPX4 and ACSL4 were examined by western blot, (A) Representative western blotting images of GPX4 and ACSL4 in each group.(B) ACSL4 (C) GPX4. Relative levels of proteins were qualified by ImageJ software and was normalized by corresponding total protein content. All data were presented as mean ± standard deviation. $^{###}p<0.001$, vs CON group; $^{*}p<0.05$,$^{**}p<0.01$,$^{***}p<0.001$, vs DSS group.

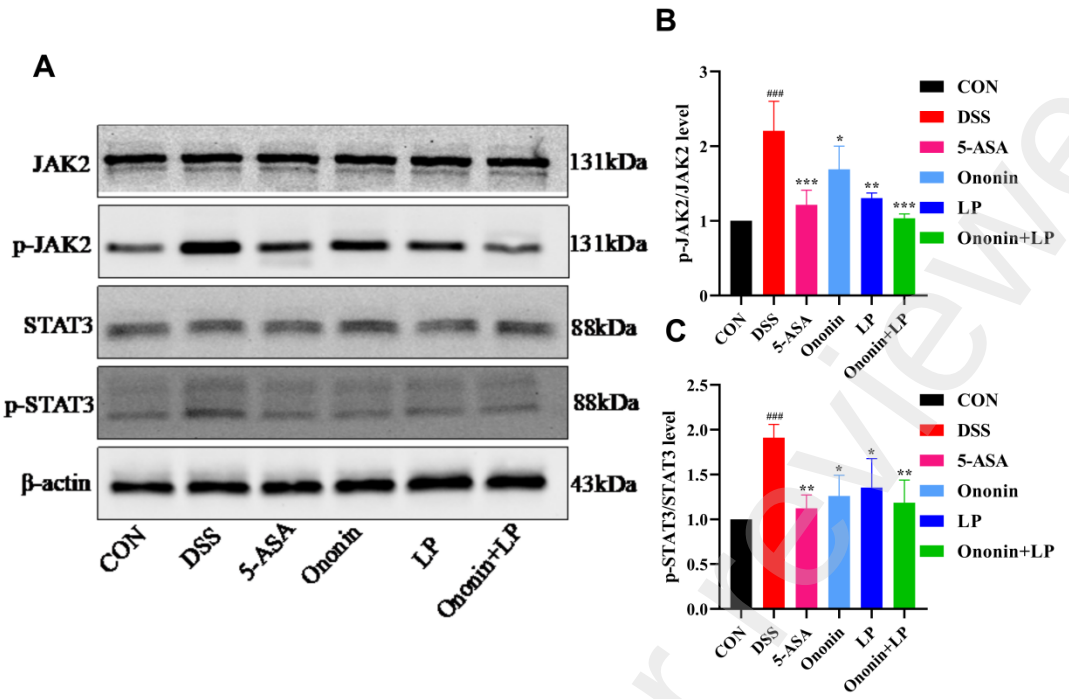

Figure 6. The effect of Ononin combined with *Lactobacillus paracasei* on the JAK2/STAT3 signaling pathway in a DSS-induced UC model in mice is presented. (A) Representative western blot images showing the levels of p-JAK2, JAK2, p-STAT3, and STAT3 in each group. (B) The ratio of p-JAK2 to JAK2. (C) The ratio of p-STAT3 to STAT3. Relative phosphorylation levels of the proteins were quantified using ImageJ software and normalized to the corresponding total protein content. All data are presented as mean ± standard deviation. $^{###}p<0.001$ vs. CON group; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. DSS group.