

Danmu, a Traditional Chinese Medicine from the stem of *Nauclea officinalis*, Alleviates DSS-Induced Colitis by Modulating Inflammatory Cytokines and Gut Microbiota

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Research Article

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Abstract

Danmu (DM), derived from the stem of *Nauclea officinalis*, is a traditional Chinese medicine renowned for its anti-inflammatory and antibacterial properties. However, its potential in treating inflammatory bowel diseases (IBD) remains unexplored. This study investigates the therapeutic effects of DM on dextran sulfate sodium (DSS)-induced colitis in mice, focusing on its modulation of inflammatory cytokines and gut microbiota. Using a DSS-induced colitis model, we evaluated the impact of DM on clinical symptoms, inflammatory cytokine expression, and gut microbiota composition. DM treatment significantly alleviated weight loss, colon shortening, and histopathological damage in DSS-treated mice. Mechanistically, DM suppressed pro-inflammatory cytokines (IL-6, IL-12, IL-17, and TL-1A) while upregulating anti-inflammatory cytokines (IL-13, TGF- β , and IL-2). Furthermore, 16S rRNA sequencing revealed that DM altered gut microbiota composition, reducing Firmicutes and increasing Actinobacteriota, with specific genera (e.g., *Bifidobacterium*) correlating with IL-6 modulation. These findings suggest that DM ameliorates colitis by regulating inflammatory cytokine expression and reshaping gut microbiota, thereby modulating intestinal immune responses. This study highlights DM as a promising therapeutic candidate for IBD, offering a dual mechanism of action through immune modulation and microbiota regulation.

Introduction

Nauclea officinalis Pierr ex Pitard is a plant of the genus *Nauclea* in the family *Rubiaceae* with a height of 4–12 m. It grows in hidden and humid mountainous forests at an altitude of 200–2200 m. Wild *Nauclea officinalis* is primarily distributed in Hainan, Yunnan, and Guangxi provinces of China [1], as well as in Southeast Asian countries such as Vietnam, Cambodia, and Laos. The cultivated *Nauclea officinalis* is mainly distributed in the Qiongzong area of Hainan province, China [2]. The stem of *Nauclea officinalis* is known as Danmu in Chinese, which gives off a unique smell. In folk traditions, Danmu has long been used to boil syrup, and the syrup made from it has shown efficacy in relieving throat inflammation [3]. Moreover, Danmu, as a traditional Chinese medicine, has a wide - ranging anti - inflammatory effect. It exhibits therapeutic effects on a variety of inflammatory diseases, including cold, fever, acute tonsillitis, sore throat, conjunctiva inflammation, enteritis, dysentery, eczema, etc. [2, 4, 5].

Inflammatory bowel disease (IBD) is a chronic gastrointestinal inflammatory disease, including Crohn's disease (CD) and Ulcerative colitis (UC). It is estimated that there are more than 1 million IBD patients in the United States, 2.5 to 3 million IBD patients in Europe, and more than 1 million IBD patients each in India and China, which pose a huge burden on global medical care [6]. The pathogenesis of IBD may be related to genetic factors, environmental changes, intestinal mucosal immune dysregulation, and intestinal microbiota dysbiosis [7–10]. In general, microbial diversity is reduced in intestine of IBD patients, resulting in significant changes in the composition, structure and function of their intestinal microbiota [11]. Current therapeutic drugs for IBD are antibodies and inhibitor for pro-inflammatory cytokines include infliximab, Ustekinumab, mesalazine, and budesonide [12–15]. In recent years, as the relationship between IBD and intestinal microbiota has become better understood [9, 16], intestinal

microbiota-based therapeutic interventions, including fecal transplantation [17], have been used in the therapy of IBD. On the one hand, numerous studies have shown that many traditional Chinese medicine also have good therapeutic effects on IBD, including Qing Hua Chang Yin, *Paeonia lactiflora* pall, Baishaoqiwu, etc. [18]. However, whether DM with anti-inflammatory effect is effective in treating IBD has not been reported.

To explore the curative effect of DM on IBD, we used a DSS-induced colitis model in mice to study the therapeutic effect of DM on UC and further studied its effect on the expression of related inflammatory cytokines in the mouse colon tissues, and also explored the effect on intestinal microbiota during the treatment of colitis.

Results and discussion

2.1 DM significantly alleviated DSS-induced colitis in mice

DM was found to be effective in the therapy of DSS-induced colitis (Fig. 1). The body weight changes of mice in different groups are shown in Fig. 1a. Both low DM and high DM could significantly attenuate the weight loss of mice caused by DSS-induced colitis. As shown in Fig. 1a that the body weights of mice in DM_treat group are significantly higher than that of mesalazine group on days 3–5; while on day 7, the body weight of mice in DM_treat group and in the mesalazine group were roughly equivalent, suggesting that the therapeutic effect of DM on colitis was more rapid compared with that of mesalazine, while the final effect was similar. It also reflected that the mechanisms for therapy of colitis may be different from each other.

Figure 1b shows that DSS-induce could shorten the length of mouse colon, and DM treatment had a good therapeutic effect on DSS-induced colitis, and could significantly slow down DSS-induced mouse colon shortening in a dose-dependent manner. We performed histopathological analysis on mouse colon tissue through HE staining (Fig. 2c), and found that the colon tissue structure in the low DM group was moderately abnormal, with a small amount of inflammatory cell infiltration. In the high DM group, only mild abnormalities were observed in the colon tissue. On the other hand, the colon tissue in the DSS group was severely abnormal, with a large number of inflammatory cells and fibroblasts infiltrated. On the contrary, the slices of the control group showed normal colon tissue. In the mesalazine group, the colon tissue was mildly abnormal, with some cells necrotic, and small amount of inflammatory cell infiltration in the mucosal layer. The results indicate that low DM could relieved DSS-induced colitis to a certain extent, and high DM had a more significant therapeutic effect on DSS-induced colitis, which is similar to mesalazine.

2.2 DM effected the expression of inflammatory cytokines in mouse colon tissue

To explore the mechanism of how DM alleviated DSS-induced colitis, we measured the transcriptional levels of inflammatory cytokines in colon tissue in different treatment groups by qRT-PCR (Fig. 2).

Compared with the control group, the expressions of pro-inflammatory cytokines IL-6, IL-1 β , IL-12, IL-17, TNF- α , and TNF-like ligand 1A (TL1A) in colon tissues treated with DSS were significantly increased (Fig. 2a, c, f, i, h, j), and the expression of anti-inflammatory cytokines IL-10 and IL-13 was significantly decreased (Fig. 2b, e), which was consistent with the previous research results [14]. Compared with the DSS group, the expression of anti-inflammatory factor IL-10 and pro-inflammatory factor TNF- α in low DM group and high DM group had no significant difference (Fig. 2b, h), the expression levels of pro-inflammatory cytokines IL-6, IL-12, IL-17, and TL-1A were significantly reduced (Fig. 2a, f, i, j), while the expressions of pro-inflammatory cytokines IL-2 and IL-13, and anti-inflammatory factor TGF- β were significantly increased (Fig. 2e, g, k). Compared with DSS group, the expression levels of IL-1 β and IFN- γ had no significant difference in low DM group (Fig. 2c, d), but the expressions of IL-1 β and IFN- γ had significantly decreased in high DM (Fig. 2c, d). Thus, DM appeared to upregulate anti-inflammatory cytokines expression and inhibit the expression of pro-inflammatory cytokines in a dose-dependent manner, thereby alleviating the inflammatory responses.

The anti-inflammatory effects observed in DM are primarily linked to its active components, where multiple compounds have been identified as possessing anti-inflammatory properties. The main active components of DM are alkaloids, and the contents of strictosamide and pumiloside in DM are the highest, accounting for 11.5% and 4.7%, respectively [5]. Several studies have shown that strictosamide had the best anti-inflammatory effect among many components of DM [7, 8, 19]. In the DSS-associated colitis model in mice, strictosamide could down-regulate TNF- α , IL-1 β and IL-6, and reduce the production of NO to inhibit inflammation. At the same time, strictosamide could also inhibit the signal transduction of NF- κ B, and had a good therapeutic effect on colitis. It has also been found that most of the other active components of DM can inhibit the production of NO, including naucleoffeine H [20], 17-O-methyl-19-(Z)-naucine [21], and 17-oxo-19-(Z)-naucine [6]. When studying the anti-inflammatory active ingredients present in DM, it was found that in addition to alkaloids, pentacyclic triterpenoids and phenolic acids also have certain anti-inflammatory activities. For example, 3 β ,19 α ,23,24-tetrahydroxyurs-12-en-28-oic acid isolated from DM has anti-inflammatory activity and can inhibit LPS-induced NO synthesis in macrophages [22].

2.3 Effect of DM on mouse intestinal microbiota

To explore the effect of DM on intestinal microbiota of mice, we used 16S rRNA gene sequencing to analyze the composition and structure of intestinal microbiota in the cecal contents of different groups of mice. We evaluated the α diversity of intestinal microbiota in different treatment groups by calculating Shannon, Shannoneven, and Sobs indices (Fig. 3a-c). The results showed that there were no significant differences in Shannon and Shannoneven indices of the intestinal microbiota in the mice treated with DM compared with those in the DSS group (Fig. 3a, b), while the Sobs index was significantly decreased in the high DM group (Fig. 3c). The results of α diversity analysis showed that DM treatment did not affect the species diversity and species evenness, but it could reduce the species richness of mouse intestinal microbiota, which may result from the bactericidal activity of DM, leading to the death of some bacteria to decrease the Sobs index (Fig. 3b). PCoA analysis showed that there were significant

differences among the intestinal microbiota of mice in different treatment groups, and this difference increased with the increase of DM concentration (Fig. 3d).

The abundances of some bacteria in the intestinal microbiota of mice were changed after DM treatment. When the intestinal microbiota of mice from different groups were analyzed at the phylum level, we found that the bacterial phyla with a total abundance greater than 0.01% include *Bacteroidota*, *Firmicutes*, *Actinobacteriota*, *Campilobacterota*, *Desulfobacterota*, *Verrucomicrobiota*, and *Proteobacteria* (in order from the highest to the lowest), and the abundance of *Firmicutes* in the mouse cecum decreased while the abundance of *Actinobacteriota* increased after DM treatment (Fig. 4a). Simultaneously, we analyzed the intestinal microbiota at the genus level (Fig. 4b), and found that compared with the DSS group, the abundances of *Lachnospiraceae* NK4A136 group, *Turicibacter*, and *Dubosiella* decreased significantly, while the abundances of *Ruminococcus torques* group, *Bifidobacterium*, and GCA-900066575 were significantly increased in DM_treated group (Fig. 4c). When the low DM group was compared with the DSS group, the *Parabacteroides* abundance was significantly decreased; while the *Lachnoclostridium* abundance was significantly increased. When the high DM group was compared with the DSS group, the abundance of *Parabacteroides* increased significantly, while the abundance of *Lachnoclostridium* decreased significantly (Fig. 4c).

We also used LEfSe to analyze strain differences at different taxonomic levels (Fig. 4d). Compared with the DSS group, the abundances of some bacteria in the low DM group and high DM group increased or decreased at the same time (strains in Fig. 4d with *). Changes in these bacteria were more likely to be induced by DM than strains whose abundances only increased or decreased in the low DM group or high DM group, and we therefore focused on these strains. In phylum *Bacteroidetes*, the abundances of family *Prevotellaceae* and its genus *Prevotellaceae*_UCG-001 in the DM_treat group were lower than those in the DSS group. In phylum *Firmicutes*, the abundances of order *Erysipelotrichales*, order *Peptostreptococcales-Tissierellales*, family *Peptostreptococcaceae*, genus *Romboutsia*, genus *Dubosiella*, and genus *Turicibacter* were all decreased in the DM_treat group then those in the DSS group, while the abundances of family *Erysipelatoclostridiaceae*, *Clostridium innocuum* group, genus *Staphylococcus*, genus GCA-900066575 (belong to family *Lachnospiraceae*), *Ruminococcus torques* group and so on were all increased in the DM_treat group compared with those in the DSS group. These shifts in bacterial abundance could arise from either the direct bactericidal effect of DM or alterations in the intestinal immune microenvironment following DM treatment.

To explore the relationship between the changes in the intestinal immune environment of mice and the changes in intestinal microbiota after DM treatment, we performed a correlation analysis between the transcript abundance of inflammatory cytokines in mouse colon tissues and the abundance of intestinal microbiota at genus level (Fig. 5), and found that 17 genera of intestinal bacteria were correlated with the expression of IL-6, among which 11 genera of bacteria were positively correlated with IL-6, and 5 genera were negatively correlated with IL-6; 15 genera of intestinal bacteria were correlated with the expression of IFN- γ , among which 10 genera of intestinal bacteria were positively correlated with IFN- γ , while 5 genera were negatively correlated with IFN- γ (Fig. 5a). But when we separated the DM_treat group from

the DM_untreat group for correlation analysis, we found that in the DM_untreat group, intestinal microbiota was regulated by a variety of inflammatory cytokines, including the important inflammatory factor nodes such as IFN- γ , IL-13, IL-17, and TGF- β (Fig. 5b); in the DM_treat group, only IL-6 was the important inflammatory factor node, but a few inflammatory cytokines were associated with individual intestinal bacteria (Fig. 5c). According to the results, we speculated that DM treatment could reshape the intestinal immune environment, thus altering the intestinal microbiota. Moreover, the structure of the intestinal microbiota could regulate intestinal inflammatory cytokines, especially IL - 6, thereby improving DSS - induced colitis in mice.

Multiple studies validate the rationality of our finding that gut microbiota changes alleviate murine colitis by downregulating IL-6. Qu et al. found kaempferol - induced gut microbiota changes reduced IL-1 β , IL-6 [23], and TNF- α levels, while Wu et al. reported *Lactobacillus plantarum* HNU082 regulated the gut microbiota to decrease these cytokines and IFN- γ [24]. Chen et al. showed palmitoleic acid - reprogrammed gut microbiota reduced TNF- α and IL-6, improving anti-TNF- α therapy [25]. Hao et al. demonstrated extracellular vesicles from *Lactobacillus plantarum* Q7 improved gut microbiota and decreased IL - 6, IL - 1 β , IL - 2, and TNF- α [26]. These studies highlight the link between gut microbiota modulation, cytokine regulation, and colitis alleviation. Building on our finding that DM treatment reshapes the intestinal microbiota to improve the intestinal environment, this effect is manifested in its distinct impact on the abundances of various bacterial species. Prior research has identified distinct microbiota alterations in IBD patients. For instance, compared to healthy individuals, the intestines of IBD patients show a significant increase in the abundance of the *Clostridium innocuum* group [27], *Staphylococcus* [28], and *Ruminococcus torques* group [29], while the abundance of *Romboutsia* [30] is notably decreased. In DSS - treated mice, reducing the abundance of *Prevotellaceae*_UCG - 001 can be counteracted by nitrate application, which improves colitis and boosts the abundance of this bacterium [31].

In our study, compared with DSS - treated mice, low - and high - dose DM - treated mouse colon tissues had increased abundances of the *Clostridium innocuum* group, *Staphylococcus*, and *Ruminococcus torques* group, but decreased abundances of *Romboutsia* and *Prevotellaceae*_UCG - 001. These changes imply DM modifies the murine intestinal microbiota structure, potentially affecting colitis development. Despite the lack of established links between these bacteria and colitis, our results call for caution. For future DM applications in treating human colitis, monitoring microbiota changes is crucial. Using probiotics and prebiotics to regulate the microbiota may optimize the intestinal environment and boost DM's therapeutic effect.

Conclusion

This study shows that Danmu (DM) effectively alleviates DSS - induced colitis. DM reduces pro - inflammatory cytokines (IL-6, IL-12, IL-17, and TL-1A) and boosts anti - inflammatory ones (IL-13, TGF- β , and IL-2), improving colonic histopathology. It also reshapes the gut microbiota, decreasing Firmicutes and increasing Actinobacteriota. Specific genera like *Bifidobacterium* strongly correlate with IL-6

expression. These suggest DM regulates intestinal immunity via the gut microbiota. DM has potential as a multi - target IBD treatment, combining anti - inflammatory and microbiota - modulating effects. Future research should explore DM - gut microbiota interaction mechanisms, validate its clinical efficacy, and consider combining it with probiotics for better IBD treatment.

Declarations

CRedit authorship contribution statement

Xianglan Lei: designed the experiments and analyzed data, supervision, conceptualization, writing-review & editing. Yuxuan Peng: analyzed data, verified the figures, methodology, data curation and wrote the original draft. Zhenguo Shen: contributed to the animal experiment. Yaqin Lin: performed the experiment and analyzed data. Yan Li: performed the experiment and analyzed data. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Ethics declaration

All animal experiments described in this study were conducted in strict accordance with the guidelines and regulations set by the Experimental Animal Centre of Huazhong Agriculture University (ID Number HZAUMO-2022-0055).

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Data availability

Data will be made available on request.

Funding Declaration

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Figures

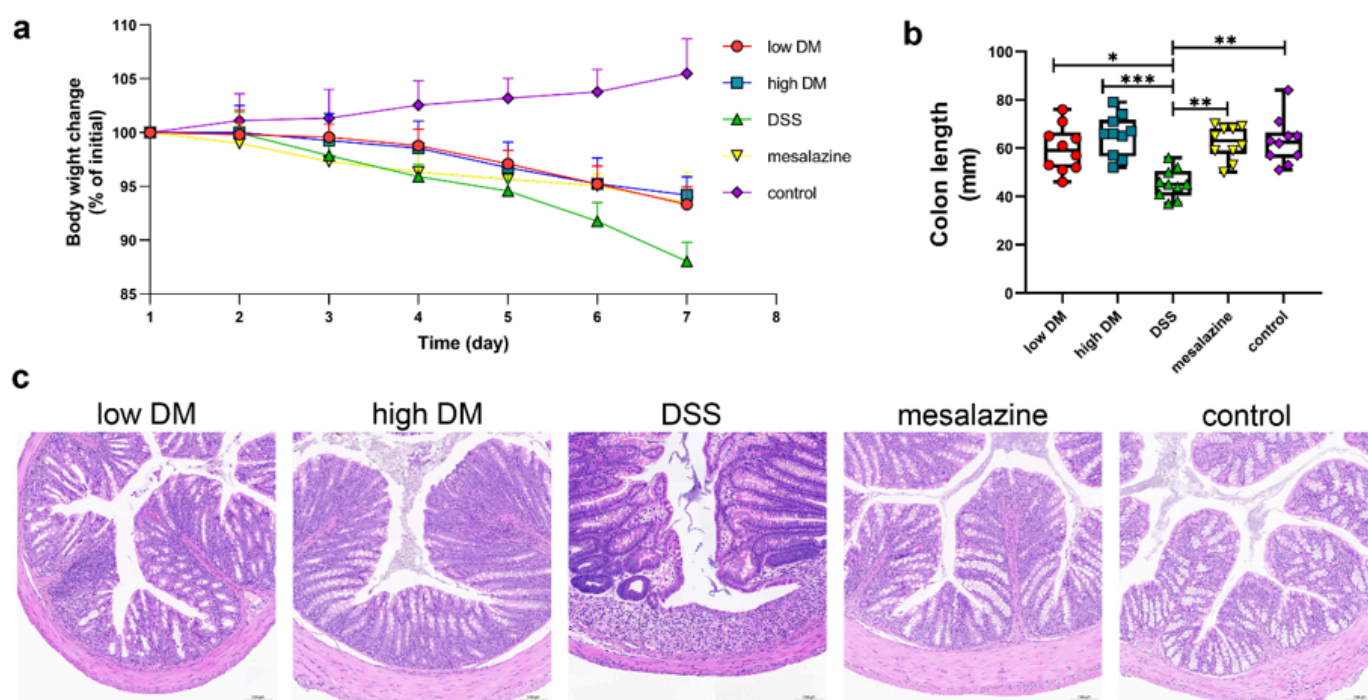


Figure 1

The therapeutic effect of DM on DSS-induced colitis in mice. **(a)**: Changes in body weight of mice in different treatment groups. **(b)**: Colon length of mice in different treatment groups, $n = 10$ for all groups, statistical analyses were performed using Kruskal-Wallis test, *: $p < 0.05$, **: $p < 0.01$, ***, $p < 0.001$. **(c)**: Histopathological analysis of mouse colon tissue sections in different treatment groups. The scale bar is $100 \mu\text{m}$. The mouse colon tissues in the DSS group were severely abnormal, those in the low DM group were moderately abnormal, and those in the high DM group and mesalazine groups were only slightly abnormal, whereas the colon tissues in the control group exhibited normal morphology.

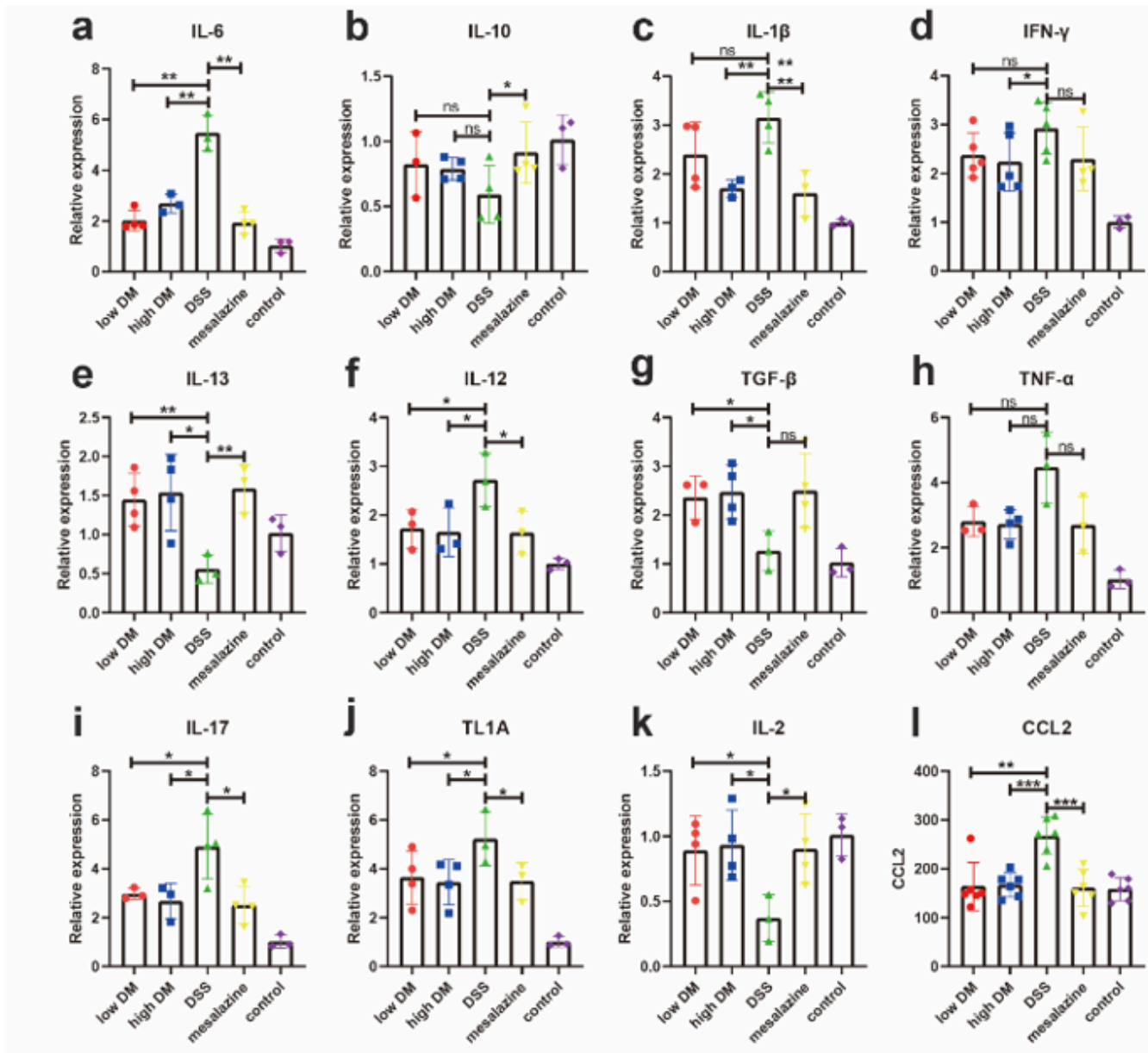


Figure 2

Effects of the expression levels of inflammatory cytokines in mouse colon tissues by different treatments. **(a-k)** qRT-PCR was used to detect the relative expression levels of inflammatory cytokines in different treatment groups. **(l)**: ELISA was used to measure the expression level of CCL2 protein. Statistical analysis was performed using a two-sided Student *t*-test. *: $p < 0.05$, **: $p < 0.01$, ***, $p < 0.001$. The values represent the mean $\pm$ standard deviation.

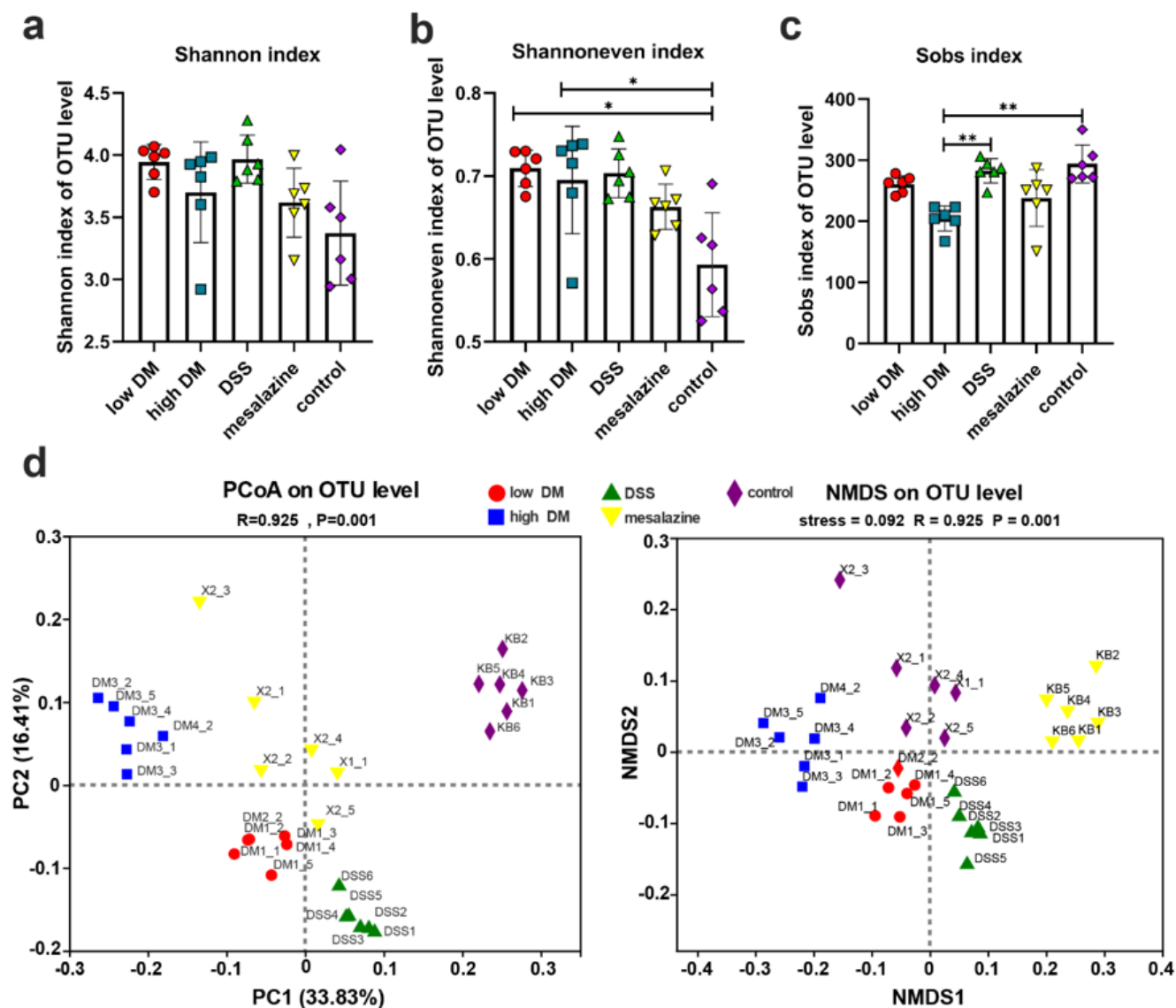


Figure 3

Analysis of DM on intestinal microbial diversity in mice. Shannon (a), Shannoneven (b), and Sobs (c) indices were calculated to assess the α diversity of intestinal microbiota. (d): PCoA analysis and NMDS analysis of intestinal microbiota in different groups, based on unweighted uniFrac distance. The values represent the mean $\pm$ standard deviation. Statistical analysis was performed using Kruskal-Wallis test. *: $p < 0.05$, **: $p < 0.01$.

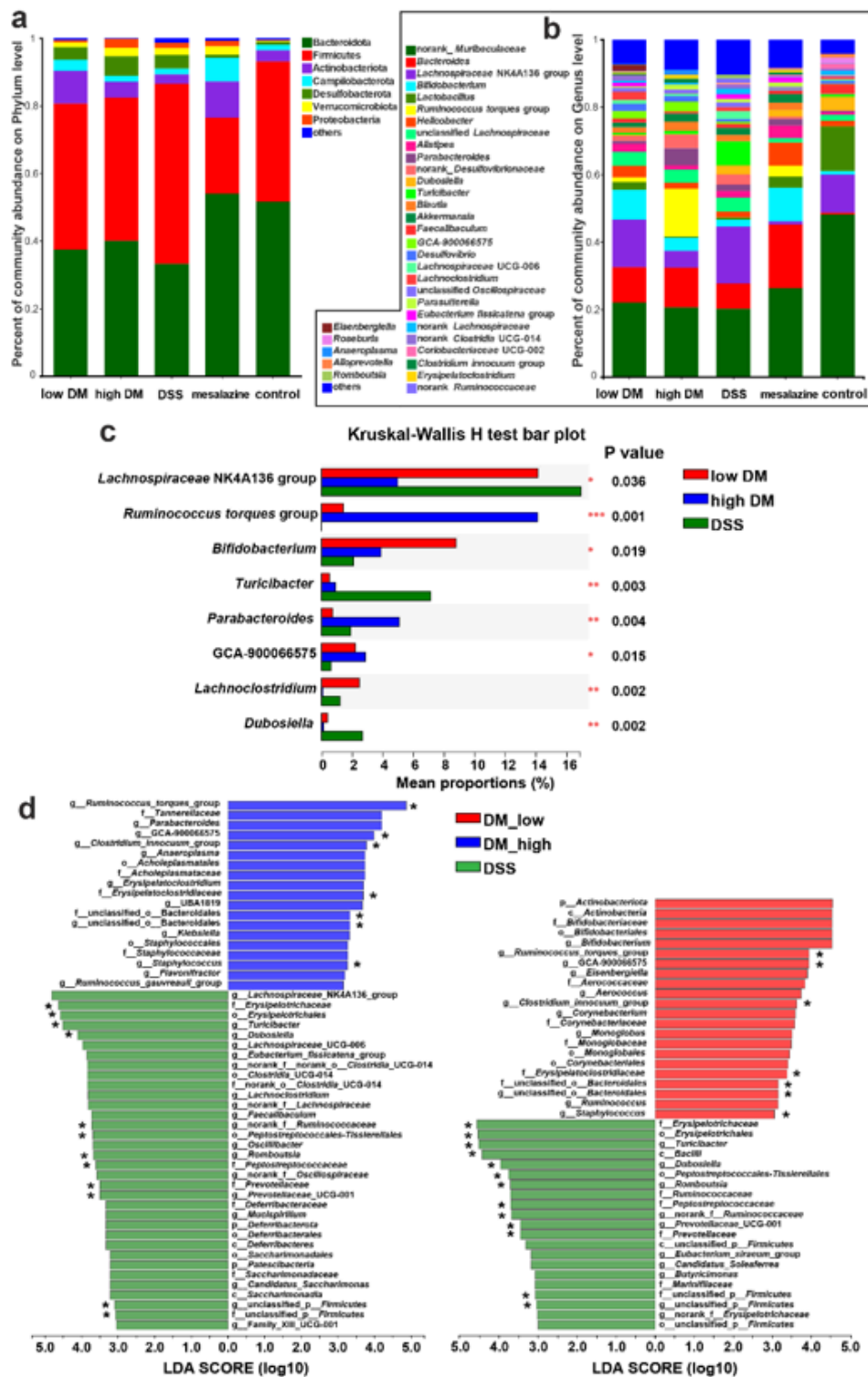


Figure 4

The composition and difference of intestinal microbiota in different treatment groups. **(a)**: The composition structure of intestinal microbiota in different treatment groups at the phylum level. **(b)**: The composition structure of intestinal microbiota in different treatment groups at the genus level. **(c)**: Differences at the genus level of intestinal microbiota between low DM, high DM and DSS groups, using the Kruskal-Wallis H test. **(d)**: The left picture shows the LefSe difference analysis between high DM and

DSS, and the right picture shows the LEfSe difference analysis between low DM and DSS, using the all-against-all analysis strategy. *: Compared with the DSS group, the bacteria whose abundances increased or decreased simultaneously in the low DM and high DM groups.

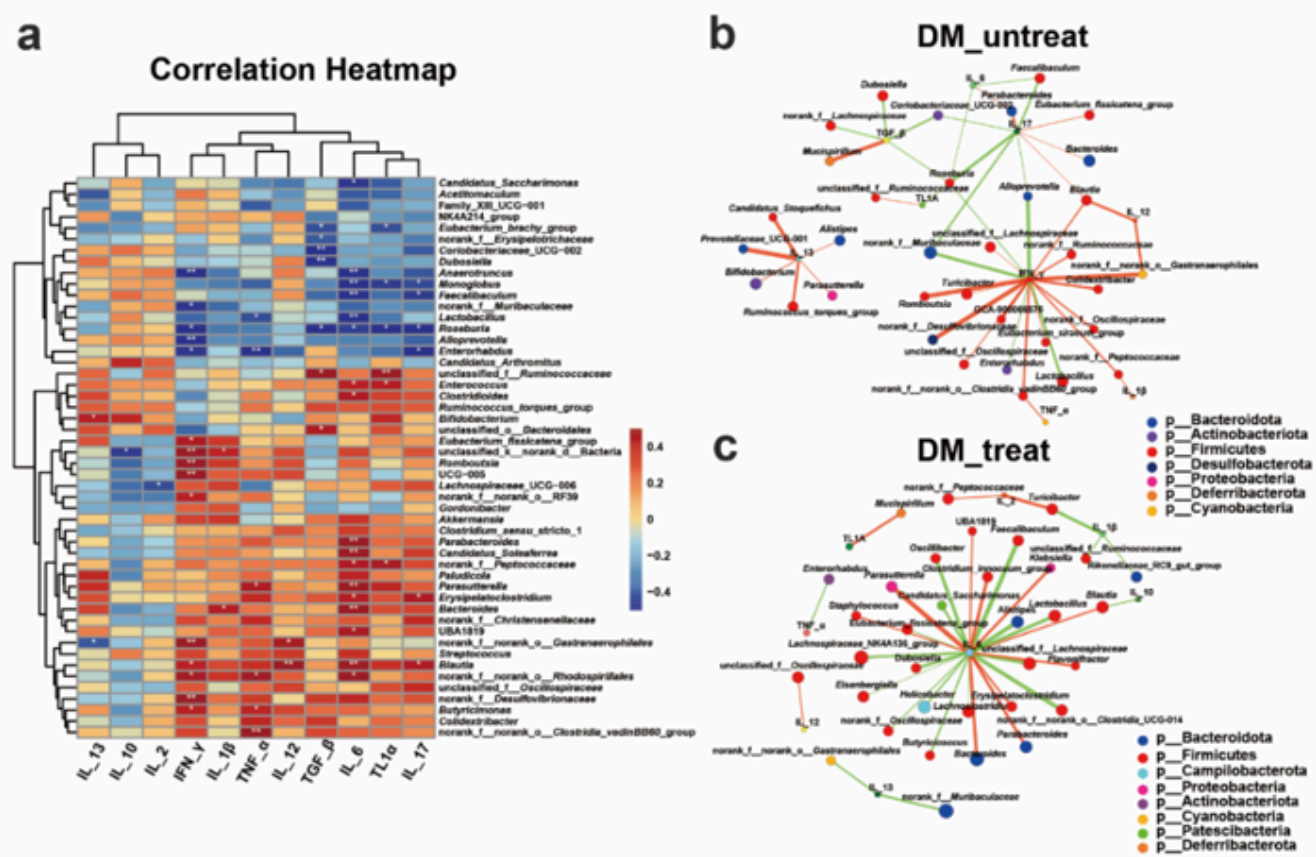


Figure 5

Analysis of the correlation between inflammatory cytokines and intestinal bacteria. **(a)**: Correlation analysis between the expression of inflammatory cytokines and intestinal microbiota in samples from all treatment groups. **(b)**: Correlation analysis between the expression of inflammatory cytokines in DM_untreat samples and intestinal microbiota. **(c)**: Correlation analysis between the expression of inflammatory cytokines in DM treat samples and intestinal microbiota. Red represents a positive correlation, green represents a negative correlation, and the thicker the line between the two points, the stronger the correlation. Correlation analysis was carried out using Spearman, and the absolute value of the correlation coefficient ≥ 0.5 , p value < 0.05 was recorded as correlation, *: $p < 0.05$, **, $p < 0.01$.

Supplementary Files

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