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Title page

Tauroursodeoxycholic acid reverses DSS-induced colitis in mice via modulation of intestinal barrier dysfunction and microbiome dysregulation

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Running title page

Running Title: TUDCA & UC: inflammation, intestinal barrier and microbiota

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DAI, disease activity index; DSS, dextran sulfate sodium; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; FXR, farnesoid X receptor; H&E, hematoxylin and eosin; IBD, inflammatory bowel disease; IL-10, interleukin 10; IL-1β, interleukin 1 beta; IL-6, interleukin 6; LEfSe, linear discriminant analysis with effect size; MPO, myeloperoxidase; OTUs, operational taxonomic units; PCoA, principal coordinate analysis; TGR5, takeda G protein-coupled receptor 5; TJ, tight junction; TNF-α, tumor necrosis factor alpha; TUDCA, tauroursodeoxycholic acid; UC, ulcerative colitis; ZO-1, zonula occludens-1.

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Abstract

Background: Ulcerative colitis (UC) is an immune-mediated inflammatory disease that can lead to persistent damage and even cancer without any intervention. Conventional treatments can alleviate UC symptoms but are costly and even cause various side effects. Tauroursodeoxycholic acid (TUDCA), a secondary bile acid derivative, possesses anti-inflammatory and cytoprotective properties for various diseases, but its potential therapeutic benefits in UC have not been fully explored.

Methods: Mice were subjected to colitis induction using 3% dextran sulfate sodium (DSS). The therapeutic effect of TUDCA was evaluated by body weight loss, disease activity index (DAI), colon length, and spleen weight ratio. Tissue pathology was assessed using H&E staining, while the levels of pro-inflammatory and anti-inflammatory cytokines in colonic tissue were quantified via enzyme-linked immunosorbent assay (ELISA). Tight junction proteins were detected by immunoblotting and intestinal permeability was assessed using fluorescein isothiocyanate (FITC)-dextran. Moreover, the gut microbiota was profiled using high-throughput sequencing of the 16S rDNA gene.

Results: TUDCA alleviated the colitis in mice, involving reduced DAI, attenuated colon and spleen enlargement, ameliorated histopathological lesions, and normalized the levels of pro-inflammatory and anti-inflammatory cytokines. Furthermore, TUDCA treatment inhibited the downregulation of intestinal barrier proteins including ZO-1 and occludin, thus reducing intestinal permeability. The analysis of

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gut microbiota suggested that TUDCA modulated the dysbiosis in mice with colitis, especially for the remarkable rise in *Akkermansia*.

Conclusion: TUDCA exerted a therapeutic efficacy in DSS-induced colitis by reducing intestinal inflammation, protecting intestinal barrier integrity, and restoring gut microbiota balance.

Key Words: Tauroursodeoxycholic acid; Ulcerative colitis; Intestinal barrier; Gut microbiota; 16S rDNA

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Significance Statement:

This study demonstrates the potential therapeutic benefits of Tauroursodeoxycholic acid (TUDCA) in ulcerative colitis (UC). TUDCA effectively alleviated colitis symptoms in mice, including reducing inflammation, restoring intestinal barrier integrity and the dysbiosis of gut microbiota. This work highlights the promising role of TUDCA as a potentially alternative treatment, offering new insights into managing this debilitating condition.

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1 Introduction

The occurrence of inflammatory bowel disease (IBD) has shown a persistent upward trend globally in recent years, placing a heavy burden on healthcare systems worldwide(Kaplan, 2015). Ulcerative colitis (UC), classified as a phenotypic manifestation of IBD(Guan, 2019), can cause long-term inflammation and superficial ulcers primarily affecting the colon(Saez et al., 2023). Currently, 5-aminosalicylic acid, corticosteroids, immunosuppressants, and biologics are usually used to treat UC, but these agents are costly and not curative. Thus, the exploration of novel alternative therapeutic strategies is of utmost importance.

The pathogenesis of UC is complicated, including imbalances in immune response, dysbiosis in the intestinal flora, genetic susceptibility, intestinal barrier disruption and environmental factors(Kobayashi et al., 2020). Some studies demonstrated that intestinal barrier dysfunction and microbial dysbiosis are closely linked to the onset and progression of UC(Ananthakrishnan et al., 2018; Jing et al., 2021). The intestinal barrier is vital for preserving gut homeostasis and protecting against the invasion of harmful pathogens as well as toxins(Laukens et al., 2014). In IBD, the compromised intestinal barrier results in increased permeability and translocation of luminal antigens into the mucosa, which triggers an exaggerated immune response and causes chronic inflammation and tissue damage(Song et al., 2022). Researches have revealed notable distinctions in the structure and function of gut microbiota between individuals with IBD and healthy individuals, including reduced microbial diversity, increased

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pathogenic bacteria, and decreased beneficial bacteria[(Guan, 2019)]. The protective barrier formed by the intestinal mucosa can be compromised by dysbiosis, leading to increased susceptibility to and worsening of inflammatory reactions[(Yang et al., 2023)]. Gut metabolites play a role in the pathogenesis of IBD by influencing various biological processes within the gut, such as inflammation, immune response, and epithelial barrier function. Moreover, the dysregulation of short-chain fatty acids, bile acids, and tryptophan metabolites, which are under the influence of gut microbiota, is notably implicated in IBD pathogenesis[(Jing et al., 2021)]. Bile acids, among these metabolites, have a crucial function in lipid absorption, metabolism, and modulation of gut microbiota composition[(Van den Bossche et al., 2017)]. They can bind to farnesoid X receptor (FXR) or takeda G protein-coupled receptor 5 (TGR5), exerting functions including maintaining intestinal barrier homeostasis and immune regulation[(Gadaleta et al., 2011; Sinha et al., 2020)]. Disruptions in bile acid synthesis, transport, and excretion can disturb the balance of bile acid composition in the intestine, thereby impairing the function of the intestinal mucosal barrier. This functional impairment triggers abnormal immune response and provokes chronic inflammation, leading to damage in the intestinal tissue[(Gadaleta et al., 2011)]. Therefore, dysregulation of bile acid metabolism plays a substantial role in the onset and advancement of IBD.

Tauroursodeoxycholic acid (TUDCA), a hydrophilic derivative of bile acid conjugated with taurine, has received FDA approval for the treatment of primary biliary cholangitis[(Zangerolamo et al., 2021)]. Initially, TUDCA was utilized for the

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management of various liver conditions, but with the accumulated clinical and experimental evidence, it has shown promise as a therapeutic option for a range of conditions, such as neurodegenerative diseases, cardiovascular diseases, gastrointestinal dysfunction, and glucose metabolism due to its cytoprotective effects[(Kusaczuk, 2019)]. As a "chemical chaperone", TUDCA exhibits the potential to attenuate endoplasmic reticulum stress and unfolded protein responses to protect cellular activity[(Kusaczuk, 2019)]. Furthermore, TUDCA is also found to reduce oxidative stress, apoptosis, and inflammatory response[(Hou et al., 2021)]. Thanks to its cell-protective and anti-inflammatory properties, TUDCA has received particular attention for its therapeutic potential on IBD. Stewart Siyan *et al*. demonstrated that TUDCA could reduce protein misfolding by complementing the requirement for $P58^{IPK}$ and *Atf6α*, thus improving colitis[(Cao et al., 2013)]. Debby Laukens *et al*. found TUDCA could prevent early intestinal epithelial cell death by blocking caspase-3 activation[(Laukens et al., 2014)]. Yet, there is limited research available regarding the regulation of gut microbiota dysbiosis by TUDCA. In an experiment on weaned piglets, TUDCA was able to modulate three genera that were closely associated with changes in serum metabolome after treatment[(Song et al., 2022)]. In patients with moderate to severe UC, dietary supplements of TUDCA could significantly improve clinical symptoms[(Huang et al., 2021)].

Therefore, we hypothesized that TUDCA could alleviate colitis by restoring impaired intestinal barrier function and modulating dysbiosis. In this study, the

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therapeutic efficacy of TUDCA on colitis was estimated by body weight loss, disease activity index (DAI), colon length, spleen weight, pathological changes, intestinal permeability, the levels of inflammation-related cytokines and tight junction proteins. Furthermore, the intestinal microbiota was profiled using high-throughput sequencing of 16S rDNA.

2 Materials and method

2.1 Chemicals and reagents

Dextran sulfate sodium (DSS) and TUDCA were provided by MP Biochemicals (Santa Ana, CA). 5-aminosalicylic acid (5-ASA) was obtained from Shanghai Aladdin Biotech Co. (Shanghai, China). An occult blood test kit was provided by Beijing Olaibo Technology Co. (Beijing, China). The ELISA kit for detection of myeloperoxidase (MPO) activity, tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and interleukin 10 (IL-10) were provided by J&L Biological Company (Shanghai, China). Fluorescein isothiocyanate (FITC)-Dextran was provided by Sigma-Aldrich (MO, USA).

2.2 Animals

C57BL/6J male mice (8 weeks old, weighing 18-20 g) were obtained from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China) [SCXK (Shan) 2018-001]. Food and water were available to the animals ad libitum in the

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climate-controlled vivarium at 25 ± 3 °C, relative humidity of 55%, and a 12-hour light-dark cycles. The experimental procedures were granted ethical clearance by the Ethics Committee and were conducted in accordance with the Laboratory Animal Care and Use Guide (permit NO. XJTU2022-0003).

2.3 DSS-induced colitis

The mice were randomly assigned to five groups (n = 10): Control group (Con), untreated colitis group (Colitis), Colitis + high dose TUDCA group (TUDCA-H, 400 mg kg^{-1} d^{-1}), Colitis + low dose TUDCA group (TUDCA-L, 200 mg kg^{-1} d^{-1}) and Colitis + 5-ASA group (5-ASA, 400 mg kg^{-1} $d^{-1)}$. From days 1-6, 3% (w/v) DSS solution was given to the models with colitis, while the control group was provided with regular water. TUDCA-H, TUDAC-L, and 5-ASA groups were gavaged the corresponding concentrations of the drug from days 6-10, for the groups (Con and Colitis), an equal volume of water was given.

Daily monitoring was conducted to closely observe the mice and assess their health status. Based on previous research[(Jing et al., 2021)], the DAI was calculated based on the scores as follows: weight loss (0: 0%, 1: 1%-5%, 2: 5%-10%, 3: 10%-15%, 4: >15%), stool characters (0: normal and well-formed feces, 1: soft and spherical feces, 2: pasty and not stick to the anus, 3: loose and stick to the anus, 4: loose and bloody feces), blood in feces (0: normal, 1: slight bleeding, 2: moderate bleeding, 3: severe bleeding, 4: gross). The group-level average DAI score was computed by

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dividing the cumulative sum of individual DAI values within the group by the total count of mice. After the experiment concluded, mice were euthanized in a humane manner, and fecal samples were collected to analyze gut microbiota composition. In addition, fresh spleen and colon tissues were harvested and weighed to facilitate subsequent bioanalysis, which included ELISA and histologic examination, respectively.

2.4 Histopathological examination

Colon specimens were obtained and immersed in a 10% formalin solution buffered with PBS for 48 hours before being subjected to paraffin embedding. Tissue slices measuring 5 micrometers in thickness were subjected to hematoxylin and eosin (H&E) staining.

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2.5 Biochemical analyses

The MPO activity and levels of TNF-α, IL-1β, IL-6 and IL-10 in the colon tissues in various groups were evaluated by specific ELISA kits.

2.6 Intestinal permeability assay

Following a four-hour period of food and water deprivation, 4 kDa FITC-dextrans were administered to mice at a dose of 60 mg per 100 g of body weight via gavage. Subsequently, retroorbital blood samples were obtained five hours later,

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centrifuged. The resulting serum samples were then analyzed for fluorescence intensity at a wavelength of 525 nm. The concentration of FITC-dextran was determined using a standard curve.

2.7 DNA extraction and Illumina sequencing

DNA of bacteria in mouse feces was extracted and then analyzed by 16S rDNA sequencing. DNA sequences were amplified with PCR using universal primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3' and then sequenced on Illumina Hiseq PE300. The raw sequences were analyzed based on the microbiome analysis platform QIIME2 2018.4 (Quantitative Insights Into Microbial Ecology, USA). Classify-sklearn algorithm was used to classify these unique sequences and the QIIME2 correlation channel was used to calculate the α-diversity index.

2.8 Statistical analysis

The analysis of the sequencing data was performed in R (v.3.5.2) and statistical analysis of the remaining data was conducted using Graphpad prism 8.4. p-value less than 0.05 was considered statistically significant, and all data were presented as mean ± standard error of the mean (SEM).

3 Results

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3.1 TUDCA administration attenuated pathological phenotypes in DSS-induced colitis

From day 1 to day 6, mice with colitis displayed wet feces, fecal blood, and gradually increasing DAI (Fig. 1 A-1), indicating successful colitis induction. From day 6 to day 10, the DAI in TUDCA-H and TUDCA-L groups consistently decreased after administration and were more effective in the higher dose group, suggesting a dose-dependent manner of TUDCA on DSS-induced colitis (Fig. 1 A-1). Similarly, the treatment groups (TUDAC-H, TUDAC-L and 5-ASA) showed a reduction in weight loss (Fig. 1 A-2).

Macroscopic examination of colon tissues revealed significant colonic shortening, congestion and swelling in the model group, but a normal colon appearance, characterized by the absence of congestion or adhesions, was observed in the control group (Fig.1 B-1). The colon weight/length ratio can serve as a quantitative indicator of the extent of colonic distension and inflammation, and as shown in Fig.1 B-2, mice with TUDCA treatment had a lower weight/length ratio, indicating that TUDCA could normalize the colonic morphology and relieved inflammation induced by colitis. Besides, the weight of the spleen is an indicator of systemic inflammation, as splenomegaly is a common occurrence in individuals with colitis(Li et al., 2021). As shown in Figures 1 B-3 and B-4, the spleens in the colitis group were larger than the control group (p<0.001), and the interventions of TUDCA and 5-ASA effectively reduced spleen enlargement. In comparison, high-dose TUDCA

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exhibited greater therapeutic efficacy than 5-ASA in alleviating colon and spleen swelling (Fig. 1 B). According to results of histopathological examination, colon tissues from colitis group mice showed extensive mucosal ulceration, neutrophil infiltration, and surface epithelial destruction, while TUDCA and 5-ASA treatment improved colonic mucosal damage and inflammatory cell infiltration, with lower histopathological scores (Fig. 2). These findings suggested that the TUDCA administration could alleviate DSS-induced colitis and the therapeutic efficacy likely depended on the dose.

3.2 TUDCA administration alleviated colonic inflammation in DSS-induced colitis

Myeloperoxidase (MPO) is a potent inflammatory cytokine produced by neutrophils, and its expression serves as a marker of infiltration of immune cells(Dong et al., 2021). We measured MPO activity in mice colon tissue using ELISA kits and the result showed that the colitis group exhibited a significant elevation in MPO activity (p<0.001), suggesting an increase in the number of infiltrating neutrophils (Fig. 3). In contrast, TUDCA and 5-ASA treatment led to a noticeable reduction in MPO activity, indicating that TUDCA could prevent infiltration of inflammatory cells and mitigate colonic inflammation within colon tissues. Besides, histopathological examination also revealed that TUDCA inhibited mucosal ulceration and neutrophil infiltration (Fig. 2A).

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The onset of UC in humans is characterized by dysregulation of pro-inflammatory and anti-inflammatory cytokines, culminating in an overactive immune response[(Park et al., 2017)]. The activity of cytokines was assessed by ELISA to determine the efficacy of TUDCA on cytokine levels. TUDCA significantly lowered the levels of pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-1β, and increased levels of anti-inflammatory cytokine, IL-10, compared to the colitis group (p<0.001, Fig. 3). As expectedly, high-dose TUDCA showed better regulatory effects on cytokine levels than low-dose TUDCA and was comparable to 5-ASA (Fig. 3). Overall, TUDCA could alleviate colonic inflammation, modulate the expression of inflammatory cytokines, and restore intestinal mucosal damage.

3.3 High-dose TUDCA administration reduced colonic epithelial permeability in DSS-induced colitis

To assess the integrity of intestinal barrier, dextran-FITC was administered orally to the mice, and the serum concentrations of FITC-dextran were subsequently measured. Mice with colitis exhibited elevated levels of FITC-dextran, suggesting an increase in colonic epithelial permeability and compromised intestinal barrier function, in contrast to the control group (p<0.001, Fig. 4A). While the intervention with TUDCA resulted in a reduction of the elevated colonic epithelial permeability, with a more pronounced effect observed in the TUDCA-H group than the 5-ASA group (Fig. 4A).

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Immunofluorescence staining was employed to evaluate the expression and activity of tight junction (TJ) proteins, serving as indicators for assessing the integrity and functionality of the intestinal epithelial barrier. There was a notable decrease in the presence of ZO-1 and occludin proteins compared with control groups in colitis mice (Fig. 4B). Conversely, after being treated with TUDCA, the increased expression of TJ proteins was observed in the spinous and granular layers of mucosa. Similarly, the TUDCA-H group exhibited superior regulatory effects compared to the TUDCA-L group, their efficacy was more pronounced than 5-ASA (Fig. 4B). So, TUDCA can limit the increase in colonic epithelial permeability, and upregulate the amount of TJ proteins, thus restoring intestinal barrier function in colitis mice.

3.4 High-dose TUDCA administration alleviated colonic microbiota dysbiosis in DSS-induced colitis

Biological barrier damage resulting from dysbiosis is one of the core factors of colitis. This study analyzed the gut microbiota composition through a 16S rDNA sequencing assay. The Shannon index is widely used in population genetics research to measure α-diversity in the gut microbiome. A higher Shannon index value indicates greater species richness, evenness, and biodiversity in the microbial community. Conversely, lower values suggest lower diversity or a higher dominance of a few species. In Fig. 5A, colitis mice had a lower Shannon index and operational taxonomic units (OTUs), while high dose of TUDCA administration reversed this

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decrease significantly (Fig. 5B). The variability or dissimilarity between the microbial communities was assessed through β-diversity analysis, with the distance between samples reflecting the similarity of the intestinal flora (Fig. 5C). Principal Coordinates Analysis (PCoA) reveals the dissimilarity between samples, and greater distances indicate larger differences in the composition of intestinal flora between samples. Based on PCoA results, the samples in the colitis group were differentiated from those in the control group, while the high-dose TUDCA group aggregated toward the control group, indicating TUDCA partially inhibited DSS-induced difference in microbial communities and restored the dysbiotic gut microbiota to normal (Fig. 5C).

Gut flora was analyzed to assess the influence of TUDCA on the intestinal microbiota. *Firmicutes* and *Bacteroidota* were identified as the predominant phyla at the taxonomic level of the gut microbiota analysis (Fig. 6A). The colitis group demonstrated a notable elevation in *Bacteroidota* ($p<0.01$) and a decrease in the *Firmicutes* as compared to the control group, but TUDCA of high dose reversed the changes in colitis group, as represented by *Firmicutes/Bacteroidota* (F/B) ratio (Fig. 6A, B). *Firmicutes* and *Bacteroidota* are considered essential to maintain intestinal homeostasis. In the colitis group, there was an elevation in *Bacteroidaceae* at both the family and genus levels, while the administration of TUDCA reduced this increase to normal levels (Fig. 6C, D). At family level, there is no significant change in *Muribaculaceae*, *Lactobacillaceae* and *lachnospiraceae* (Fig. 6C). *Muribaculaceae*, *Lactobacillus and Clostridia*_UCG-014 were similar in three groups in genus level,

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Faecalibaculum and *Eubacterium_coprostanoligenes_group* increased remarkably in TUDCA-H group compared to the other two groups (Fig. 6D). Notably, *Akkermansiaceae* had a significant increase in TUDCA-H group (Fig. 6C) in both family and genus level. Linear discriminant analysis with effect size (LEfSe) was employed to further compare the gut microbe's differentiation in the TUDCA-H and colitis group, which is commonly employed to analyze differences in microbial composition between different groups and identify key microbial features that can distinguish between these groups. LEfSe analysis identified 22 bacterial clades that exhibited statistically significant and biologically consistent differences and linear discriminant analysis (LDA) with Lefse analysis demonstrated that the family *Bacteroidaceae*, *Streptococcus* and genus *Bacteroides*, *Streptococcaceae* exhibited high LDA scores in colitis mice. However, the family *Akkermansiaceae*, as well as the *Eubacterium_coprostanoligenes_group*, and the genera *Akkermansia* and the *Eubacterium_coprostanoligenes_group* showed high LDA scores after TUDCA treatment, highlighting their important role in TUDCA-anti colitis (Fig. 6E).

The findings indicated that that TUDCA effectively restored the reduced diversity of intestinal microbiota and reversed the decline in the F/B ratio, while also increasing the relative abundance of *Akkermansia*, thereby alleviating colonic microbiota dysbiosis induced by DSS-induced colitis.

4 Discussion

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The increased global incidence of IBD, makes it a common digestive system disorder that inflicts a huge financial and resource burden on the healthcare system[(Kaplan, 2015)]. Exploring new therapeutic options that address unmet medical needs and improve patient outcomes is imperative. Previous studies have reported that TUDCA has therapeutic potential for colitis[(Yang et al., 2016; Van den Bossche et al., 2017; Song et al., 2022)], and could attenuate intestinal inflammatory response, oxidative stress, and dampen Crohn's disease-like ileitis[(Van den Bossche et al., 2017)]. Our study provides evidence that TUDCA can ameliorate pathological phenotypes in colitis, including DAI, enlargement of the colon and spleen, as well as histopathological lesion of the colon. In addition, daily administration of TUDCA can alleviate colonic inflammation, restore intestinal-barrier disruption, and improve colonic microbiota dysbiosis.

The functional integrity of the intestinal barrier relies on an intricate interplay among physical, chemical, immune, and microbial components. The intestinal barrier protects the host from intestinal microorganisms, food antigens, and toxins. ZO-1 and occludin are integral components of tight junctions, pivotal for the regulation of paracellular permeability and the prevention of translocation of potentially harmful substances across the intestinal epithelium[(Garg et al., 2016)]. The depletion of TJ proteins increases colonic epithelial permeability, consequently stimulating the mucosal immune system[(Garg et al., 2016)]. The dysregulated mucosal immune response (in particular, proinflammatory cytokines released from resident macrophages) is a major contributor to intestinal barrier impairment[(Ahluwalia et al., 2018)]. Increased levels of pro-

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inflammatory cytokines in the colon, IL-6, TNF-α, and IL-1β, lead to TJ protein-mediated barrier dysfunction, thus triggering immune activation and tissue inflammation(Lee, 2015). IL-1β and TNF-α have been shown to markedly enhance the permeability of the intestinal epithelial barrier and the inhibition of IL-1β-induced intestinal permeability increase can prevent DSS-induced intestinal inflammation(Kaminsky et al., 2021). In our research, we have discovered that TUDCA can regulate the expression of ZO-1, occludin and the levels of inflammatory factors. However, the relationship between the two during TUDCA treatment of UC remains to be further investigated. It is still unclear whether TUDCA acts by upregulating the levels of TJ proteins (especially ZO-1 and occludin), enhancing intestinal barrier permeability, preventing the entry of antigens and toxins into cells, inhibiting immune dysregulation, and subsequently regulating the levels of inflammatory factors. Alternatively, TUDCA may exert its effects by reducing the levels of pro-inflammatory cytokines. This, in turn, could enhance intestinal epithelial TJ barrier permeability and inhibit immune dysregulation. Further experimental exploration is needed to elucidate these mechanisms.

In addition to inflammatory response and physical barrier damage in the intestinal mucosa, numerous studies propose a link between intestinal microbiota alterations and the development of IBD, but whether a causal relationship exists is uncertain(Ni et al., 2017; Nishida et al., 2018). Gut microbes serve vital functions in the human body, including nutrition, defense, and immune development(Lee and Chang, 2021). Alterations in the

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composition and functionality of the intestinal flora have been implicated in the dysregulation of small molecule metabolites, such as short-chain fatty acids, secondary bile acids, and tryptophan derivatives. These changes can compromise the microbiota's capacity to uphold the integrity of the intestinal barrier and modulate immune responses(Li et al., 2022). Patients with IBD and animal models frequently exhibit diminished gut microbial diversity and a decreased F/B ratio(Stojanov et al., 2020). Our data also demonstrate a notable reduction in the diversity of intestinal microbiota and F/B ratio in mice with colitis, but TUDCA treatment can effectively reverse this trend. The findings from the analysis of α and β-diversity indicate that TUDCA can increase intestinal flora diversity, normalize intestinal flora, and restore microbial barriers in colitis mice, hinting at the prebiotic properties of this compound.

Many studies have confirmed that beneficial bacterium is essential in relieving or treating IBD. The beneficial bacteria have the capacity to reinforce the self-protective mechanism of the intestinal mucosa, counteract the pathogenic impact of harmful bacteria, regulate the release of inflammatory factors, and expedite the restoration of the intestinal flora within a brief timeframe(Jakubczyk et al., 2020; Martyniak et al., 2021). *Akkermansia* has been proven to be a promising candidate in IBD management(Cheng and Xie, 2021). It has been found to protect the colonic epithelial tight junctions, reduce intestinal permeability and rectify mucosal barrier damage, and decrease the expressions of serum and colonic pro-inflammatory cytokines (such as TNF-α, IL-6, IL-12a, IFN-γ and IL-1α) and increase the anti-inflammatory factors output(Bian et al.,

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2019; Rodrigues et al., 2022). In this paper, we observe that *Akkermansia* has a noticeable decrease in the colitis mice groups, which is also consistent with the reduced presence of this bacterium in the gut microbiota of patients with IBD(L et al., 2020), indicating the impaired protective function of *Akkermansia* in colitis. However, TUDCA administration significantly increases the relative abundance of *Akkermansiaceae* and *Akkermansia*, consistent with previous studies. In addition, TUDCA can regulate the level of inflammatory factors and increase the amount of tight junction proteins based on the previous observation. Given the reported remarkable functionality of *Akkermansia* in regulating the expression of TJ proteins and inflammatory factors(Bian et al., 2019; Rodrigues et al., 2022), we speculate that TUDCA may increase the relative abundance of *Akkermansia*, thereby enhancing the protective function of *Akkermansia* on intestinal epithelial tight junctions and repairing the damaged intestinal barrier. Meanwhile, the increase of *Akkermansia* can also regulate inflammatory factors and alleviate inflammation. However, the mechanisms by which TUDCA influences the abundance of *Akkermansia* have not been thoroughly investigated. TUDCA may help preserve the integrity of the intestinal barrier, preventing the infiltration of harmful substances, thus providing a favorable environment for beneficial bacteria. Additionally, TUDCA may indirectly promote an increase in the abundance by reducing the detrimental effects of inflammation on *Akkermansia*.

5 Conclusion

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Taken together, we find that TUDCA treatment can alleviate intestinal inflammation, increase the expression of tight junction proteins, restore the damaged physical barrier and function of the intestinal mucosa, upregulate the relative abundance of *Akkermansia* and reverse the intestinal bacteria disarray in DSS-induced colitis mice. These results demonstrate TUDCA's potential as an effective agent for treating UC, yet its exact mechanisms of action and its applicability as a viable drug in humans still need comprehensive exploration and investigation.

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Data Availability Statement

The authors declare that all the data supporting the findings of this study are contained within the paper.

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Authorship Contribution

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Wrote or contributed to the writing of the manuscript: L.B. Luo, Y. Zhao, Guangji Zhang,W.H. Jing, H. Luo.

Conflict of interest

All authors declare that they have no conflicts of interest.

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References

Ahluwalia B, Moraes L, Magnusson MK and Öhman L (2018) Immunopathogenesis of inflammatory bowel disease and mechanisms of biological therapies. *Scand J Gastroenterol* **53**:379-389.

Ananthakrishnan AN, Bernstein CN, Iliopoulos D, Macpherson A, Neurath MF, Ali RAR, Vavricka SR and Fiocchi C (2018) Environmental triggers in IBD: a review of progress and evidence. *Nat Rev Gastroenterol Hepatol* **15**:39-49.

Bian X, Wu W, Yang L, Lv L, Wang Q, Li Y, Ye J, Fang D, Wu J, Jiang X, Shi D and Li L (2019) Administration of Akkermansia muciniphila Ameliorates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Mice. *Front Microbiol* **10**:2259.

Cao SS, Zimmermann EM, Chuang BM, Song B, Nwokoye A, Wilkinson JE, Eaton KA and Kaufman RJ (2013) The unfolded protein response and chemical chaperones reduce protein misfolding and colitis in mice. *Gastroenterology* **144**:989-1000.e1006.

Cheng D and Xie MZ (2021) A review of a potential and promising probiotic candidate-Akkermansia muciniphila. *J Appl Microbiol* **130**:1813-1822.

Dong S, Zhu M, Wang K, Zhao X, Hu L, Jing W, Lu H and Wang S (2021) Dihydromyricetin improves DSS-induced colitis in mice via modulation of fecal-bacteria-related bile acid metabolism. *Pharmacol Res* **171**:105767.

Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, Klomp LW, Siersema PD, Schipper ME, Danese S, Penna G, Laverny G, Adorini L, Moschetta A and van Mil SW (2011) Farnesoid X receptor activation inhibits

inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut* **60**:463-472.

Garg A, Zhao A, Erickson SL, Mukherjee S, Lau AJ, Alston L, Chang TK, Mani S and Hirota SA (2016) Pregnane X Receptor Activation Attenuates Inflammation-Associated Intestinal Epithelial Barrier Dysfunction by Inhibiting Cytokine-Induced Myosin Light-Chain Kinase Expression and c-Jun N-Terminal Kinase 1/2 Activation. *J Pharmacol Exp Ther* **359**:91-101.

Guan Q (2019) A Comprehensive Review and Update on the Pathogenesis of Inflammatory Bowel Disease. *J Immunol Res* **2019**:7247238.

Hou Y, Luan J, Huang T, Deng T, Li X, Xiao Z, Zhan J, Luo D, Hou Y, Xu L and Lin D (2021) Tauroursodeoxycholic acid alleviates secondary injury in spinal cord injury mice by reducing oxidative stress, apoptosis, and inflammatory response. *J Neuroinflammation* **18**:216.

Huang K, Deng RS, Liu TC, Gremida A, Deepak P, Chen CH, Davidson N, Kaufman R and Ciorba M (2021) A TRANSLATIONAL PHASE I STUDY OF TAUROURSODEOXYCHOLIC ACID (TUDCA) TO REDUCE SYMPTOMS AND ER STRESS IN ACTIVE ULCERATIVE COLITIS. *Inflammatory Bowel Diseases* **27**:S5-S6.

Jakubczyk D, Leszczyńska K and Górska S (2020) The Effectiveness of Probiotics in the Treatment of Inflammatory Bowel Disease (IBD)-A Critical Review. *Nutrients* **12**.

Jing W, Dong S, Luo X, Liu J, Wei B, Du W, Yang L, Luo H, Wang Y, Wang S and Lu H (2021) Berberine improves colitis by triggering AhR activation by microbial tryptophan catabolites. *Pharmacol Res* **164**:105358.

Kaminsky LW, Al-Sadi R and Ma TY (2021) IL-1β and the Intestinal Epithelial Tight Junction Barrier. *Front Immunol* **12**:767456.

Kaplan GG (2015) The global burden of IBD: from 2015 to 2025. *Nat Rev Gastroenterol Hepatol* **12**:720-727.

Kobayashi T, Siegmund B, Le Berre C, Wei SC, Ferrante M, Shen B, Bernstein CN, Danese S, Peyrin-Biroulet L and Hibi T (2020) Ulcerative colitis. *Nat Rev Dis Primers* **6**:74.

Kusaczuk M (2019) Tauroursodeoxycholate-Bile Acid with Chaperoning Activity: Molecular and Cellular Effects and Therapeutic Perspectives. *Cells* **8**.

L W, L T, Y F, S Z, M H, C Z, G Y, J Z, S C, Q W, L L and Z Z (2020) A purified membrane protein from Akkermansia muciniphila or the pasteurised bacterium blunts colitis associated tumourigenesis by modulation of CD8 + T cells in mice. *Gut* **69**:1988-1997.

Laukens D, Devisscher L, Van den Bossche L, Hindryckx P, Vandenbroucke RE, Vandewynckel YP, Cuvelier C, Brinkman BM, Libert C, Vandenabeele P and De Vos M (2014) Tauroursodeoxycholic acid inhibits experimental colitis by preventing early intestinal epithelial cell death. *Lab Invest* **94**:1419-1430.

Lee M and Chang EB (2021) Inflammatory Bowel Diseases (IBD) and the Microbiome-Searching the Crime Scene for Clues. *Gastroenterology* **160**:524-537.

Lee SH (2015) Intestinal permeability regulation by tight junction: implication on inflammatory bowel diseases. *Intest Res* **13**:11-18.

Li H, Chen X, Liu J, Chen M, Huang M, Huang G, Chen X, Du Q, Su J and Lin R (2021) Ethanol extract of Centella asiatica alleviated dextran sulfate sodium-induced colitis: Restoration on mucosa barrier and gut microbiota homeostasis. *J Ethnopharmacol* **267**:113445.

Li M, Yang L, Mu C, Sun Y, Gu Y, Chen D, Liu T and Cao H (2022) Gut microbial metabolome in inflammatory bowel disease: From association to therapeutic perspectives. *Comput Struct Biotechnol J* **20**:2402-2414.

Martyniak A, Medyńska-Przęczek A, Wędrychowicz A, Skoczeń S and Tomasik PJ (2021) Prebiotics, Probiotics, Synbiotics, Paraprobiotics and Postbiotic Compounds in IBD. *Biomolecules* **11**.

Ni J, Wu GD, Albenberg L and Tomov VT (2017) Gut microbiota and IBD: causation or correlation? *Nat Rev Gastroenterol Hepatol* **14**:573-584.

Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y and Andoh A (2018) Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clin J Gastroenterol* **11**:1-10.

Park JH, Peyrin-Biroulet L, Eisenhut M and Shin JI (2017) IBD immunopathogenesis: A comprehensive review of inflammatory molecules. *Autoimmun Rev* **16**:416-426.

Rodrigues VF, Elias-Oliveira J, Pereira Í S, Pereira JA, Barbosa SC, Machado MSG and Carlos D (2022) Akkermansia muciniphila and Gut Immune System: A Good

Friendship That Attenuates Inflammatory Bowel Disease, Obesity, and Diabetes. *Front Immunol* **13**:934695.

Saez A, Herrero-Fernandez B, Gomez-Bris R, Sánchez-Martinez H and Gonzalez-Granado JM (2023) Pathophysiology of Inflammatory Bowel Disease: Innate Immune System. *Int J Mol Sci* **24**.

Sinha SR, Haileselassie Y, Nguyen LP, Tropini C, Wang M, Becker LS, Sim D, Jarr K, Spear ET, Singh G, Namkoong H, Bittinger K, Fischbach MA, Sonnenburg JL and Habtezion A (2020) Dysbiosis-Induced Secondary Bile Acid Deficiency Promotes Intestinal Inflammation. *Cell Host Microbe* **27**:659-670.e655.

Song M, Zhang F, Fu Y, Yi X, Feng S, Liu Z, Deng D, Yang Q, Yu M, Zhu C, Zhu X, Wang L, Gao P, Shu G, Ma X, Jiang Q and Wang S (2022) Tauroursodeoxycholic acid (TUDCA) improves intestinal barrier function associated with TGR5-MLCK pathway and the alteration of serum metabolites and gut bacteria in weaned piglets. *J Anim Sci Biotechnol* **13**:73.

Stojanov S, Berlec A and Štrukelj B (2020) The Influence of Probiotics on the Firmicutes/Bacteroidetes Ratio in the Treatment of Obesity and Inflammatory Bowel disease. *Microorganisms* **8**.

Van den Bossche L, Borsboom D, Devriese S, Van Welden S, Holvoet T, Devisscher L, Hindryckx P, De Vos M and Laukens D (2017) Tauroursodeoxycholic acid protects bile acid homeostasis under inflammatory conditions and dampens Crohn's disease-like ileitis. *Lab Invest* **97**:519-529.

Yang Y, He J, Suo Y, Zheng Z, Wang J, Lv L, Huo C, Wang Z, Li J, Sun W and Zhang Y (2016) Tauroursodeoxycholate improves 2,4,6-trinitrobenzenesulfonic acid-induced experimental acute ulcerative colitis in mice. *Int Immunopharmacol* **36**:271-276.

Yang Y, Wang Y, Zhao L, Wang F, Li M, Wang Q, Luo H, Zhao Q, Zeng J, Zhao Y, Du F, Chen Y, Shen J, Wei S, Xiao Z and Wu X (2023) Chinese herbal medicines for treating ulcerative colitis via regulating gut microbiota-intestinal immunity axis. *Chin Herb Med* **15**:181-200.

Zangerolamo L, Vettorazzi JF, Rosa LRO, Carneiro EM and Barbosa HCL (2021) The bile acid TUDCA and neurodegenerative disorders: An overview. *Life Sci* **272**:119252.

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Figure Legends.

Fig. 1. TUDCA intervention attenuated pathological symptoms in DSS-induced colitis mice. (A) Daily changes in disease activity index (DAI) (A-1, n ≥ 7) and body weight (A-2, n= 8) of different groups; (B) Macroscopic observation of colon (B-1) and spleen (B-3) and ratios of colon weight to length (B-2) (n ≥ 6) and spleen weight to body weight (B-4, n = 7); Data are presented as the mean ± SEM. **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 vs control group; #*p* < 0.05， ##*p* < 0.01，###*p* < 0.001 vs colitis group.

Fig. 2. Histological examination and histological assessment on mice colon with inflammation. (A) Representative images of hematoxylin and eosin (H&E)-stained colon tissue. Scale bar = 100 μm. (B) Histological scores of mice colon in different groups. n=3, Data are presented as the mean ± SEM. *****p* < 0.0001 vs control group, ###*p* < 0.001 vs Colitis group.

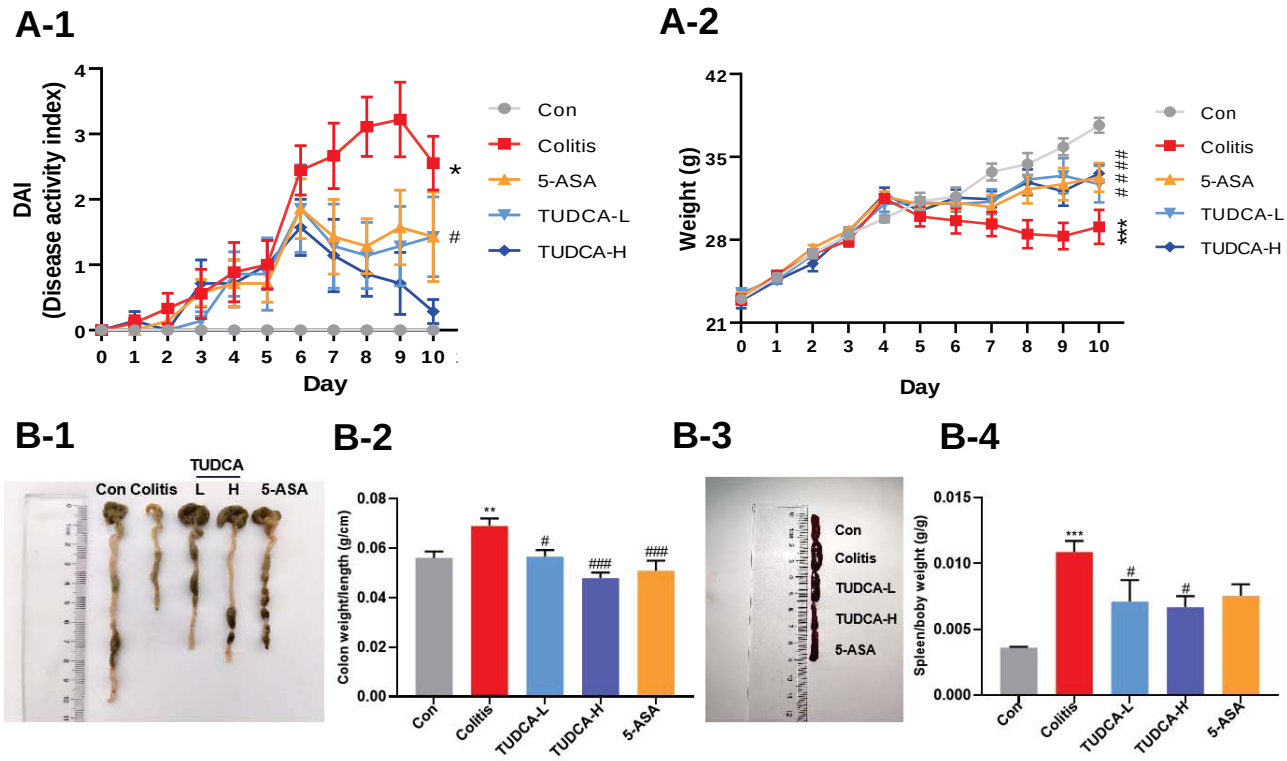
Fig. 3. TUDCA intervention attenuated colonic inflammation in DSS-induced colitis mice. Myeloperoxidase (MPO) activity and inflammatory cytokine levels in colonic tissues (n = 7). Data are presented as the mean ± SEM. ****p* < 0.001 vs control group, ###*p* < 0.001 vs Colitis group.

Fig. 4. TUDCA intervention restored gut-barrier function in DSS-induced colitis mice. (A) Epithelial permeability of FITC-dextran (n = 3); (B) Representative immunofluorescence images showing in situ expression of ZO-1 and occludin. (Scale bar = 100 μm). Data are presented as the mean ± SEM. ***p < 0.001 vs control group; ##*p* < 0.01，###*p* < 0.001 vs colitis group.

Fig. 5. High dose of TUDCA intervention normalized the disordered intestinal flora in DSS-induced colitis mice. (A-B) Shannon index and observed OTUS of all samples; (C) PCoA analysis of all samples.

Fig. 6. High dose of TUDCA intervention alleviated gut dysbiosis in DSS-induced colitis mice. (A) Relative abundance at phylum level; (B) Firmicutes/Bacteroidota (F/B) ratio of all samples; (C-D) Relative abundance at family and genus level; (E) LefSe analysis of all samples. n=4, ***p* < 0.01 vs control group, ##*p* < 0.01 vs colitis group.

Fig. 1.



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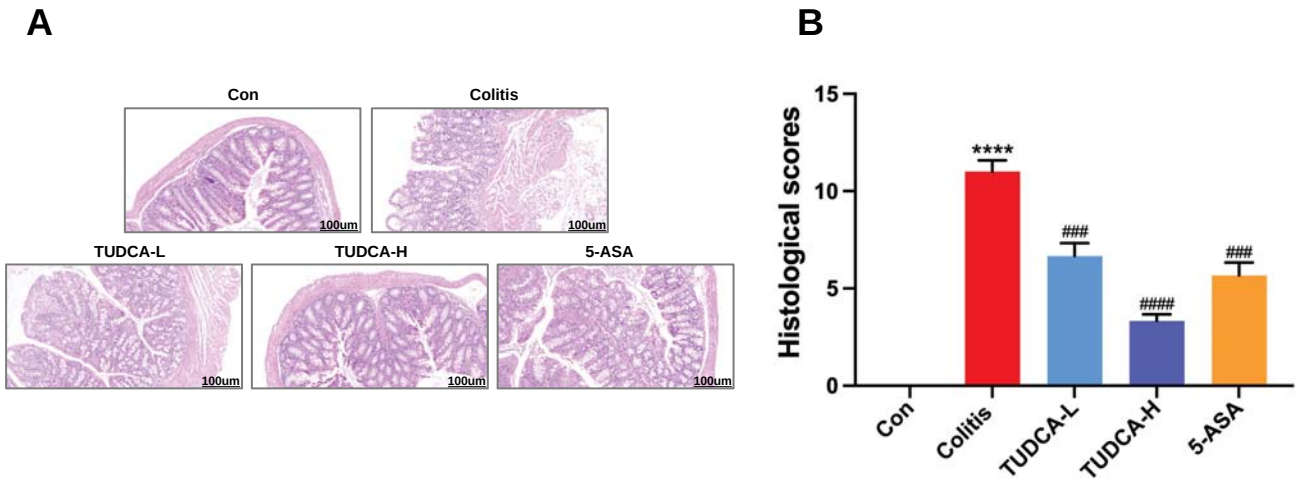

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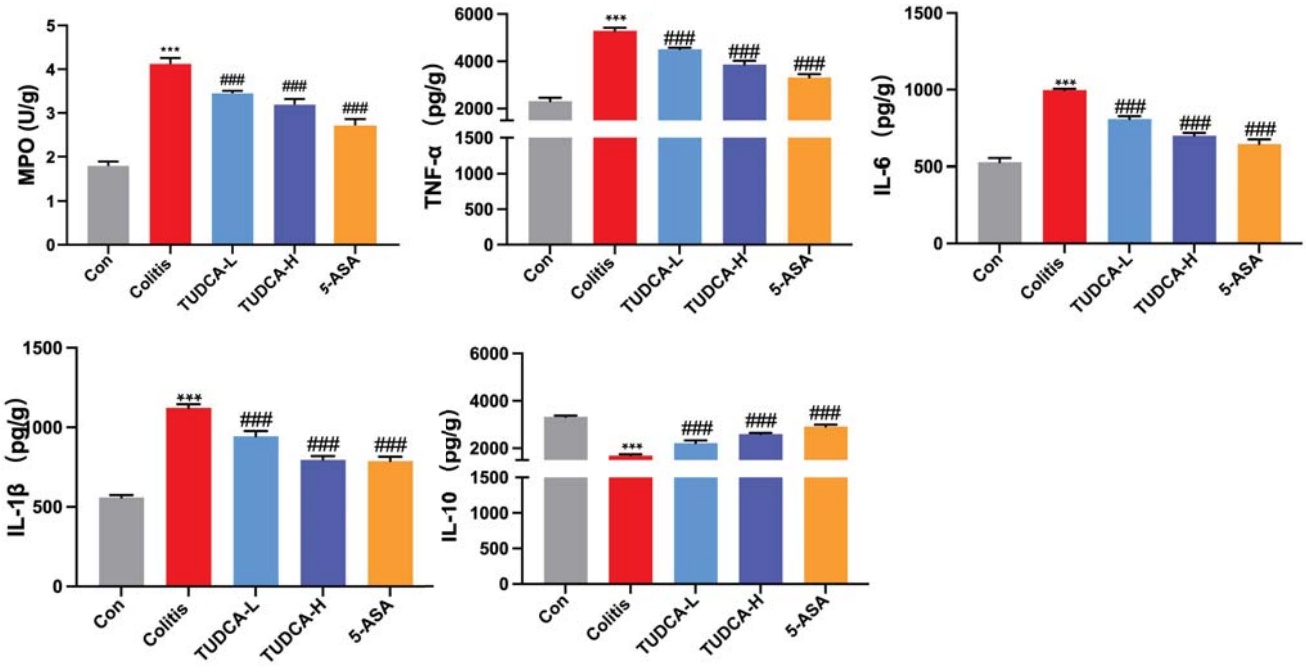

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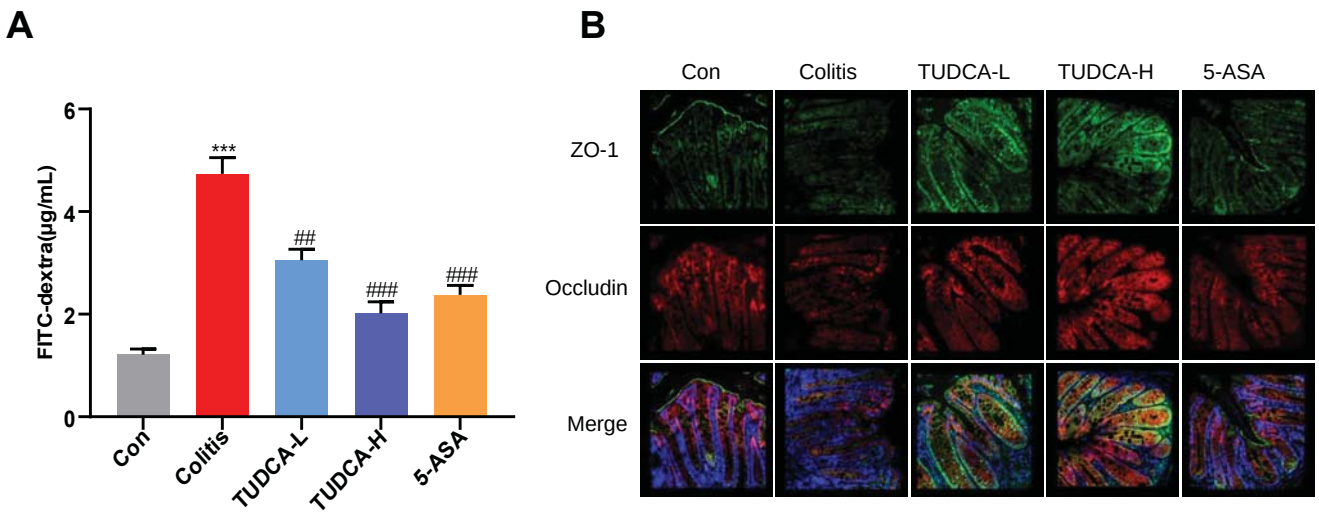

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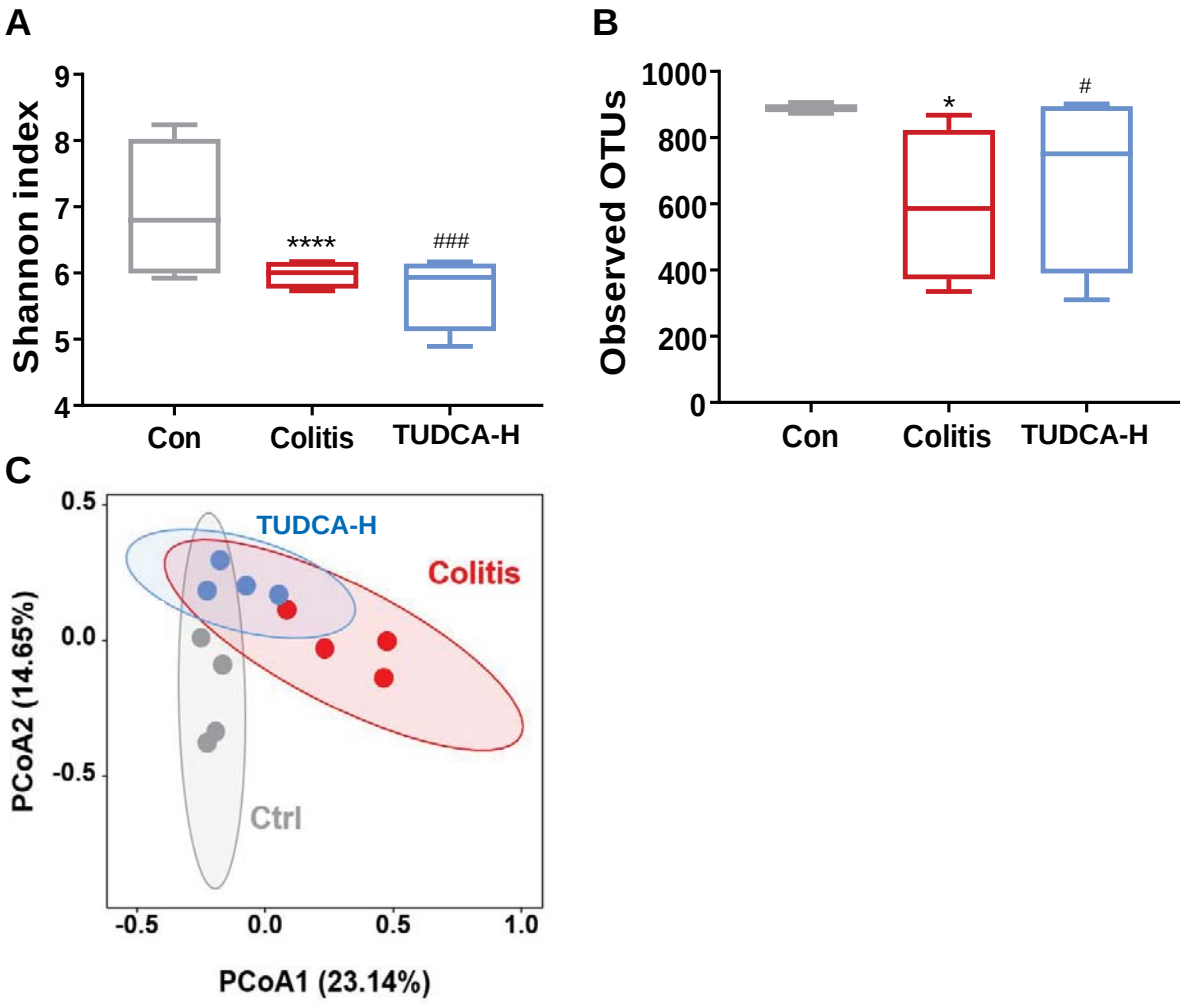

Fig. 6.