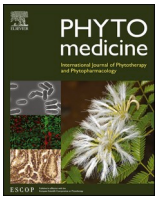
Phytomedicine 112 (2023) 154702

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed



Original Article

Improved colonic inflammation by nervonic acid *via* inhibition of NF-κB signaling pathway of DSS-induced colitis mice

Sheng-Nan Yuan [a], Mu-xuan Wang [a], Jin-Long Han [a], Cai-Yun Feng [a], Meng Wang [b], Min Wang [c], Jin-Yue Sun [a,f,*], Ning-yang Li [d,*], Jesus Simal-Gandara [e,*], Chao Liu [a,f,*]

[a] *Key Laboratory of Novel Food Resources Processing, Ministry of Agriculture and Rural Affairs/Key Laboratory of Agro-Products Processing Technology of Shandong Province/Institute of Agro-Food Science and Technology, Shandong Academy of Agricultural Sciences, 202 Gongye North Road, Jinan 250100, China*
[b] *Shanxi Functional Food Engineering Center Co. Ltd, Xian 710000, China*
[c] *College of Food Science and Engineering, Northwest A&F University, Xianyang 712100, China*
[d] *Key Laboratory of Food Processing Technology and Quality Control in Shandong Province, College of Food Science and Engineering, Shandong Agricultural University, Taian, China*
[e] *Universidade de Vigo, Nutrition and Bromatology Group, Analytical Chemistry and Food Science Department, Faculty of Science, E-32004 Ourense, Spain*
[f] *Shandong Huatai Nutrition and Health Industry Technology Research Institute Co. Ltd, Jinan 250100, China*

ARTICLE INFO

Keywords:
Nervonic acid
Macrophages
Inflammatory factors
Ulcerative colitis
NF-κB signaling pathway

ABSTRACT

Background: Nervonic acid (C24:1$^{\Delta 15}$, 24:1 ω-9, *cis*-tetracos-15-enoic acid; NA), a long-chain monounsaturated fatty acid, plays an essential role in prevention of metabolic diseases, and immune regulation, and has anti-inflammatory properties. As a chronic, immune-mediated inflammatory disease, ulcerative colitis (UC) can affect the large intestine. The influences of NA on UC are largely unknown.

Purpose: The present study aimed to decipher the anti-UC effect of NA in the mouse colitis model. Specifically, we wanted to explore whether NA can regulate the levels of inflammatory factors in RAW264.7 cells and mouse colitis model.

Methods: To address the above issues, the RAW264.7 cell inflammation model was established by lipopolysaccharide (LPS), then the inflammatory factors tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), Interleukin-1β (IL-1β), and Interleukin-10 (IL-10) were detected by Enzyme-linked immunosorbent assay (ELISA). The therapeutic effects of NA for UC were evaluated using C57BL/6 mice gavaged dextran sodium sulfate (DSS). Hematoxylin and eosin (H&E) staining, Myeloperoxidase (MPO) kit assay, ELISA, immunofluorescence assay, and LC-MS/MS were used to assess histological changes, MPO levels, inflammatory factors release, expression and distribution of intestinal tight junction (TJ) protein ZO-1, and metabolic pathways, respectively. The levels of proteins involved in the nuclear factor kappa-B (NF-κB) pathway in the UC were investigated by western blotting and RT-qPCR.

Results: In vitro experiments verified that NA could reduce inflammatory response and inhibit the activation of key signal pathways associated with inflammation in LPS-induced RAW264.7 cells. Further, results from the mouse colitis model suggested that NA could restore intestinal barrier function and suppress NF-κB signal pathways to ameliorate DSS-induced colitis. In addition, untargeted metabolomics analysis of NA protection against UC found that NA protected mice from colitis by regulating citrate cycle, amino acid metabolism, pyrimidine and purine metabolism.

Conclusion: These results suggested that NA could ameliorate the secretion of inflammatory factors, suppress the NF-κB signaling pathway, and protect the integrity of colon tissue, thereby having a novel role in prevention or treatment therapy for UC. This work for the first time indicated that NA might be a potential functional food ingredient for preventing and treating inflammatory bowel disease (IBD).

Abbreviations: ALA, alpha-linolenic acid; AP-1, Activator Protein-1; Bax, Bcl-2-associated X; Bcl-2, B-lymphoma-2; DEX, dexamethasone; DHA, docosahexaenoic acid; DSS, dextran sodium sulfate; ELISA, Enzyme-linked immunosorbent assay; EPA, eicosapentaenoic acid; IBD, inflammatory bowel disease; IKK, IκB kinase; IL-1R, interleukin-1 receptor; LPS, lipopolysaccharide; MAPK, Mitogen-activated protein kinase; MyD88, myeloid differentiation factor 88; NA, Nervonic acid; NF-κB, nuclear factor kappa-B; PCD, programmed cell death; PUFAs, poly-unsaturated fatty acids; PRRs, pattern-recognition receptors; UC, ulcerative colitis; SPSS, stroke-physiological saline solution; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor alpha.

* Corresponding authors.
E-mail addresses: moon_s731@hotmail.com (J.-Y. Sun), nigyangli@126.com (N.-y. Li), jsimal@uvigo.es (J. Simal-Gandara), liuchao555@126.com (C. Liu).

https://doi.org/10.1016/j.phymed.2023.154702
Received 22 October 2022; Received in revised form 14 January 2023; Accepted 3 February 2023
Available online 4 February 2023

Introduction

Recently, the prevalence of ulcerative colitis (UC) has been increasing globally, and UC occurs widely in people of all ages, which is a chronic, immune-mediated inflammatory disease affecting the large intestine. However, the pathogenesis and etiology of UC have not been fully defined (Laura et al., 2020; Bergemalm et al., 2021). Current studies believe that UC is a kind of autoimmune disease mainly mediated by immune response and closely associated with environmental and genetic factors (Kuwada et al., 2021). The immune system *in vivo* is activated under the stimulation of different microbial antigens, which results in chronic inflammatory response of intestinal tissues, activation of various inflammatory cells, as well as the imbalance of cytokines, and ultimately leads to UC (Williams et al., 2020; Vipul et al., 2016; Q. Chen et al., 2019). During the progression of UC, myeloid differentiation factor 88 (MyD88), Toll-like receptor 4 (TLR4), tumor necrosis factor alpha (TNF-α), and interleukin-1 receptor (IL-1R) were considered to be the main participation factors (Yang et al., 2017; Hug et al., 2018; Bruning et al., 2021; Q. Chen et al., 2019). Presently, pharmaceuticals are the primary approach to treating colitis, and the conventional ones include corticosteroids, aminosalicylates, azathioprine/6-mercapto purine, anti-tumor necrosis factor and cyclosporine. However, some defects of medicines mentioned above are continually highlighted, including high rates of recurrence, increased resistance, serious adverse effects and so on. Given that food first enters into the digestive system, it could be deduced that diet affects the prevalence of UC to some extent, thus a combined strategy of drug therapy and nutritional therapy may better alleviate or cure the disease. Medicinal and edible plants and their secondary metabolites, such as terpenoids, flavonoids, alkaloids and steroids, have been identified as valuable sources of natural drugs to treat different diseases according to their prominent curative effects and low-toxicity (Hirten et al., 2021; Luo et al., 2022). Traditional Chinese medicine has been used to treat UC for thousands of years, including Yunnan Baiyao, Baitouweng decoction, Qingchang suppository Xilei powder and so on, and their great potential deserves attention (Zhang et al., 2013). In addition, unsaturated fatty acids have recognised anti-inflammatory properties. In previous reports, researchers have studied the effects of poly-unsaturated fatty acids (PUFAs) including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic Acid (DHA) on intestinal inflammatory diseases by supplementing fish oil or plant seed oil (Kim et al., 2020; Marton et al., 2019). Therefore, it is possible to alleviate UC with functional factors in natural products.

As a very long-chain fatty acid, the name of nervonic acid (C24:$1^{\Delta 15}$, cis-15-tetracosenoic acid, NA) is attributed to that it is initially discovered in mammalian nerve tissues (Fan et al., 2018). NA plays an important role in human health, specifically for the brain (Hu et al., 2021; Lewkowicz et al., 2019). NA not only has a protective effect on brain tissue, but also has other physiological functions of unsaturated fatty acids. NA mainly maintains the composition, structure and function of biomembranes through sphingomyelin and sphingolipid, thus improving cell vitality and enhancing the ability of normal human activities (Keppley et al., 2020). NA can promote the transformation and proliferation of splenic lymphocytes; increase the number of antibody-producing cells and the activity of NK cells, thus improving immune function. NA supplementation can decrease the yield of pro-inflammatory chemokines and cytokines (Fan et al., 2018; Yamazaki et al., 2014; Borish et al., 2003). In recent years, numerous studies have attempted to determine the mechanisms involved in regulating the intestinal inflammatory response, but this is still unclear.

In this work, the effect and mechanism of NA in eliminating inflammation and regulating immunity were firstly elucidated *in vitro*. Then the colitis mouse model induced by dextran sodium sulfate (DSS) was designed and employed to assess the therapeutic effect and relevant molecular mechanism of NA on UC. Additionally, in view of the metabolic diseases in colitis, the metabolic changes in plasma composition during the process of UC treatment were also determined. This study will also provide some validated and reliable evidence for utilization of NA to develop health promoting products.

Material and methods

Materials

NA (98%), EPA, lipopolysaccharide (LPS), dexamethasone (DEX), dextran sodium sulfate (DSS), and all other chemicals were provided by Sigma Chemical Co. (St. Louis, MO, USA). The chemical structure of NA was presented in Fig. 1.

In vitro study

Cell culture

Macrophages (RAW264.7) were provided by the Chinese Academy of Sciences Cell Bank (Shanghai, China). Cells were cultivated in DMEM medium containing 1% penicillin-streptomycin, 1% l-glutamine and 10% fetal bovine serum (Hyclone, Logan, UT, USA) at 37 °C in a humidified 5% (v/v) CO_2 incubator. The cells were maintained by passaging every two days.

Cell viability assay

A CCK-8 cell counting kit (Vazyme, Nanjing, China) was utilized to determine Cell viability. The RAW264.7 cells listed above were inoculated into 96-well plates with 1×10^5 cells per well, and cultivated under conditions of 5% CO_2 and 37 °C for 24 h. Subsequently NA at the concentrations of 200 μM, 100 μM, 75 μM, 50 μM, 25 μM, 12.50 μM, and 6.25 μM were placed into the wells, respectively, and incubated for 24 h. Then each well was added 10 μl of CCK-8 solution, and the plates were incubated at 37 °C for 2 h. The absorbance of each well was measured at 450 nm with a microplate reader (BioRad, Hercules, CA, USA), and GraphPad Prism 5.0 software was used to calculate the survival rate. LPS was used as an inflammatory inducing drug, while DEX (50 μM), an anti-inflammatory drug, served as the positive control,. All experiments were carried out in triplicate, and cell viability was described as a percentage of the control cells.

Anti-inflammatory activity assay

The RAW264.7 cells in the exponential phase were inoculated into 96-well plates with 1×10^5 cells per well, and cultivated with 5% CO_2 at 37 °C for 24 h. After pretreatment using the corresponding concentration of NA (50 μM, 25 μM and 12.50 μM) for 2 h, cells were stimulated using 1 μg/ml LPS for 24 h. The supernatant was harvested by centrifuge. NO concentration in the cell supernatant was detected by Griess colorimetry to evaluate the anti-inflammatory activity of NA.

Enzyme-linked immunosorbent assay (ELISA)

The RAW264.7 cells in the exponential phase were inoculated into 24-well plates with 1×10^6 cells per well, and treated with LPS, DEX (50 μM) and NA (50 μM, 25 μM and 12.50 μM) at the corresponding concentration. The supernatant was collected by centrifugation. The following experimental mice were anaesthetized with isoflurane to

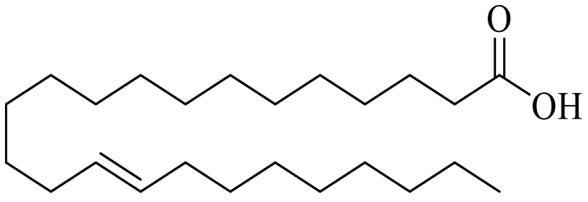


Fig. 1. The chemical structure of nervonic acid.

collect blood from eyeballs. The obtained blood was left to rest for 15 min, and centrifugated at 2000 g for 15 min to get the serum. ELISA kits were used to measure the production of TNF-α, IL-6, IL-1β, and IL-10 in the supernatant of RAW264.7 cells and serum of mice based on the manufacturer's recommendations (Beyotime Biotechnology, Shanghai, China).

Animals and study design

Male C57BL/6 mice of 7 – 8 weeks, weighing 20 – 22 g, were provided by Huafukang Biological Technology Co. Ltd (Beijing, China). Mice were raised at 24 to 25 °C, humidity of 50 to 60%, ventilation rate of 10 to 20 times/h and light/dark alternately for every 12 h. All procedures in the experiments were performed in accordance with the Guidelines of the Institutional Animal Care Committee (USA) and were approved by the Animal Research Committee of Shandong Academy of Agricultural Sciences (SCXK20170005). Three mice were fed and watered freely in each cage, and the cage was washed twice a week. After 7 days of adaptation, mice were randomly separated into 7 groups (Table 1), and each group contained 6 mice. An overview of the design can be seen in Fig. 1. The positive control was the anti-inflammatory drug DEX. DSS was used as the inflammatory inducing drug. EPA has been reported to significantly attenuate DSS-induced colitis in C57BL/6 and was used as a positive control as well (Zhang et al., 2021). Different doses of NA was dissolved in corn oil and administered to mice by gavage. The same amounts of stroke-physiological saline solution (SPSS) were given to the mice in normal group.

Clinical observations

During medication treatment, changes in body weight, feces and blood in stool of mice were monitored daily, and scores were given according to the stated scoring criteria in Table 2. Disease activity index (DAI) = (weight loss score + fecal traits score + blood in stool score) /3.

Histological evaluation

The mice were sacrificed by cervical dislocation after anaesthetization using isoflurane. Formaldehyde (4%) was used to fix the distal portions of the colons overnight, which were then inserted into paraffin, sliced into samples with a thickness of 6 μm and placed onto microscopic slides for staining using haematoxylin-eosin (H&E) for histological analysis.

MPO activity assay

MPO activity of serum and colonic tissue was determined with MPO assay kit (Nanjing Jiancheng Bioengineering Institute, China). Concisely, colon tissues were homogenized in the stocking buffer from manufacturer. Homogenate or serum was mixed and incubated at 37 °C for 30 min in the reaction solution. The mixture was subsequently kept for 10 min at 60 °C, followed by adding hydrogen peroxide (50 μl). A multimode microplate reader (BioTek Instruments, VT, USA) was used to measure the absorbance of mixture at 460 nm. One unit of MPO activity was designated as the quantity of enzyme capable of degrading 1 μM hydrogen peroxide at 37 °C. The results were determined as units per liter serum or per gram colon tissue.

Table 1
Experimental grouping and administration scheme.

Group	Number of mice	Intervention	Therapeutic Does(mg/kg)
Normal	6	SPSS	SPSS
Model	6	3% DSS+ SPSS	SPSS
Positive	6	3% DSS+ DEX	1 mg/kg
EPA	6	3% DSS + EPA	50 mg/kg
Low dose	6	3% DSS + NA	5 mg/kg
Medium dose	6	3% DSS + NA	50 mg/kg
High dose	6	3% DSS + NA	100 mg/kg

Table 2
Disease activity index.

Score	Percentage weight loss (%)	Stool	Stool blood content
0	0	Normal	Negative haemoccult test-no blood
1	0 – 5	Moist/ sticky	Negative haemoccult test-no blood
2	5 – 10	Soft	Positive haemoccult test in > 30 s
3	10 – 15	diarrhea	Positive haemoccult test in < 30 s
4	≥ 15	diarrhea	Gross observable blood

Immunofluorescence assay

Formaldehyde (4%) was used to fix the distal colon of each mouse, which was then sectioned after embedding in paraffin and frozen in liquid nitrogen. Then the section of colon was washed thrice using cold PBS, followed by incubation at room temperature for 30 min with blocking buffer composed of 10% goat serum, 1% bovine serum albumin (BSA), and 0.5% triton X-100. After removing the blocking buffer, ZO-1 antibody was incubated with the colon section at 4 °C overnight. After rinsing the specimens thrice with PBS buffer, the corresponding fluorescent secondary antibody was added and developed for 2 h at room temperate. Finally, 4′, 6-diamidino-2-phenylindole (DAPI) was used to counterstain the specimens in darkness for 5 min. The fluorescent microscope (Leica, Tokyo, Japan) was used to capture the images.

Real-time fluorescent quantitative polymerase chain reaction (RT-qPCR) analysis

TRIzol reagent (Life Technologies, Carlsbad, CA, USA) was used to extract total RNA from RAW264.7 cells or colons in light of the manufacturer's protocol. A reverse transcription kit (Vazyme, Nanjing, China) was used to conduct the first-stand cDNA synthesis. A fluorescence ration PCR instrument (Life Technologies) was used to carry out RT-qPCR with SYBR® Premix ExTaq II (TLiRNaseH Plus) (Takara) using the relative quantity ($2^{-\Delta\Delta Ct}$) method. The primers used in this study were listed in Table 3. Relative gene expression levels were normalised to *GAPDH* for mouse gene expression.

Western blot analysis

RIPA lyses buffer (Beyotime Biotechnology, Shanghai, China) with phenylmethanesulfonyl were used to extract the protein of collected cells or colons, and a BCA protein assay kit (Beyotime Biotechnology, Shanghai, China) was utilized to quantify the total protein concentration. SDS-PAGE (10%) was utilized to separate the protein samples, followed by transferring onto polyvinylidene fluoride membranes. Skim milk (5%) in TBST was utilized to block the membranes at room temperature for 2 h. Then the membranes were incubated at 4 °C overnight with specific primary antibodies to p65, GAPDH, and p-p65 provided by Servicebio Technology (Servicebio Technology, Wuhan, China), JNK1/2/3, p-JNK1/2/3, p38, p-p38, IκBα and p-IκBα purchased from ABclonal Biotechnology (ABclonal, Wuhan, China), followed by incubation with HRP-conjugated secondary antibodies (Proteintech Group Inc., Chicago, USA). An enhanced chemiluminescence system (Vazyme, Nanjing, China) was utilized to develop the Signals.

Table 3
Names and sequences of primers used for quantitative reverse transcription PCR.

Name	Species	Sense	Antisense
iNOS	Mouse	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGA AGGGGTCGT
COX-2	Mouse	ATAGACGAAATCAACAACCCCG	GGATTGGAAGTTCTATTGGCAG
TNF-α	Mouse	ATGTCTCAGCCTCTTCTCATTCCT	GGGTCTGGGCCATAGAACTGA
IL-6	Mouse	CCCCAATTTCCAATGCTCTCC	CGCACTAGGTTTGCCGAGTA
IL-10	Mouse	TTTAAGGGTTACTTGGGTTGCC	AATGCTCCTTGATTTCTGGGC
GAPDH	Mouse	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT

LC-MS/MS data acquisition

Extraction of metabolites: 900 μl of precooled extraction solvent (methanol: acetonitrile: water = 4:4:1, v/v/v) was mixed with 100 μl of plasma samples in the 1.5-ml tubes. The samples were sonicated for 60 min in ice water, and incubated at −20 °C for 1 h to precipitate proteinion. Then the samples were collected by centrifugation (16,000 g) at 4 °C for 20 min. The supernatants were dried in a high-speed vacuum centrifuge after being transferred into 1.5-ml tubes. Subsequently, 100 μl of extraction solvent (acetonitrile: water = 1:1 v/v) was mixed with the samples, which were centrifuged (12,000 g) at 4 °C for 15 min. Finally, the supernatant was taken for analysis.

LC-MS/MS analysis: An UHPLC system (DHIMADZU-LC30, Shimadzu) equipped with an UPLC BEH Amide column (1.7 μm, 2.1 mm × 100 mm, Waters) was used to carry out the LC-MS/MS analysis. The mobile phase composed of H_2O and 25 mM NH_4OH in water (A) and acetonitrile (B) was eluted at 0.3 ml/ min with the following gradient: 0 – 1 min, 95% B; 1 – 7 min, 65% B; 7 – 9 min, 35% B; 9 – 10.5 min, 35% B; 10.5 – 11 min, 95% B. The samples were injected with a volume of 3 μl for separation, which were then determined with a QE Plus mass spectrometer (Thermo Scientific) and ionized using an electrospray ionization (ESI) source. The ESI source conditions were listed below: capillary temperature: 320 (±) °C; spray voltage: 3.8 kv (+) and 3.2 kv (-); aux gas: 5 (±) arb; sheath gas: 30 (±) arb; S-lens RF level: 50; probe heater temp: 350 (±) °C. The mass spectrum acquisition settings were as follows: mass spectrum acquisition time 12 min, primary mass spectrometry resolution 70,000 @ *m/z* 200, precursor ion scanning range 80 – 1200 *m/z*, primary maximum IT 100 ms, AGC target $3e^6$. The MS^2 analysis was acquired as follows: MS^2 scan was triggered after each full scan for the 10 most intense precursor ions, AGC target $1e^5$, MS^2 resolution 17,500 @ *m/z* 200, MS^2 activation type HCD, secondary maximum IT 50 ms, normalized collision energy (Setpped) 10, 20, 30, isolation window 2 *m/z*.

Raw data were peak aligned, retention time was corrected and peak areas were extracted using MSDIAL software. Metabolite structure identification adopts accurate mass matching (mass deviation mass tolerance < 20 ppm) and secondary spectrum matching (mass deviation mass tolerance < 0.02 Da), searching public databases including MassBank, HMDB and our self-built Metabolites Standard Library. The systematic stability of the experiment in this study was evaluated and analyzed by using two strategies, mass base peak map alignment of QC samples and PCA statistical analysis of QC samples.

Statistical analysis

GraphPad Prism 5.0 software (Graphpad Software Inc., California) was utilized to analyze data using analysis of variance. All the experiments were carried out in triplicate. One-way ANOVA and student's *t*-test were utilized to determine the significance of parametric data. The *p*-values less than 0.05 were considered as statistically significant differences between groups.

Results

The production of pro-inflammatory cytokines was reduced by NA in vitro

To verify the primary anti-inflammatory effect of NA *in vitro*, cell experiments were first carried out using RAW264.7 cells. The effect of various concentrations of NA on RAW264.7 cell activity was firstly evaluated to determine the concentrations used in the following experiments. Results demonstrated that the NA concentrations lower than 50 μM had no significant toxicity to RAW264.7 cells (Fig. 2A). Therefore, NA concentrations lower than 50 μM were selected in the following cellular experiments. Next, the LPS-induced inflammatory model cells were incubated with NA at concentrations of 6.25 μM, 12.50 μM, 25 μM, and 50 μM, and the production of NO was measured to detect the anti-inflammatory action of NA preliminarily and rapidly. It was found that NA at the concentrations from 6.25 μM to 50 μM could inhibit the yield of NO in RAW264.7 cells induced by LPS, with significant inhibitory effect at 12.50 μM, 25 μM and 50 μM compared with the LPS group (Fig. 2B). Therefore, the three NA concentrations were used in the following experiments. The influence of NA on the excretion of inflammatory cytokines in LPS-stimulated RAW264.7 cells was also evaluated. The results showed that NA inhibited the excretion of pro-inflammatory factors IL-6, IL-1β and TNF-α but promoted the release of IL-10, compared with the LPS group, as measured by ELISA (Fig. 2C). Then RT-qPCR analysis verified that NA strongly inhibited the LPS-induced expression of cyclooxygenase-2 (COX-2), IL-6, TNF-α and inducible nitric oxide synthase (iNOS) on the transcriptional level (Fig. 2D). COX-2 and iNOS are two kinds of inflammatory enzymes. All the above results suggested that NA could efficiently reduce the inflammatory response in RAW264.7 cells induced by LPS.

NA suppressed NF-κB and MAPK signaling pathways in LPS-induced macrophages

Nuclear factor kappa-B (NF-κB) and Mitogen-activated protein kinase (MAPK) are two key signaling pathways in inflammatory responses. To investigate the anti-inflammatory mechanism of NA, the protein and phosphorylated protein levels related to NF-κB and MAPK signaling pathways were examined using western blotting. As demonstrated in Fig. 3A, LPS stimulation increased the expression level of TLR4, a kind of trans-membrane protein recognizing pathogens and activating immune activity. However, the expression of TLR4 decreased when the cells were treated with NA in a dose-dependent manner, compared with the LPS group. LPS stimulation increased the phosphorylation of JNK and P38 in RAW264.7 cells, which were decreased by NA in a dose-dependent manner, suggesting that the MAPK signaling pathway was involved in the anti-inflammatory response of NA. The phosphorylation level of IκBα was increased by LPS stimulation in RAW264.7 cells, which was reduced by NA in a dose-dependent manner as well, meaning that the NF-κB signaling pathway also played a significant role in the anti-inflammatory response of NA.p65, one of the proteins in the NF-κB complex,plays an

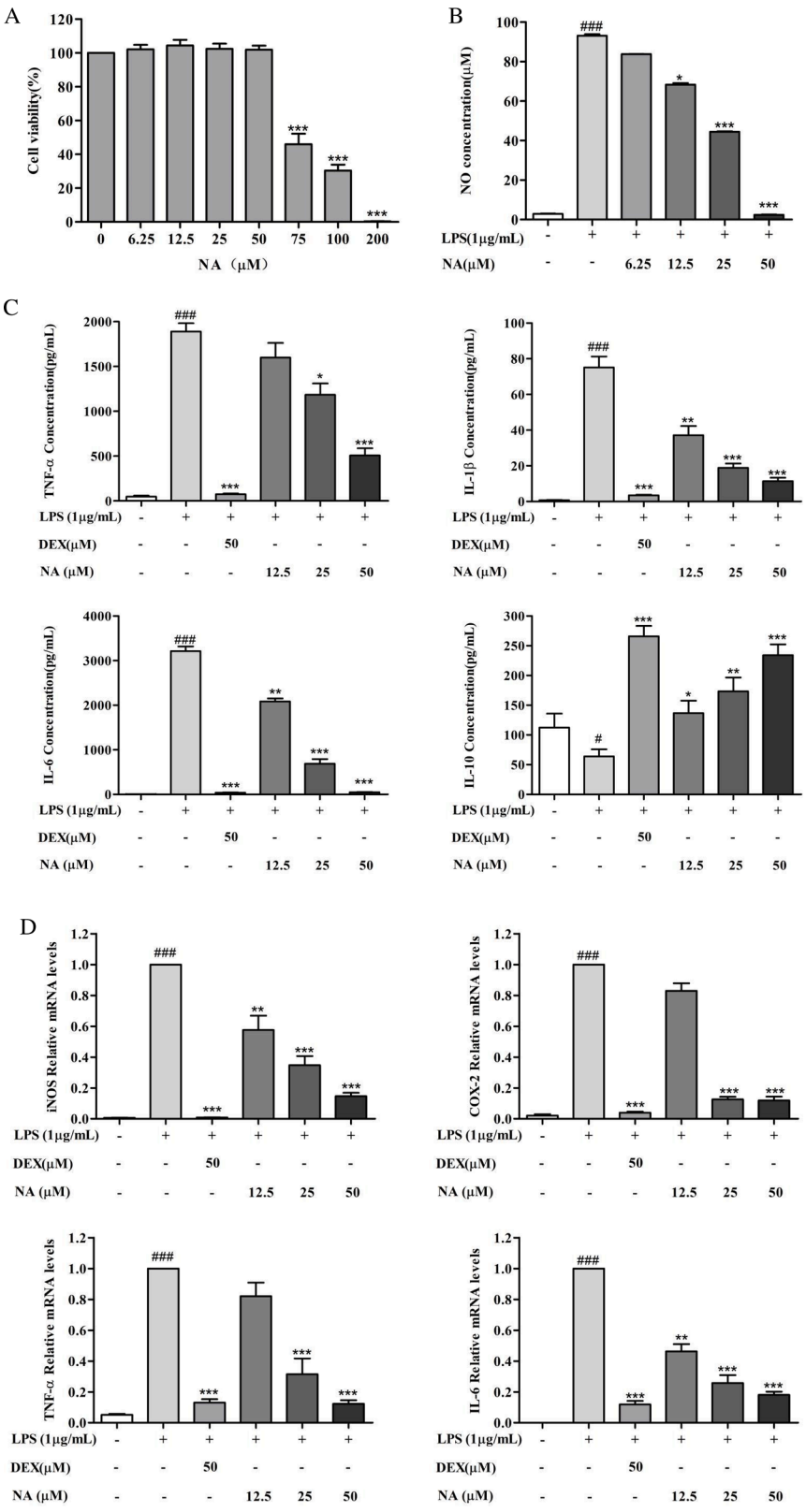


Fig. 2. Nervonic acid inhibited pro-inflammatory cytokine production in LPS-stimulated macrophages.

essential role in adjusting the function and activation of NF-κB by translocating into the nuclear and regulating gene expression of TNF-α, IL-6, COX-2, iNOS. . As shown in Fig. 3B, NA inhibited the nuclear translocation of p65 subunit in a dose-dependent manner in comparison to the LPS group. In short, NA suppressed the activation of both NF-κB and MAPK signaling pathways, which are closely related to inflammatory factors release in RAW264.7 cells.

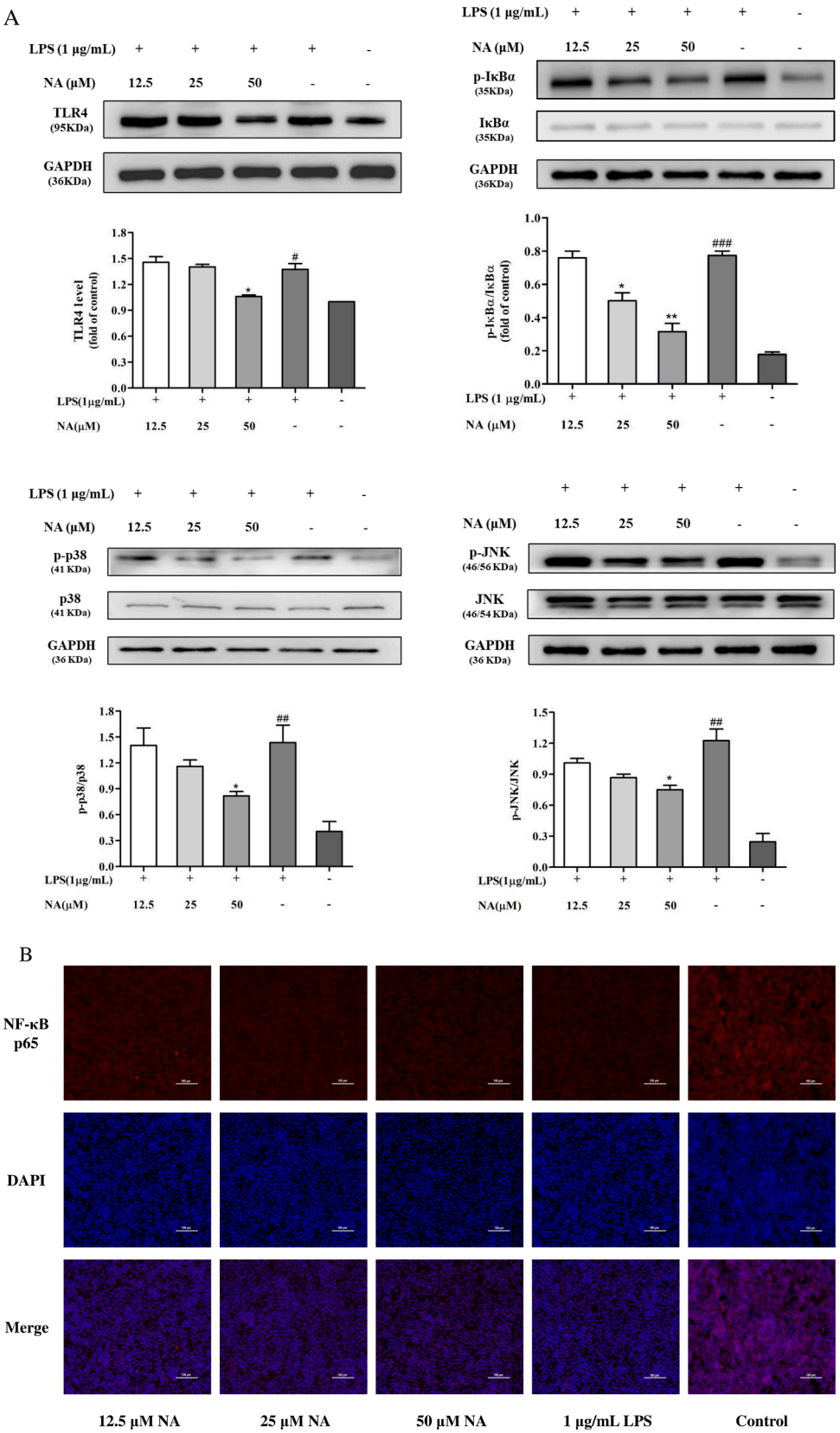


Fig. 3. Nervonic acid inhibited the activation of MAPK and NF-κB signaling pathway in RAW264.7 cells.

NA attenuated the colitis induced by DSS in mice

The cellular results demonstrated that NA played a key role in the anti-inflammatory activity. To study the alleviating influence of NA on colitis, C57BL/6 mice were administered with 3% DSS, EPA (50 mg/kg), DEX (1 mg/kg), and NA (5 mg/kg, 50 mg/kg, 100 mg/kg) separately or combined for 7 days (Fig. 4A). The DAI score, body weight and colon length reflect the severity of UC in animal models directly and are commonly utilized to assess the severity of colitis preliminarily. The body weight and DAI scores of each mice group during days 0 – 14 are presented in Fig. 4B-C. After consuming DSS for four days, the body weight of the model mice group decreased significantly and continued in the following days, with severe hematochezia and diarrhea. There were remarkable differences ($p < 0.01$) in mice weight between the model and the normal groups. However, the symptoms of diarrhea, weight loss and fecal occidental blood were improv ed in all the low-, medium- and high-dose NA groups. The overall effects of NA on colon condition were also observed (Figure 4D) in this study. The colon of the normal group had no obvious congestion and edema. While the colon length of the model mice group was significantly shorter than these of normal mice group, with different degrees of edema. The colon condition of the DEX, EPA and NA groups was improved, with significantly increased colonic length and less edema. All these results suggested that UC could be clearly alleviated by NA in mice.

The infiltration of inflammatory cells into colon was inhibited by NA in DSS-induced mice

The eroded intestinal epithelial cells induced by treatment with DSS could increase the colonic mucosal permeability. Compared with the normal group, the pathological section of colon tissue of mice in the model group showed intestinal wall edema and thickening, with a considerable number of inflammatory cells infiltration, severely deformed crypt structure, and destroyed or even disappeared goblet cells (Fig. 5A). However, the colon tissue injury mentioned above was reduced in the NA treatment groups compared with the model group. COX-2 and iNOS are the main causes of prostaglandins (PGs, mainly PGE2) and NO, respectively. Excessive PGE2 and NO could cause vascular dilation and increased permeability, which result in inflammatory cell infiltration and mucosal edema. In this study, western blot was utilized to determine the expression level of inflammatory enzyme COX-2 and iNOS extracted from colon. The expression levels of iNOS (Fig. 5B) and COX-2 (Fig. 5C) in the NA treatment groups reduced in a dose-dependent manner compared with the model group. All the above results suggested the protective effect of NA on the colon damage induced by DSS in mice.

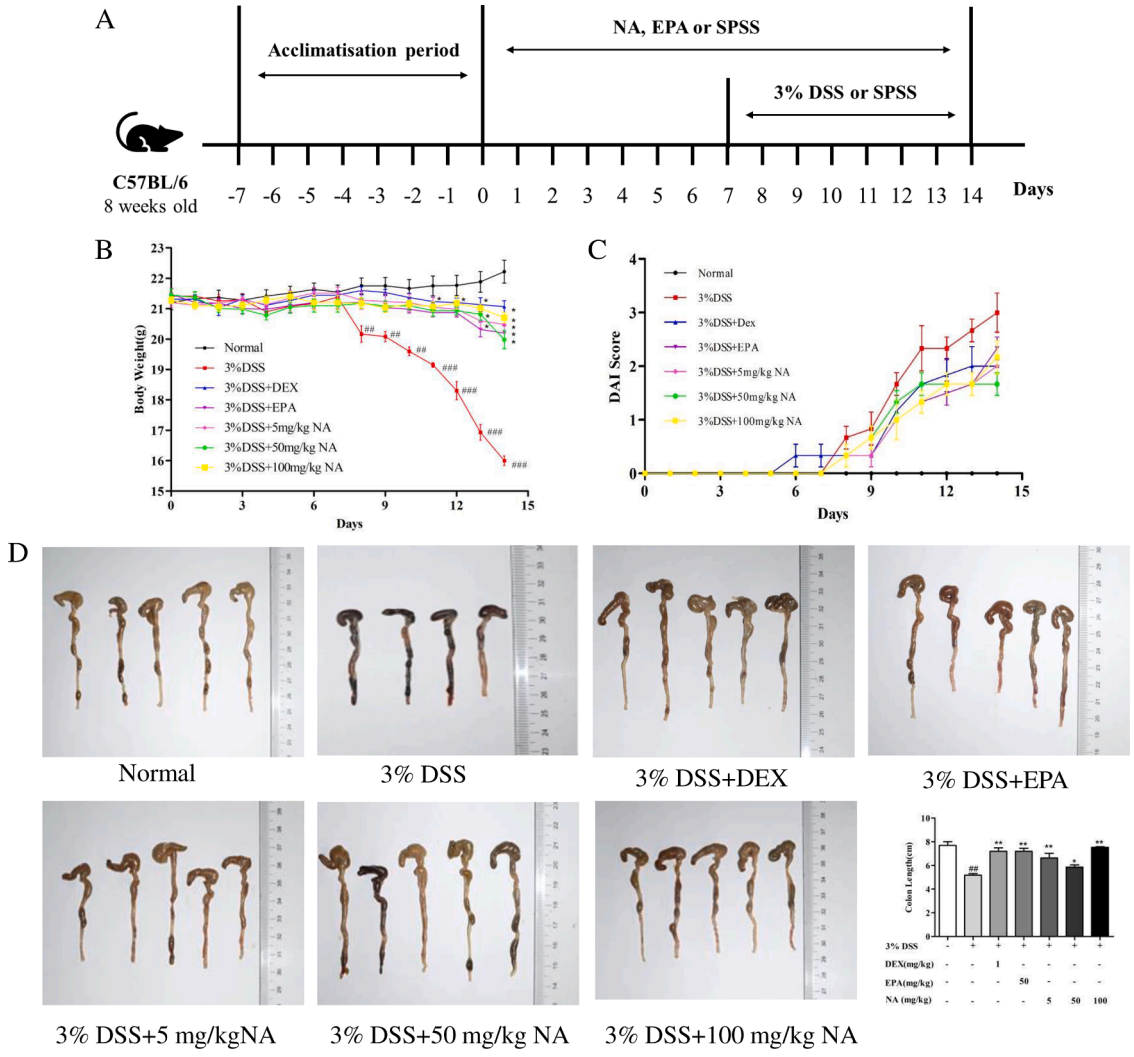


Fig. 4. Nervonic acid ameliorated DSS-induced acute colitis in mice.

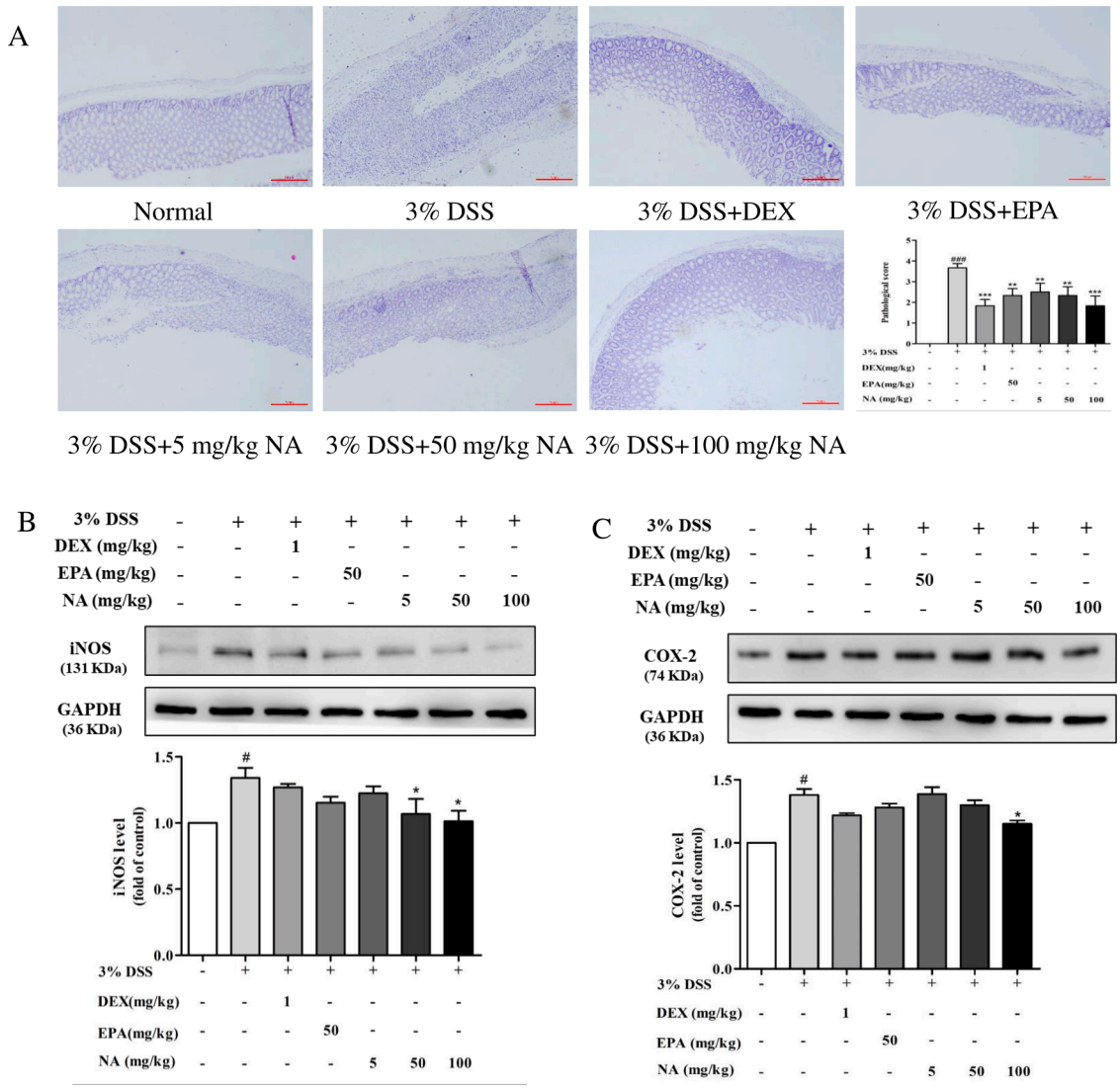


Fig. 5. Nervonic acid prevented DSS-induced colon damage in mice.

NA decreased the levels of pro-inflammatory cytokines in mice treated with DSS

Cytokines are mainly produced by lymphocytes, monocytes, macrophages and epithelial cells, with pro-inflammatory effects, such as TNF-α, IL-6 and IL-1β, or anti-inflammatory effects, such as IL-10. There is a dynamic balance in the cytokine network in healthy individuals which plays a critical role in intestinal barrier function, host immune regulation, tissue repair, and intestinal homeostasis (Ramani et al., 2015; Leppkes et al., 2020; Williams et al., 2020). As demonstrated in Fig. 6A, in comparison to the normal group, the secretion levels of inflammatory cytokines including IL-6, TNF-α and IL-1β of serum in DSS model mice were remarkably improved while IL-10 was significantly reduced. Compared with the model group, different concentrations of NA treatment reduced the secretion of IL-1β, IL-6 and TNF-α significantly, and the most significant reduction was observed in the 100 mg/kg NA treatment group. The expression of some cytokines on transcriptional level in colon tissue was determined using RT-qPCR. The mRNA expression of COX-2, iNOS, IL-6 and IL-1β in colon tissue decreased after NA intervention in a dose-related manner, compared with the model group (Fig. 6B), with the most significant inhibitory effect at the concentration of 100 mg/kg NA. MPO activity is an inflammatory indicator of neutrophils infiltrating into colon tissue and was utilized to assess the effect of NA on neutrophils infiltration. Test results exhibited that MPO activity in the serum and colon was significantly reduced in the NA-treated mice groups compared with that of the model group (Fig. 6C), which indicated that NA could inhibit neutrophils infiltration and inflammation in mice. Collectively, NA decreased the inflammatory response in DSS induced mice.

NA suppressed NF-κB signaling pathway in colitis mice induced by DSS

Previous reports have shown that the development of colitis is accompanied by the strong expression and activation of transducers associated with the NF-κB signaling pathway, and the activation degree of transducers is significantly related to the severity of intestinal inflammation. The phosphorylation level of p65 and IκBα, the representative transducers of NF-κB signaling pathway, was significantly increased during the progress of inflammation (Li et al., 2020). As an important component of innate pattern-recognition receptors (PRRs), TLR4 activates the NF-κB signaling pathway by recognizing pathogens. As demonstrated in Fig. 7A-C, the expression of TLR4, p65 and p-IκBα tended to decrease in a concentration-dependent manner when the C57BL/6 mice were treated with NA (100 mg/kg, 50 mg/kg, and 5 mg/kg) in comparison to the DSS group. These results suggested that the alleviation caused by NA on intestinal inflammation may be related to

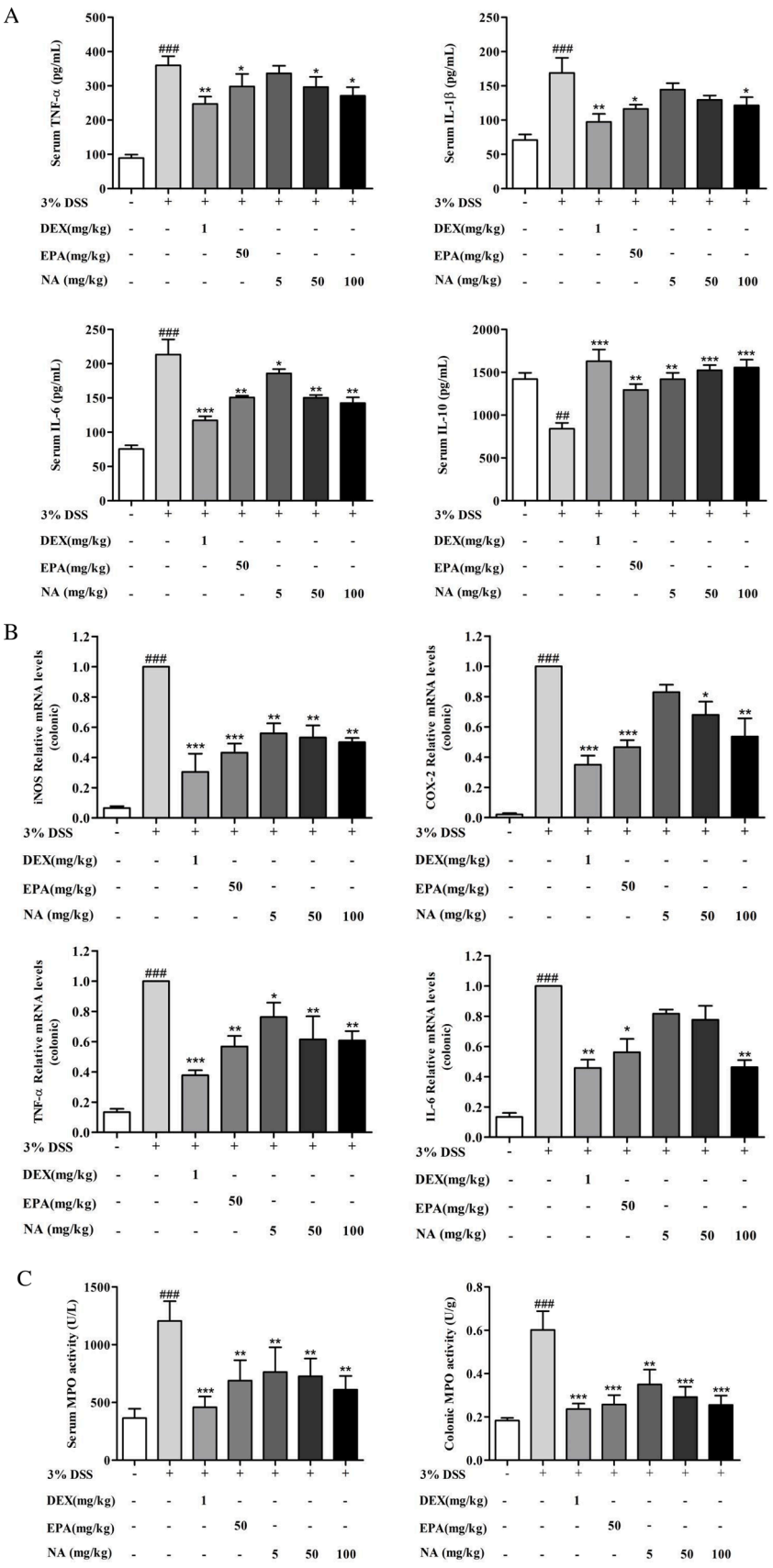


Fig. 6. Nervonic acid inhibited pro-inflammatory cytokine production in serum and colon tissues from DSS-induced colitis mice.

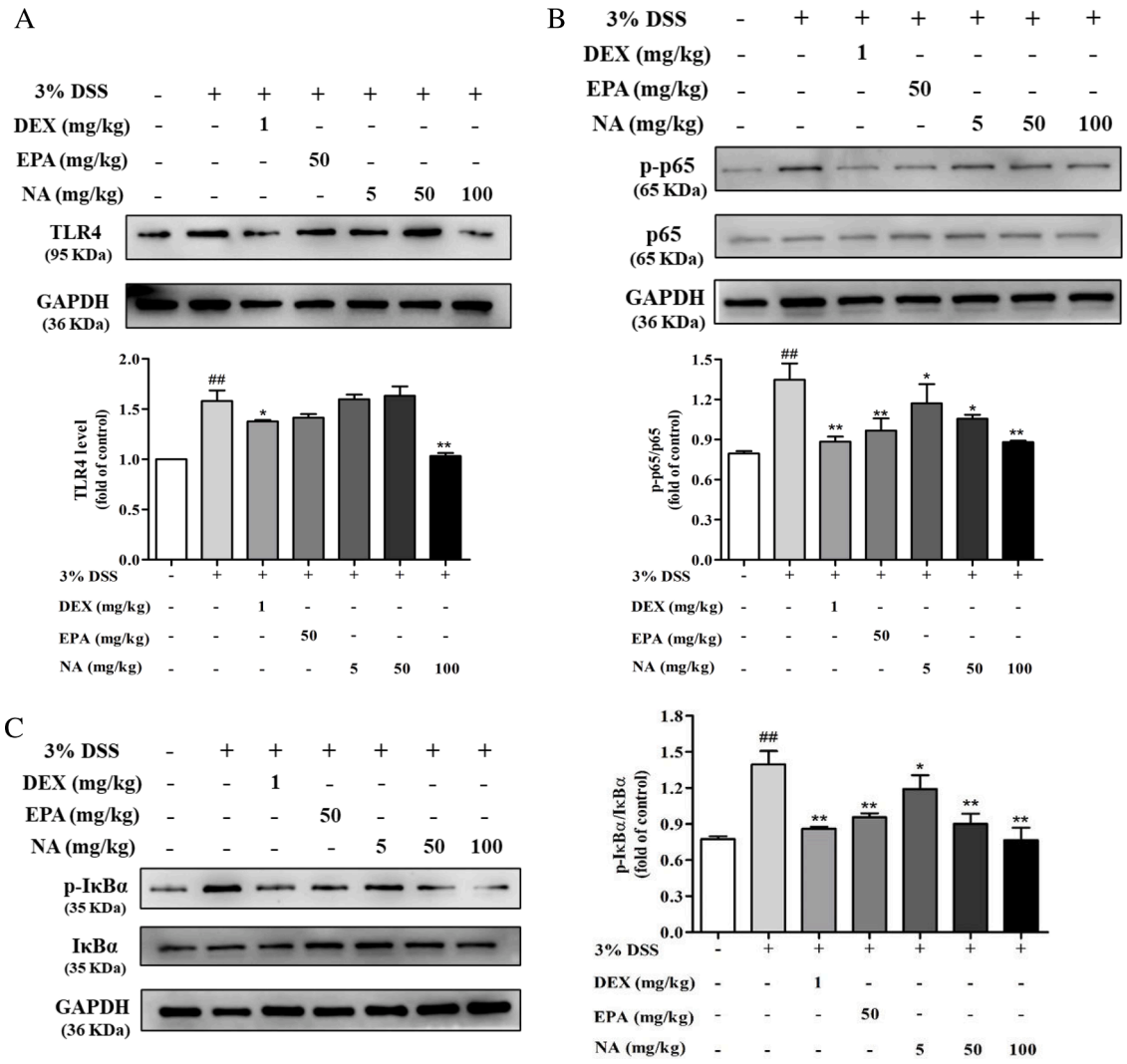


Fig. 7. Nervonic acid inhibited the activation of NF-κB signaling pathway in DSS-induced colitis.

the inhibition of NF-κB signaling pathway.

NA inhibited apoptosis induced by DSS and enhanced expression of tight junction protein in mice colonic tissues

Apoptosis, also known as programmed cell death (PCD), is one of the important mechanisms affecting intestinal mucosal injury and immune disorder in UC patients. Apoptosis of colonic epithelial cells plays a crucial role in the UC pathogenesis. B-lymphoma-2 (Bcl-2) is a type of proto-oncogene inhibiting apoptosis; conversely, Bcl-2-associated X (Bax) is the most important apoptotic gene promoting apoptosis. The combination of Bcl-2 with Bax form a stable heterodimer, which can regulate apoptosis. As shown in Fig. 8A, compared with the model group, NA enhanced the expression of Bcl-2 while inhibited the expression of Bax in the colon, as detected by western blot analysis, which suggested the alleviation of DSS-induced apoptosis of colonic tissues. In addition, when colitis occurs the intestinal barrier function is impaired, and intestinal permeability is increased (Ungaro et al., 2017). In this work, the immunofluorescence method was utilized to determine the expression level of tight junction (TJ) protein ZO-1 in colon tissues. Results showed that NA enhanced the expression and distribution of intestinal TJ protein ZO-1, and reduced the permeability of intestinal mucosa in DSS-treated mice (Fig. 8B).

Differential metabolites and metabolic pathways in different mice groups NA

To further study the mechanism of anti-inflammatory effects of NA, mice blood sample collected at different time points were used for metabolite analysis as Fig. 9A shown. The OPLS-DA model was utilized to obtain the Variable Importance for the Projection (VIP). The explanatory ability and influence intensity of each metabolite expression pattern on the discrimination and classification of each group of samples were determined, and the different metabolites with biological significance were investigated. The screening criterion was set as VIP > 1 to screen the differences between groups preliminarily. Furthermore, univariate statistical analysis was utilized to validate whether there were remarkable differences in metabolites or not. Metabolites with significant differences were defined by metabolites of both univariate statistical analysis P value ⟨ 0.05 and VIP ⟩ 1 with multivariate statistical analysis. The differential metabolites in the serum of the model, control and different concentrations NA groups were shown in Fig. 9B. The pathway enrichment analysis was performed by MetaboAnalyst system, and the results were demonstrated in Fig. 9C.

In the first sampling, the 181 biomarkers identified were observed to be associated with four metabolic pathways, including taurine and hypotaurine metabolism, D-arginine and D-ornithine metabolism,

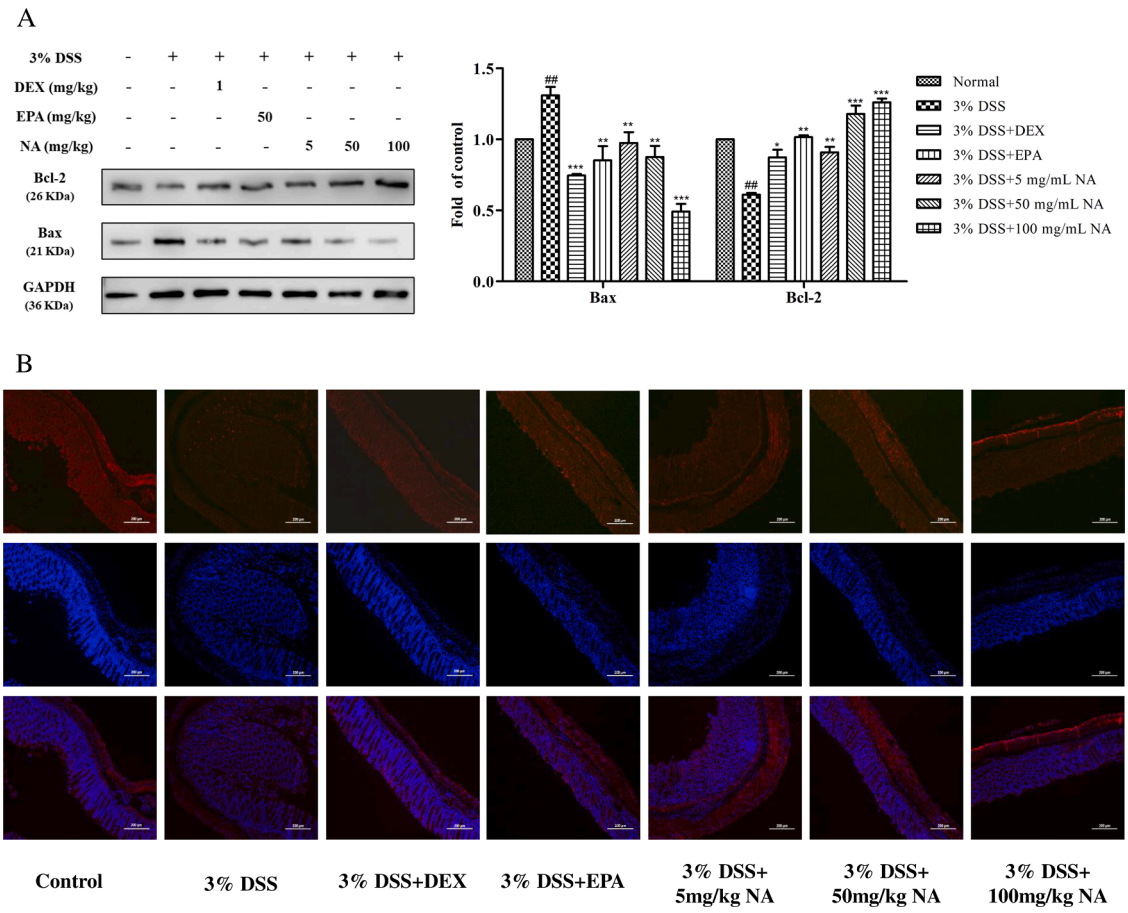


Fig. 8. Nervonic acid attenuated DSS-induced apoptosis and deletion of tight junction protein in mice colonic tissues.

arginine and proline metabolism, and glutathione metabolism. In the second sampling, the 116 biomarkers were detected to be related to four metabolic pathways, including citrate cycle (TCA cycle), biosynthesis of amino acids, arginine biosynthesis, thiamine metabolism, and glycine, serine and threonine metabolism. In the third sampling, the i116 biomarkers identified were found to be involved in four metabolic pathways, including arginine and proline metabolism, purine metabolism, D-arginine and D-ornithine metabolism, pyrimidine metabolism, and arginine biosynthesis and biosynthesis of cofactors. The metabolomics research demonstrated that the disturbance of some metabolites and metabolisms was caused by UC disease, which could be recovered by the NA treatment.

Discussion

UC is a chronic inflammatory bowel disease (IBD), and its therapeutic goals are to induce the relief of active disease and avoid repetition. With the gradually increased prevalence of UC, the current therapies are definitely not optimal and usually cause serious side effects, thus more and more attentions are paid to natural products with anti-UC functions.

A complex inflammatory response is involved in the pathogenesis of UC. DSS is a polyanionic derivative of dextran, and its modeling is relatively simple compared with other animal models of UC. Its symptoms, signs and pathological features are basically consistent with human UC (Glick et al., 2020), therefore the DSS-induced UC mice models are quite suitable to study the inflammatory mechanism and evaluate the effects of anti-inflammatory drugs. It is well known that unsaturated fatty acids have a prominent role in anti-inflammatory and immune regulation, and they can be obtained from natural resources, so more and more researchers are paying attention to them. Belluzziet et al. studied the effect of fish oil on IBD and observed fish oil had an anti-inflammatory influence and could reduce the frequency of IBD recurrence (Belluzzi et al., 1996). ALA alleviated the UC induced by DSS through inhibiting colon injury (Kim et al., 2020). The intake of DHA and EPA reduced the risk of UC in mice induced by DSS, and EPA was more effective for the treatment of UC than DHA (Zhang et al., 2021). Therefore, in the current work, the efficiency of NA in colitis mice induced by DSS was evaluated with EPA as a reference. The results showed that NA treatment (100 mg/kg body weight) ameliorated UC symptoms, increased colonic length, DAI, body weight, and histological scores as effectively as EPA and DEX in DSS-induced mice critically, which are primary parameters for assessing the severity of UC. NA also notably hindered the expression or activity of COX-2, iNOS, IL-1β, TNF-α, MPO and IL-6 and increased the expression of IL-10 in DSS-induced mice. Moreover, similar effects of NA on the enhancement of anti- inflammatory cytokine and the expressional inhibition of pro-inflammatory cytokines were observed *in vitro*. These results indicated that NA supplementation decreased the gut inflammation related to UC effectively.

A previous work has suggested that the UC pathogenesis is mainly concentrated in the colonic mucosa or submucosa (Guo et al., 2019). Therefore, it is speculated that a scaffold will exist to keep the gut stable and tightly connected to function effectively. This is called cell TJ, and it is made up of a variety of proteins, of which ZO-1 is the most important. The tightly connected transmembrane structures allow cells to join together precisely and efficiently to protect the mucosal barrier (Otani et al., 2020). In the present study, the NA treatment clearly repaired the damaged intestinal barrier in mice induced by DSS. Regulation of apoptosis might also be an important therapeutic goal in UC (Ungaro

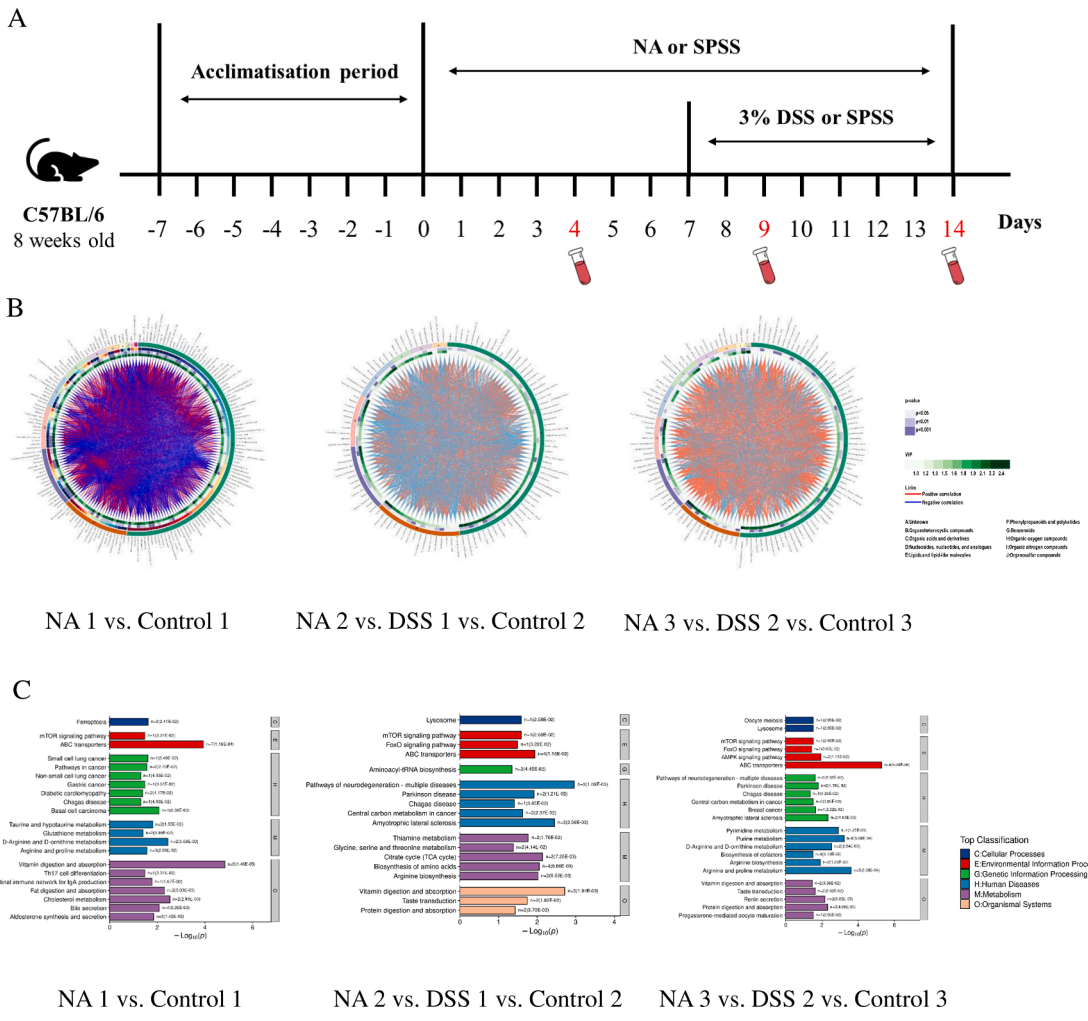


Fig. 9. The key differential metabolites and KEGG pathway analysis.

et al., 2017). It was also found that NA played an essential role in sustaining the mucosal barrier integrity through restraining the apoptosis of intestinal epithelial cells, which was judged by the ratio change of Bcl-2/Bax, thus regulating gut mucosal inflammation. However, the molecular mechanisms of NA promoting ZO-1 expression and inhibiting apoptosis in the intestine have not been elucidated precisely. Thus, further studies are greatly needed.

TLRs belong to the PRRs family and are powerful molecular modulators. TLR4, one member of TLRs, plays an important role in the process of inflammatory development, which recognizes and binds to LPS and activates the MyD88-mediated signaling pathway (Molteni et al., 2016; Yang et al., 2016). The MyD88-dependent pathway involves the activation of IκB kinase (IKK) and MAPKs.IKK and MAPKs complexes induce the translocation and activation of transcription factors including Activator Protein-1 (AP-1) and NF-κB in the nucleus and promote the yield of pro-inflammatory cytokines (Li et al., 2017; Yeom et al., 2015). TLR4/NF-κB signaling is a key pathway to regulate immune inflammatory response, which participates in the progression of many inflammatory diseases. According to previous studies, a large number of immune inflammatory mediators released by activation of TLR4/NF-κB signaling pathway are closely associated with the happening and progress of UC (Chen et al., 2021; Kim et al., 2010). DSS enhances the expression of pathogen-recognition receptor TLR4, phosphorylates IκBα (NF-κB inhibitor) through MyD88-dependent pathway, and is degraded by ubiquitination protease, which will force the release of NF-κB dimer, expose the nuclear sequence of p65, and promote the release of inflammatory factors (Qu et al., 2021). This research found that NA impeded the expression level of TLR4, and activated the transcription of the gene encoding the self-suppressing inhibitor IκBα significantly induced by NF-κB. The newly synthesized IκBα entered the nucleus where it dissociated NF-κB dimer from DNA, and then it is released from the nucleus. Treatment with NA counters colitis induced by DSS through inhibiting the NF-κB pathway activation *in vivo*. Additionally, NA

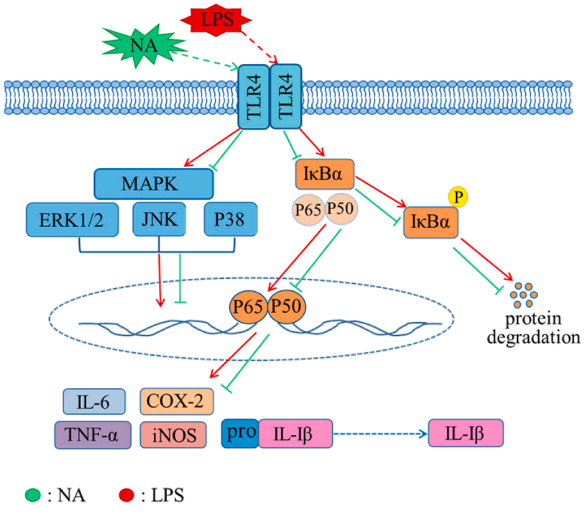


Fig. 10. The molecular mechanisms on the anti-inflammatory action of NA.

treatment reduced the nuclear translocation of NF-κB-p65 in macrophages stimulated by LPS *in vitro*. These results explicitly demonstrated that NF-κB played a significant role in the anti-inflammation and UC-protective effect of NA. The anti-inflammation mechanisms of NA were demonstrated in Fig. 10.

In the metabolomic study, the analysis of differential metabolites in the blood of mice at different stages found that these metabolites were primarily associated with the metabolism of amino acids, pyrimidines and purines. Amino acids play a key role in life activity, providing materials and energy for numerous biosynthesis processes in mice (Jha et al., 2015; Zhang et al., 2018). It was concluded that the treatment with histidine, glycine and arginine alleviated the inflammation of colitis, and the metabolites of histidine and arginine promoted the growth of body and muscles (Gallagher et al., 2021; Bao et al., 2017). Conversely, less abilities of anti-oxidation and anti-inflammation was demonstrated by lower levels of these amino acids or their intermediate metabolites in UC. These metabolites were remarkably affected by NA treatment, suggesting that the inflammation of UC might be alleviated by NA through enhancing the capacity of anti-oxidation and energy supply and maintaining the balance of amino acids metabolism. As the hub coupled with amino acid, lipid and carbohydrate metabolism, citrate cycle is the most effective approach to obtain energy through oxidizing sugar and other substances (Ryan et al., 2020). When colitis occurred, citrate cycle was remodeled and an intracellular metabolic intermediate was accumulated (Williams et al., 2018). Interestingly, the core metabolites in citrate cycle enhanced to the normal level with the NA treatment. Therefore, it is suggested that NA has a role of increasing energy supply in DSS-induced UC and repairing the metabolic imbalance in citrate cycle. Purine metabolism, which is related to amino acid metabolism *via* purine nucleotide cycle (Wu et al., 2020), plays a key role in metabolism regulation, coenzyme production, and energy supply, suggesting that NA may modulate purine metabolism to protect the mice from the colitis. These studies suggest that the metabolism of amino acids, pyrimidines and purines are obviously affected by NA treatment, which could contribute to amelioration UC in DSS-induced mice. However, the linking of ameliorated metabolism caused by NA treatment, inflammation and UC *in vivo* needs to be clearly stated to evaluate the anti-UC effect of NA comprehensively and thoroughly.

Conclusion

This is the first study demonstrating that NA has a protective effect on colitis induced by DSS in mice. Such therapeutic effect of NA is predominantly associated with the inhibition on NF-κB activity and the expressional prevention of pro-inflammatory cytokines in macrophages, intestinal and/or other immune cells. In addition, NA was found to protect the integrity of gut barrier *via* regulating the expression of TJ proteins for the first time. In view of the shortcomings of current treatments for UC, there is an urgent need for safer and more effective palliative measures. . Collectively, this work could supply a theoretical basis for developing effective functional products ameliorating intestinal inflammation using NA, a natural and safe food resource with good anti-inflammatory and immune activities.

Author contributions

All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

CRediT authorship contribution statement

Sheng-Nan Yuan: Data curation, Writing – review & editing. **Muxuan Wang:** Data curation, Writing – review & editing. **Jin-Long Han:** Visualization, Conceptualization, Writing – review & editing. **Cai-Yun Feng:** Visualization, Conceptualization, Writing – review & editing. **Meng Wang:** . **Min Wang:** Visualization, Conceptualization, Writing – review & editing. **Jin-Yue Sun:** Formal analysis, Writing – review & editing. **Ning-yang Li:** Formal analysis, Writing – review & editing. **Jesus Simal-Gandara:** Conceptualization, Formal analysis, Supervision, Writing – review & editing. **Chao Liu:** Conceptualization, Formal analysis, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that there are no financial conflicts of interest in regard to this work.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2021YFE0109200); the Provincial Major Scientific and Technological Innovation Project of Shandong, China (NO. 2022TZXD0029; 2022TZXD0032; 2021SFGC0904; 2021TZXD004); the Natural Science Foundation of Shandong, China (No. ZR2020MH401; ZR2021QH351); National Wheat Industry Technology System of China (No. CARS-03–22); the Taishan Scholars's Program of Shandong for Jin-Yue Sun. Funding for open access charge: Universidade de Vigo/CISUG.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2023.154702.

References

Bao, X., Feng, Z., Yao, J., Li, T., Yin, Y., 2017. Roles of dietary amino acids and their metabolites in pathogenesis of inflammatory bowel disease. Mediat. Inflamm. 2017, 6869259.

Belluzzi, A., Brignola, C., Campieri, M., Pera, A., Boschi, S., Miglioli, M., 1996. Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. NEJM. 334 (24), 1557–1560.

Bergemalm, D., Andersson, E., Hultdin, J., Eriksson, C., Rush, S.T., D'Amato, M., Gomollon, F., Jahnsen, J., Ricanek, P., Satsangi, J., Repsilber, D., Karling, P., Halfvarson, J., 2021. Systemic inflammation in preclinical ulcerative colitis. Gastroenterology 161 (5), 1526–1539.

Borish, L.C., Steinke, J.W., 2003. Cytokines and chemokines, Allergy. J. Clin. Immun. 111 (2), S460–S475.

Bruning, E.E., Coller, J.K., Wardill, H.R., Bowen, J.M., 2021. Site-specific contribution of toll-like receptor 4 to intestinal homeostasis and inflammatory disease. J. Cell. Physiol. 236 (2), 877–888.

Chen, Q., Li, J., Yu, X., Gao, J.M., 2019a. A mini review of nervonic acid: source, production, and biological functions. Food Chem. 301, 125286.

Chen, S., Liu, H., Li, Z., Tang, J., Huang, B., Zhi, F., Zhao, X., 2021. Epithelial PBLD attenuates intestinal inflammatory response and improves intestinal barrier function by inhibiting NF-κB signaling. Cell. Death. Dis. 12 (6), 563.

Fan, Y., Meng, M., Hu, H.G.R., Li, F.L., 2018. Biosynthesis of nervonic acid and perspectives for its production by microalgae and other microorganisms. Appl. Microbiol. Biot. 102 (7), 3027–3035.

Gallagher, K., Catesson, A., Griffin, J.L., Holmes, E., Williams, H., 2021. Metabolomic analysis in inflammatory bowel disease: a systematic review. J. Crohns. Colitis. 15 (5), 813–826.

Glick, L.R., Cifu, A.S., Feld, L., 2020. Ulcerative colitis in adults. JAMA. 324 (12), 1205–1206.

Guo, C., Wu, K., Liang, X., Liang, Y., Li, R., 2019. Infliximab clinically treating ulcerative colitis: a systematic review and meta-analysis. Pharmacol. Res. 148, 104455.

Hirten, R.P., Sands, B.E., 2021. New therapeutics for ulcerative colitis. Annu. Rev. Med. 72, 199–213.

Hu, D., Cui, Y., Zhang, J., 2021. Nervonic acid amends motor disorder in a mouse model of Parkinson's disease. Transl. Neurosci. 12 (1), 237–246.

Hug, H., Mohajeri, M.H., La Fata, G., 2018. Toll-like receptors: regulators of the immune response in the human gut. Nutrients 10 (2), 203.

Jha, A.K., Huang, S.C., Sergushichev, A., Lampropoulou, V., Ivanova, Y., Loginicheva, E., Chmielewski, K., Stewart, K.M., Ashall, J., Everts, B., Pearce, E.J., Driggers, E.M., Artyomov, M.N., 2015. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarisation. Immunity 42 (3), 419–430.

Keppley, L., Walker, S.J., Gademsey, A.N., Smith, J.P., Keller, S.R., Kester, M., Fox, T.E., 2020. Nervonic acid limits weight gain in a mouse model of diet-induced obesity. FASEB J. 34 (11), 15314–15326.

Kim, E.K., Choi, E.J., 2010. Pathological roles of MAPK signaling pathways in human diseases. Biochim. Biophys. Acta. 1802 (4), 396–405.

Kim, J., Ahn, M., Choi, Y., Kang, T., Kim, J., Lee, N.H., Kim, G.O., Shin, T., 2020. Alpha-linolenic acid alleviates dextran sulfate sodium-induced ulcerative colitis in mice. Inflammation 43 (5), 1876–1883.
Kuwada, T., Shiokawa, M., Kodama, Y., Ota, S., Seno, H.J.G., 2021. Identification of an anti-integrin αvβ6 autoantibody in patients with ulcerative colitis. Gastroenterology 160 (7), 2383–2394.
Laura, R.G., Adam, S.C., Lauren, F., 2020. Ulcerative colitis in adults. JAMA. 324 (12), 1205–1206.
Leppkes, M., Neurath, M.F., 2020. Cytokines in inflammatory bowel diseases-Update 2020. Pharmacol. Res. 158, 104835.
Lewkowicz, N., Piątek, P., Namiecińska, M., Domowicz, M., Bonikowski, R., Szemraj, J., Przygodzka, P., Stasiołek, M., Lewkowicz, P., 2019. Naturally occurring nervonic acid ester improves myelin synthesis by human oligodendrocytes. Cells 8 (8), 786.
Li, C., Chen, Y., Zhu, H., Zhang, X., Han, L., Zhao, Z., Wang, J., Ning, L., Zhou, W., Lu, C., Xu, L., Sang, J., Feng, Z., Zhang, Y., Lou, X., Bo, X., Zhu, B., Yu, C., Zheng, M., Li, Y., Shen, Z., 2020. Inhibition of histone deacetylation by ms-275 alleviates colitis by activating the vitamin D receptor. J. Crohns. Colitis. 14 (8), 1103–1118.
Li, Y., He, S., Tang, J., Ding, N., Chu, X., Cheng, L., Ding, X., Liang, T., Feng, S., Rahman, S.U., Wang, X., Wu, J., 2017. Andrographolide inhibits inflammatory cytokines secretion in LPS-stimulated RAW264.7 cells through suppression of NF-κB/MAPK signaling pathway. Evid. Based Complement. Altern. Med. 2017, 8248142.
Luo, H., Cao, G., Luo, C., Tan, D., Vong, C.T., Xu, Y., Wang, S., Lu, H., Wang, Y., Jing, W., 2022. Emerging pharmacotherapy for inflammatory bowel diseases. Pharmacol. Res. 178, 106146.
Marton, L.T., Goulart, R.A., Carvalho, A., Barbalho, S.M., 2019. Omega fatty acids and inflammatory bowel diseases: an overview. Int. J. Mol. Sci. 20 (19), 4851.
Molteni, M., Gemma, S., Rossetti, C., 2016. The role of toll-like receptor 4 in infectious and noninfectious inflammation. Mediat. Inflamm. 2016, 6978936.
Otani, T., Furuse, M., 2020. Tight junction structure and function revisited. Trends. Cell. Biol. 30 (10), 805–817.
Qu, Y., Li, X., Xu, F., Zhao, S., Wu, X., Wang, Y., Xie, J., 2021. Kaempferol alleviates murine experimental colitis by restoring gut microbiota and inhibiting the LPS-TLR4-NF-κB axis. Front. Immunol. 12, 679897.
Ramani, T., Auletta, C.S., Weinstock, D., Mounho-Zamora, B., Ryan, P.C., Salcedo, T.W., Bannish, G., 2015. Cytokines: the good, the bad, and the deadly. Int. J. Toxicol. 34 (4), 355–365.
Ryan, D.G., O'Neill, L., 2020. Krebs cycle reborn in macrophage immunometabolism. Annu. Rev. Immunol. 38, 289–313.
Ungaro, R., Mehandru, S., Allen, P.B., Peyrin-Biroulet, L., Colombe, l J.F., 2017. Ulcerative colitis. Lancet 389 (10080), 1756–1770.
Vipul, Y., Felipe, V., Roberto, B., Esther, F., Daniela, B., Abdul, W.B., 2016. Inflammatory bowel disease: exploring gut pathophysiology for novel therapeutic targets. Transl. Res. 176, 38–68.
Williams, N.C., O'Neill, L., 2018. A role for the krebs cycle intermediate citrate in metabolic reprogramming in innate immunity and inflammation. Front. Immunol. 9, 141.
Williams, L.M., Gilmore, T.D., 2020. Looking down on NF-κB. Mol. Cell. Biol. 40 (15), e00104–e00120.
Wu, J., Wei, Z., Cheng, P., Qian, C., Xu, F., Yang, Y., Wang, A., Chen, W., Sun, Z., Lu, Y., 2020. Rhein modulates host purine metabolism in intestine through gut microbiota and ameliorates experimental colitis. Theranostics 10 (23), 10665–10679.
Yamazaki, Y., Kondo, K., Maeba, R., Nishimukai, M., Nezu, T., Hara, H., 2014. Proportion of nervonic acid in serum lipids is associated with serum plasmalogen levels and metabolic syndrome. J. Oleo. Sci. 63 (5), 527–537.
Yang, Y., Lv, J., Jiang, S., Ma, Z., Wang, D., Hu, W., Deng, C., Fan, C., Di, S., Sun, Y., Yi, W., 2016. The emerging role of Toll-like receptor 4 in myocardial inflammation. Cell. Death. Dis. 7 (5), e2234.
Yang, Y., Weng, W., Peng, J., Hong, L., Yang, L., Toiyama, Y., Gao, R., Liu, M., Yin, M., Pan, C., Li, H., Guo, B., Zhu, Q., Wei, Q., Moyer, M.P., Wang, P., Cai, S., Goel, A., Qin, H., Ma, Y., 2017. Fusobacterium nucleatum increases proliferation of colorectal cancer cells and tumor development in mice by activating toll-like receptor 4 signaling to nuclear factor-κB, and up-regulating expression of microRNA-21. Gastroenterology 152 (4), 851–866 e24.
Yeom, M., Kim, J.H., Min, J.H., Hwang, M.K., Jung, H.S., Sohn, Y., 2015. Xanthii fructus inhibits inflammatory responses in LPS-stimulated RAW 264.7 macrophages through suppressing NF-κB and JNK/p38 MAPK. J. Ethnopharmacol. 176, 394–401.
Zhang, C., Jiang, M., Lu, A., 2013. Considerations of traditional Chinese medicine as adjunct therapy in the management of ulcerative colitis. Clin. Rev. Allerg. Immu. 44 (3), 274–283.
Zhang, X.J., Yuan, Z.W., Qu, C., Yu, X.T., Huang, T., Chen, P.V., Su, Z.R., Dou, Y.X., Wu, J.Z., Chen, J.N., 2018. Palmatine ameliorated murine colitis by suppressing tryptophan metabolism and regulating gut microbiota. Pharmacol. Res. 137, 34–46.
Zhang, Z., Xue, Z., Yang, H., Zhao, F., Liu, C., Chen, J., Lu, S., Zou, Z., Zhou, Y., Zhang, X., 2021. Differential effects of EPA and DHA on DSS-induced colitis in mice and possible mechanisms involved. Food Funct. 12 (4), 1803–1817.