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L-Fucose ameliorates DSS-induced acute colitis *via* inhibiting macrophage M1 polarization and inhibiting NLRP3 inflammasome and NF-kB activation

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ABSTRACT

Previous studies reported that L-fucose had anti-inflammatory effects in respiratory and cutaneous system. However, the effect of L-fucose on colitis and the underlying mechanism is poorly understood. We studied the anti-inflammatory effects of L-fucose on Dextran sulfate sodium (DSS)-induced acute colitis *in vivo* and on LPS/ATP-induced bone marrow derived macrophages (BMDMs) damage *in vitro*. Our results show that L-fucose significantly alleviated weight loss and disease activity index (DAI) scores in colitis and reduced the infiltration of macrophages and neutrophils. In addition, L-fucose can inhibit macrophage M1 polarization, inactivate the NLRP3 inflammasome and reduce the release of TNFα, IL1β, IL6 pro-inflammatory cytokines. *In vitro* studies showed that L-fucose ameliorated cell damage resulting from the administration of LPS with ATP in BMDMs, inhibited NLRP3 inflammasome activation and reduced the release of corresponding pro-inflammatory cytokines. Finally, L-fucose can inhibit the expression of p-NF-kB *in vivo* and *in vitro*. Overall, our results show that L-fucose can attenuate colitis by inhibiting macrophage M1 polarization, inhibiting NLRP3 inflammasome and NF-kB activation, and down-regulation of pro-inflammatory cytokines.

1. Introduction

Ulcerative colitis (UC) is an idiopathic inflammatory bowel disease (IBD) characterized by chronic and relapsing inflammation in the gastrointestinal tract [1,2]. Although the precise pathological mechanism of UC remains elusive, it is widely accepted that disruption of the epithelial barrier causes invasion of bacterial antigens into the mucosal layer, leading to activation of pathogenic mucosal immune system that ultimately misdirects the immune responses to finally attack the colon [3–5]. Dextran sulfate sodium (DSS)-induced colitis is frequently used to elucidate molecular and cellular pathways involved in the pathogenesis of acute colitis because of model simplicity and high similarities with human UC [6,7]. DSS-induced colitis has been considered to be driven by activated intestinal macrophages which release proinflammatory cytokines and chemokines, cause tissue damage, and induce migration of neutrophils and dendritic cells (DCs) in the colon [8].

NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome is a large protein complex consisting of NLRP3, the adaptor apoptosis-associated speck-like protein containing a CARD domain (ASC), and pro-caspase-1 [9], predominantly activated in macrophages [10]. Activation of NLRP3 inflammasome results in the activation of caspase-1 [11], and release of mature IL1β [12], which participates in the generation of systemic and local responses to infection by activating lymphocytes and promoting leukocytes infiltration at sites of injury or infection [13]. It was reported that NLRP3 gene polymorphism was associated with a significantly increased risk of developing IBD in humans [14].

Nuclear factor-kappa B (NF-kB), a key transcription factor, promotes transcription of the genes encoding proinflammatory cytokines, such as TNFα, IL1β and IL6, which are considered to be related to the development and pathogenesis of UC [15]. Furthermore, activation of NF-kB induces the transcription of NLRP3 [16]. The activation of NF-kB was founded in mucosal macrophages and colonic epithelial cells of IBD patients [17]. These suggest that both NF-kB and NLRP3 axis could be potential targets for the development of new treatments for patients with inflammatory bowel disease.

L-Fucose is a natural monosaccharide present in mammals, it is now widely used as food additive and shows no adverse effect [18,19]. The anti-inflammatory effects of L-Fucose were mostly noted and attracted scholar's attention. Keiko et al. had previously proven that under oxidative environment, L-Fucose could block the priming of alveolar macrophages [20]. Another study demonstrated that L-Fucose can inhibit the cutaneous immune reaction [21]. Furthermore, free L-Fucose can be utilized by fucosyltransferases, which play an important role in immune cell development, including macrophage polarization and function regulation [22–24]. However, the effect of L-Fucose in

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gastroenterology has not been explored at present.

Therefore, we assessed the potential of L-fucose on the development and progression of DSS-induced acute colitis. We demonstrate that L-fucose ameliorates clinical symptoms and histological features of DSS-induced colitis *via* inhibiting NF-kB and NLRP3 inflammasome activation in macrophage *in vivo* and *in vitro*.

2. Materials and methods

2.1. Animal study protocol

Male C57BL/6 mice (aged 6 weeks, weighed 18–22 g) were purchased from Beijing Huafukang Biosciences Co., Ltd. (Beijing, China). They were housed in specific pathogen-free conditions with a fixed 12 h light/dark cycle at 23 °C. Mice had free access to water and food during the experiment. All experimental procedures were performed in accordance with the ethical guidelines of the animal Management Rules of the Chinese Ministry of Health (Document No.55,2001), and approved by the Animal Care and Use Committee, Union Hospital, Tongji Medical College, HUST, China (Approval ID 2016-0057).

2.2. Experimental design

Mice were randomly divided into three groups (n = 6 mice per group): (1) control group, (2) DSS-induced colitis group, (3) DSS-induced colitis mice treated with L-fucose. Acute colitis was induced by administration of 3% (w/v) DSS (MW 36,000–50,000 Da; MP Biomedicals, Solon, OH, USA) in drinking water for one DSS cycle and then followed by 3 days with normal drinking water. L-Fucose (250 mg/kg), dissolved in saline, was given to mice by gavage once a day for 10 days. In the control and DSS groups, the same volume of saline was added by gavage as vehicle control. All mice were sacrificed after 10 days. Tissues and blood were collected for subsequent analysis.

During the course of the experiment, the body weight, stool consistency and bleeding were measured every day to assess the disease activity index (DAI) [25]. Briefly, DAI scores were defined as follows, for weight loss, 0: no loss; 1: 1–5% loss; 2: 5–10% loss; 3: 10–20% loss and 4: > 20% weight loss; for stool consistency, 0: normal; 2: loose stool; 4: diarrhea; and for stool bleeding, 0: no blood; 2: presence and 4: gross blood.

2.3. Histological analysis

For histological analysis, distal colon specimens were fixed in 4% formalin for 24 h and embedded in paraffin, stained with hematoxylin and eosin (H&E), and then analyzed by a pathologist without prior knowledge of experimental procedures. The histological analysis was performed as previously described [26]. In brief, the sections were graded as to inflammation severity, inflammation extent and crypt damage as shown in Table 1, scores were calculated by multiplying the score for these grades by their percentage involvement, giving a minimum score of 0 and a maximum score of 40.

2.4. Immunofluorescence staining

Paraffin section of colonic tissues (5 μm) were dewaxed and rehydration and conducted antigen heat retrieval in citrate buffer followed by blocking with 10% donkey serum for 30 min at room temperature. Next, sections were incubated with rabbit anti-F4/80 (1:200, Abcam, Cambridge, MA, USA) primary antibodies overnight at 4 °C. After washing with PBS for 3 times, slides were stained with Alexa Fluor 488 conjugated donkey anti-rabbit (Antgene Biotech Co., Ltd. Wuhan, China) secondary antibodies for 1 h at room temperature. Sections were washed three times and nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) for 10 min at room temperature. Images were captured using confocal laser scanning microscope (Olympus, Tokyo, Japan).

Table 1
Histological colitis scoring method.

Feature scored	Score	Description
Inflammation severity	0	None
	1	Mild
	2	Moderate
	3	Severe
Inflammation extent	0	None
	1	Mucosa
	2	Mucosa and sub-mucosa
	3	Transmural
Crypt damage	0	None
	1	Basal 1/3 damaged
	2	Basal 2/3 damaged
	3	Crypts lost; surface epithelium present
	4	Crypts and surface epithelium lost
Percent involvement (%)	0	0
	1	1–25
	2	26–50
	3	51–75
	4	76–100

2.5. Preparation of colonic lamina propria mononuclear cells (LPMCs)

Colons were dissected, carefully cleaned with ice-cold PBS and cut into large fragments (0.5–1 cm). Colon fragments were placed in 40 ml of epithelial cell dissociation solution [Ca2 + and Mg2 + free PBS with 5 mM EDTA (Sigma-Aldrich) and 1 mM dithiothreitol (Roche)] and shaken at 100 g for 30 min at 37 °C. The fragments were then collected and digested in RIPM 1640 supplemented with 1 mg/ml Collagenase IV (Roche) at 37 °C, shaken at 80 g for 1 h. The cell suspension was passed through a 70 μm filter and then placed over a 70 μm nylon mesh and centrifuged at 600*g* for 5 min. Filtered cells were layered on a Percoll gradient (40%–75%) and centrifuged at 1000*g* for 20 min. The separated colon-infiltrating leucocyte fraction was collected and re-suspended in PBS for subsequent flow cytometry analysis.

2.6. Flow cytometry analysis of LPMCs

The colonic LPMCs were incubated for 40 min at 4 °C with the fluorophore-conjugated primary antibodies anti-mouse F4/80-PE (BD biosciences, New Jersey, USA), anti-mouse CD11b-APC (eBioscience, Thermo Fisher Scientific, USA), anti-mouse CD11c-FITC (BD biosciences, New Jersey, USA), anti-mouse Ly6G-PECY7 (eBioscience, Thermo Fisher Scientific, USA) for analysis of infiltration of macrophages, neutrophils and dendritic cells.

For macrophage polarization analysis, the colonic LPMCs were stained with anti-mouse F4/80-PE (BD biosciences, New Jersey, USA), anti-mouse CD206-FITC (eBioscience, Thermo Fisher Scientific, USA) and anti-mouse CD 11c-APC (eBioscience, Thermo Fisher Scientific, USA). Cells were washed twice with cold PBS and then analyzed on a BD Flow Cytometry (BD Pharmingen, San Diego, CA, USA). Data were analyzed by FlowJo V10 software (Tree Star, Ashland, OR).

2.7. Preparation of primary bone marrow derived macrophages (BMDMs)

The isolation and culture of BMDMs were performed as previous reported [27]. Briefly, mice were anesthetized and sacrificed and soaked in 75% ethanol for 5 min. Femurs were harvested and cleaned of tissue, bone marrow were flushed out with PBS by inserting a 1 ml sterile syringe and collected by centrifugation (310*g*, 5 min). Cells were suspended in high glucose DMEM containing 10% FBS [(Gibco® Cell Culture, Melbourne, VIC, Australia) with 1% penicillin and streptomycin (Gibco, Carlsbad, CA, USA), and 20 ng/ml of M-CSF (R&D Systems, Inc. Minneapolis, MN, USA)], and plated in different plates

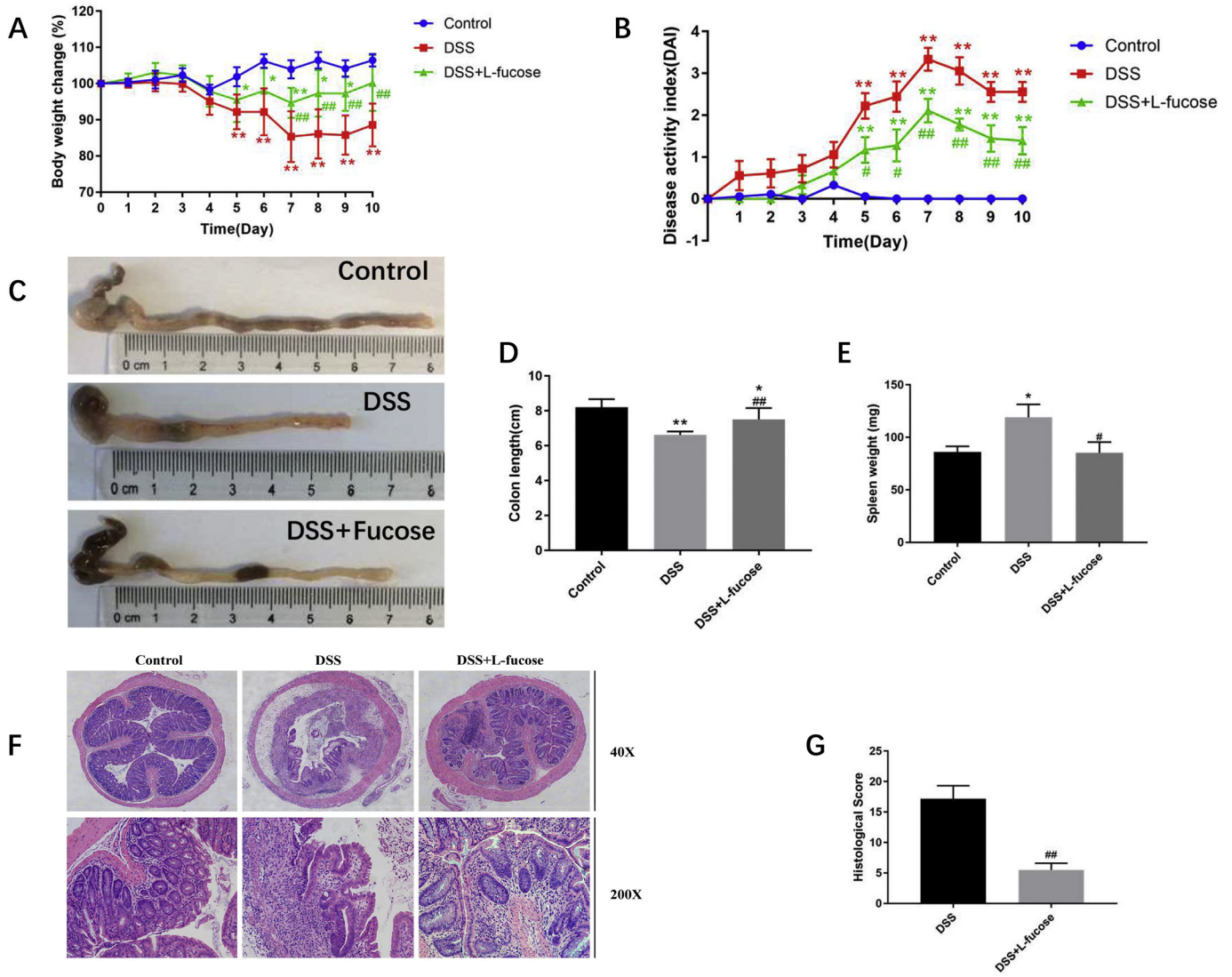

Fig. 1. L-Fucose ameliorated DSS-induced acute colitis. Colitis was induced by orally administrated 3% DSS from day 1 to day 7. L-Fucose was given by gavage each day from day 1 to day 10, mice were sacrificed on day 10. (A) Body weight change during the disease process. (B) Disease activity index (DAI) evaluations of mice. (C, D) The length of colons from each group of mice. (E) Weight of spleen from each group of mice. (F) Serial sections from colon tissue stained with H&E. (G) Histopathological (HAI) scores of colons from mice with DSS-induced colitis. This experiment was repeated three times with similar results. The results are expressed as mean ± SEM (n = 6 per group). The paired *t*-test was used for comparisons of the histological scores, and the other data were analyzed by analysis of variance with the LSD *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the DSS group. The data come from three independent experiments. DSS, dextran sulfate sodium; H&E, hematoxylin and eosin.

according to experiment protocols. After 3 days, 1 ml of fresh culture media were added to each well and on day 7, cells were washed with PBS and fresh differentiation medium was added for further experiments. The identification and purity were determined by microscope observation and FACS analysis using antibody anti-mouse F4/80-PE and anti-mouse CD11b-PerCP CY5.5 (Supplementary Fig. 1).

2.8. ELISA analysis

Serous and cell-free supernatants concentrations of TNFα, IL1β and IL6 were measured by enzyme-linked immune sorbent assay (ELISA) (eBioscience, Thermo Fisher Scientific, USA) according to the manufacturer's manuals. The absorbance was obtained at relative nanometer wavelength using a microplate reader (Biotek Instruments, Inc., Winooski, VT, USA).

2.9. RNA extraction and quantitative real-time PCR (qRT-PCR)

The total RNA was isolated from colonic tissues using Trizol reagent (Life Technologies, Carlsbad, USA). The reverse transcription (cDNA) was synthesized from 1 μg of total RNA and Prime Script RT Master Mix (Takara Biotechnology, Dalian, China). qRT-PCR was performed using 1 μl first-strand cDNA with the LightCycler® 480 SYBR I Master Mix (Roche, Switzerland), in a final volume of 20 μl. All samples were run in triplicate and underwent 45 amplification cycles in a Roche LightCycler R480 (Roche, Switzerland). The expression level of the relative genes was normalized relative to levels of the housekeeping gene GAPDH and calculated by using the comparative cycle threshold ($2-\triangle\triangle C_t$) method. The primer sequences were as follows:

TNFα forward, 5′-GGTCTGGGCCATAGAACTGA-3′ and reverse, 5′-CAGCCTCTTCTATTCCTGC-3′
IL1β forward, 5′-TTGTTGATGTGCTGCTGTGA-3′ and reverse, 5′-TGTGAAATGCCACCTTTTGA

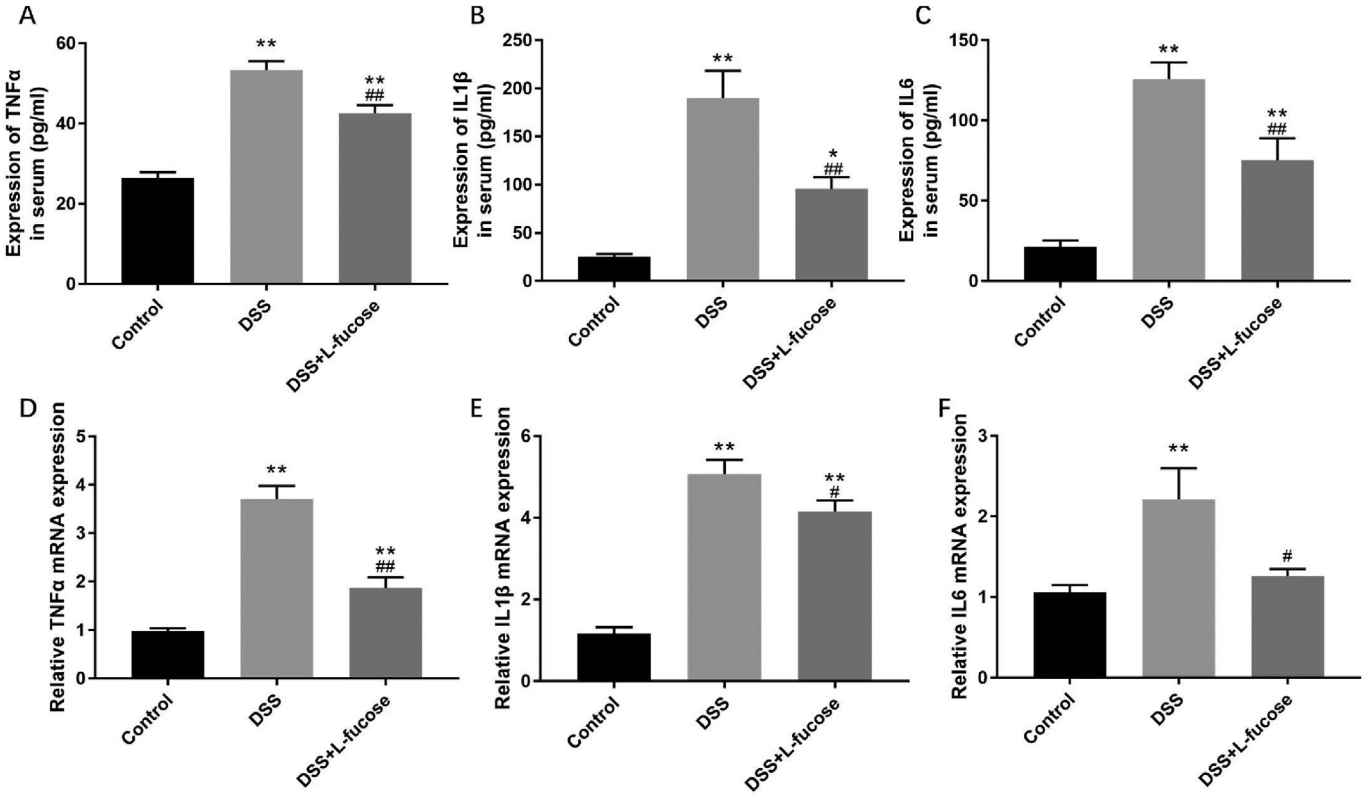

Fig. 2. L-Fucose decreased the serum and colon levels of pro-inflammatory cytokines following DSS exposure. (A–C) The expression of TNFα, IL-1β and IL6 proteins was detected by ELISA and the data were generated from the serum. (D–F) The relative gene expression of TNFα, IL-1β and IL6 was detected by qPCR and the data were generated from colon tissues. Data are expressed as mean ± SEM (n = 6 per group), the results were analyzed by analysis of variance with the LSD *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the DSS group. The data come from three independent experiments.

IL6 forward, 5′-CCGGAGAGGAGACTTCAG-3′ and
reverse, 5′-CAGAATTGCCATTGCACAAC-3′
GAPDH forward, 5′-AGGAGCGAGACCCCACTAACA-3′ and
reverse, 5′-AGGGGGGCTAAGCAGTTGGT-3′.

2.10. Western blot analysis

Proteins were harvested from colon tissues and cells with RIPA Lysis Buffer (Beyotime, Hainan, Jiangsu, China) supplemented with phenylmethyl sulfonyl fluoride (PMSF) protease inhibitor and phosphatase inhibitor. Total protein content in the supernatant were determined by the bicinchoninic acid (BCA) assay, denatured protein samples of appropriate quality of proteins were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then electro transferred to a PVDF membrane (Millipore Corp., MA, USA), proteins on the membrane were immunodetected with specific antibodies for rabbit anti-NLRP3 (15101; dilution, 1:1000; Cell signaling Technology, Massachusetts, USA), rabbit anti-Caspase-1 (ab179515; dilution, 1:1000; Abcam, Cambridge, MA, USA), mouse anti-IL1β (12242; dilution, 1:1000; Cell signaling Technology, Massachusetts, USA), rabbit anti-phospho-NF-kB p65 (3033; dilution, 1:500; Cell signaling Technology, Massachusetts, USA) and GAPDH (A01020; dilution, 1:2000; Abbkine, Inc., Redlands, CA, USA) overnight at 4 °C. Protein bands were visualized by enhanced chemiluminescence (ECL) kit (Beyotime, China).

2.11. Statistical analysis

The SPSS 20.0 and Graph-pad prism software were used for the statistical analysis. Data were presented as mean values ± SEM for independent experiments. For comparison between two groups, a paired *t*-test was performed. Multiple group comparisons were assessed by one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) *post hoc* test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. L-Fucose ameliorated DSS-induced acute colitis

For *in vivo* experiments, preliminary studies were performed to assess whether L-fucose (250 mg/kg) alone had any effect on mice. As shown in Supplementary Fig. 2, group treated only with L-fucose did not show any injury in colon (Sup Fig. 2A). Meanwhile, there was no difference in levels of systemic and colonic pro-inflammatory cytokines TNFα, IL-1β and IL6 between control and L-fucose groups (Sup Fig. 2B–G).

In subsequent studies, a DSS-induced acute colitis mice model was established to assess the effects of L-fucose on colitis. The body weight loss and the DAI score was increased with time and increased continuously in DSS-induced acute colitis mice (Fig. 1A and B) compared with healthy control mice ($p < 0.01$), reaching statistical significance from day 5. Attenuation of body weight loss and a lower DAI score was observed in the L-fucose treated mice ($p < 0.01$). Moreover, as vital markers of colitis, colonic shortening (Fig. 1C and D) and splenomegaly (Fig. 1E) were significantly protected by L-fucose ($p = 0.005$, $p = 0.029$ respectively). Consistent with the remarkable alleviation in clinical signs, L-fucose treated group showed a less glandular defects, mucosal ulceration and inflammatory cell infiltration in the H&E colon sections compared with the damage in the colon of DSS-treated group (Fig. 1F). These results were further confirmed by histological scoring ($p < 0.01$) (Fig. 1G).

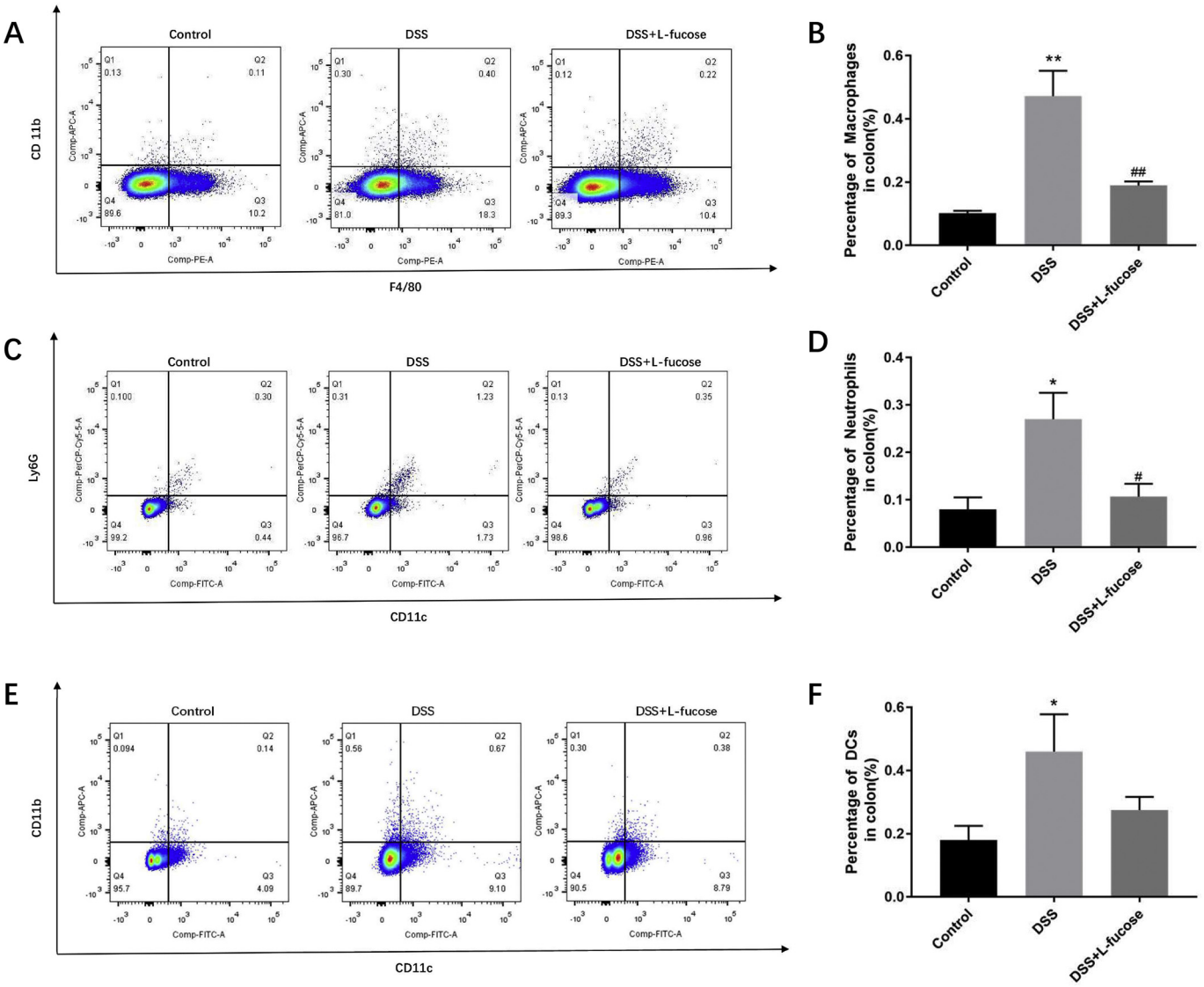

Fig. 3. L-Fucose inhibited inflammatory cell infiltration in the colon tissue. Representative flow cytometric analysis and the percentage of (A–B) macrophages, (C–D) neutrophils, (E–F) DCs in colon lamina propria mononuclear cells. Data are expressed as mean ± SEM (n = 3–4 per group), the results were analyzed by analysis of variance with the LSD *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the DSS group. The data come from three independent experiments.

3.2. L-Fucose decreased the release of pro-inflammatory cytokines in DSS-induced acute colitis

The expressions of cytokines in mice sera were shown in Fig. 2A–C, secretion of TNFα, IL-1β and IL6 in serum was greater in the DSS group than in the control group ($p < 0.01$). These cytokines were significantly decreased by L-fucose ($p < 0.01$).

We further evaluate the expression of TNFα, IL-1β and IL6 by qPCR in colonic tissues. Similarly, mRNA results suggested that TNFα, IL-1β and IL6 levels were higher in the DSS group compared with the control group ($p < 0.01$), but was lower in L-fucose treated group compared with the DSS group (for IL6 $p = 0.011$, all others $p < 0.01$) (Fig. 2D–F).

Collectively, these results demonstrated that L-fucose reduces the release of pro-inflammatory cytokines in DSS induced acute colitis.

3.3. L-Fucose inhibited inflammatory cell infiltration in the colonic tissues

Since pro-inflammatory chemokines production can recruit inflammatory cells into the colonic mucosa, the effect of L-fucose on immune cells was assessed. Fig. 3A–F showed the percentage of colonic macrophages (CD11c-Ly6G-F4/80 + CD11b +), neutrophils (F4/80-CD11c-Ly6G +) and inflammatory DCs (Ly6G-F4/80-CD11c + CD11b +) were higher in DSS group compared with the control group (for macrophages $p < 0.01$, all others $p < 0.05$). L-Fucose revealed a significant inhibition of infiltration of macrophages and neutrophils ($p = 0.004$, $p = 0.019$, respectively). There was also a reduction of inflammatory DCs after L-fucose treatment, but the difference was not significant ($p = 0.06$).

3.4. L-Fucose inhibited macrophage M1 polarization in colitis-induced mice

Given flow cytometry analysis showing declined macrophages infiltration in L-fucose treatment after DSS exposure, and it is well documented that macrophages polarization plays a pivotal role in inflammation progress, we then focused on the effect of L-fucose on colonic macrophages. Immunofluorescence staining suggested that plenty of macrophages (F4/80 +) were infiltrated in the mucosa of the lesion site, L-fucose resulted in fewer macrophages in colon ($p < 0.01$) (Fig. 4A and B). Flow cytometry analysis showed that the percentage of

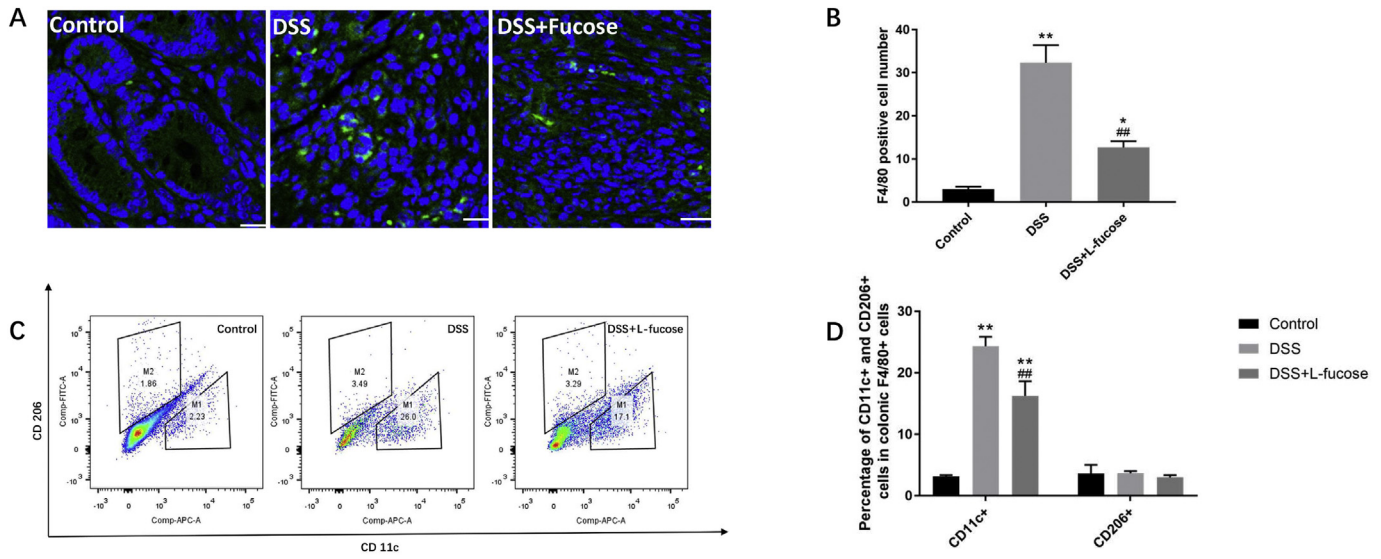

Fig. 4. L-Fucose inhibited macrophages recruitment into colon tissue and inhibited pro-inflammation phenotype M1 macrophages polarization. (A–B) Immunofluorescence of F4/80 + for macrophages (green) and DAPI staining in the colon tissues (Scare bar, 10 μm) (n = 6 per group). (C–D) M1 (F4/80 + CD11c +) and M2(F4/80 + CD206 +) cells were detected by flow cytometry (n = 3–4 per group). The results were analyzed by analysis of variance with the LSD *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the DSS group. The data come from three independent experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

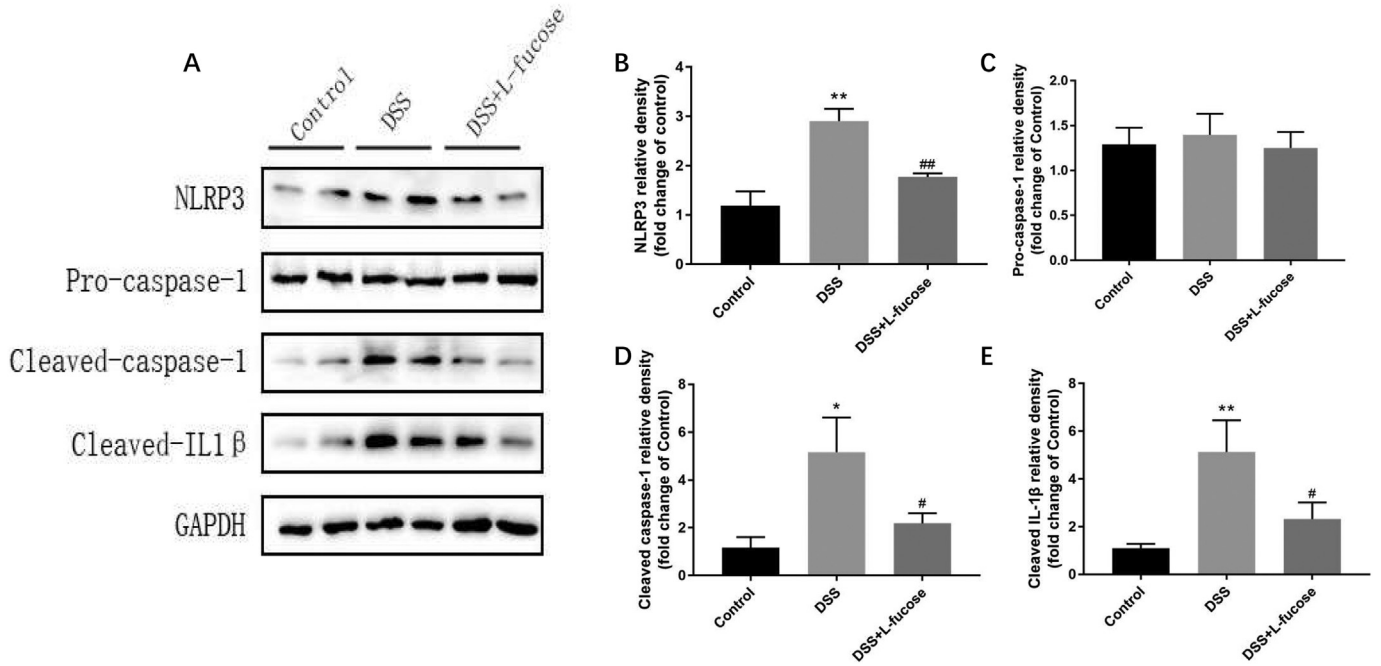

Fig. 5. L-Fucose inhibited the expression of NLRP3 inflammasome protein in the colon tissues. (A) Representative immunoblot bands for the NLRP3, pro-caspase-1, cleaved-caspase-1 and cleaved-IL1β proteins. GAPDH was used as a loading control. (B–E) Histogram of relative expression of NLRP3, pro-caspase-1, cleaved-caspase-1 and cleaved-IL1β (n = 6 per group). The results were analyzed by analysis of variance with the LSD *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the DSS group. The data come from three independent experiments.

M1 (F4/80 + CD11c +) in the colon of DSS-induced group was significantly upregulated as compared to that of control mice ($p < 0.01$), while this upregulation was significantly reduced by L-fucose treatment ($p < 0.01$). However, L-fucose had no effect on macrophages M2 (F4/80 + CD206 +) polarization ($p > 0.05$) (Fig. 4C and D).

3.5. Effect of L-fucose on NLRP3 inflammasome during colitis

The NLRP3 inflammasome is an intracellular complex triggering inflammatory responses during ulcerative colitis, which is predominantly activated in macrophages.

Therefore, we examined the effects of L-fucose on NLRP3 inflammasome complexes. In accordance with previous report [28], the protein expression of NLRP3 increased, followed by increasing level of cleaved-caspase-1 and cleaved IL1β in the DSS group, compared with the control group (for cleaved caspase-1 $p = 0.016$, all others $p < 0.01$) (Fig. 5A–E).

L-Fucose significantly suppressed the protein level of NLRP3, cleaved caspase-1 and cleaved IL1β (for NLRP3 $p < 0.01$, all others $p < 0.05$). Pro-caspase-1 did not change significantly in the three

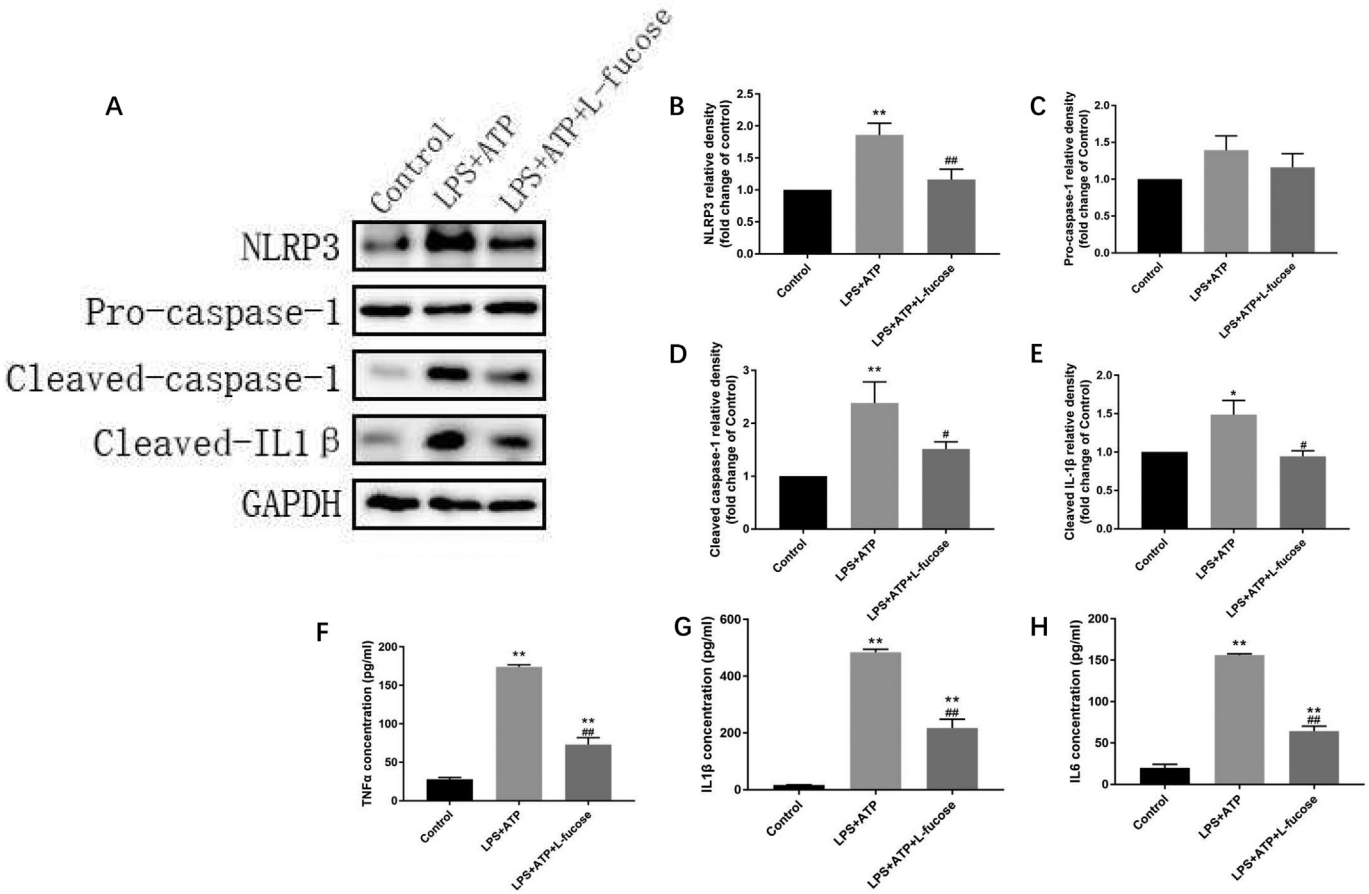

Fig. 6. L-Fucose inhibited NLRP3 inflammasome protein and the secretion of pro-inflammatory cytokines in bone marrow derived macrophages (BMDMs). BMDMs were primed with LPS (2 μg/ml) for 4 h, followed by incubation with ATP (5 mM) for 40 min. L-Fucose (5 mg/ml) was added to BMDMs at the same time with LPS. (A) Representative immunoblot bands for the NLRP3, pro-caspase-1, cleaved-caspase-1 and cleaved-IL1β proteins. GAPDH was used as a loading control. (B–E) Histogram of relative expression of NLRP3, pro-caspase-1, cleaved-caspase-1 and cleaved-IL1β (n = 4 per group). (F–H) The content of TNFα, IL-1β and IL6 in the cell supernatant was detected by ELISA kit (n = 4 per group). The results were analyzed by analysis of variance with the LSD *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the LPS + ATP group. The data come from three independent experiments.

groups ($p > 0.05$).

These results suggest that L-fucose alleviates DSS-induced colitis by suppressing the NLRP3 signaling pathway.

3.6. L-Fucose inhibited the release of pro-inflammatory factors in vitro

In order to have a more comprehensive understanding of the anti-inflammatory effect of L-fucose in macrophages, *in vitro* experiments were performed using BMDMs. The expression of NLRP3, cleaved-caspse-1 and cleaved-IL-1β proteins were significantly increased in LPS + ATP-stimulated BMDMs compared with the control (for IL1β $p = 0.02$, all others $p < 0.01$), while it was significantly decreased with L-fucose compared with LPS + ATP-stimulated group ($p < 0.05$). Pro-caspase-1 did not change significantly ($p > 0.05$) (Fig. 6A–E).

Similarly, ELISA assays of inflammatory cytokines in cell supernatants were also performed and it was found that TNFα, IL6 and IL-1β were significantly lower in the L-fucose treat group than those in the LPS + ATP group ($p < 0.01$) (Fig. 6F–H). The parallel activation of the key proteins and key inflammatory factors are consistent with the *in vivo* experiment.

3.7. L-Fucose inhibited colitis through NF-kB pathway

It is widely accepted that mRNA transcription of inflammatory cytokines depends on NF-kB activation. The priming step of NLRP3 inflammasome activation triggered by the first signal activates NF-kB signaling which induces the transcription of pro-IL1β and NLRP3. Since TNFα, IL6, pro-IL-1β and NLRP3 were up-regulated in the inflamed colonic tissues and profoundly decreased by L-fucose, we assessed the effect of L-fucose on the activity of phosphorylation level of NF-kB p65 (p-NF-kB) protein. The p-NF-kB expression levels were significantly increased in DSS group and in LPS + ATP stimulated group, which were markedly reduced by L-fucose administration ($p < 0.05$) (Fig. 7A–D).

4. Discussion

In the current study, prominent anti-inflammatory effects of L-fucose were demonstrated in a DSS induced acute colitis mouse model and in a BMDM cell-injury model. L-Fucose showed beneficial effects on acute colitis through inhibiting macrophage M1 polarization and inhibiting NF-kB and NLRP3 signaling pathways.

L-fucose, a natural monosaccharide of mammals, is now widely used as food additive [18,19], and has been reported to have anti-inflammatory effects [20,21]. Keiko et al. [20] noted that L-fucose could block alveolar macrophages priming, Baba et al. [21] demonstrated that L-fucose could inhibit skin immune reaction. However, the effects of L-fucose in colitis haven't been explored before. As shown in this study, oral administration of L-fucose significantly attenuates acute colitis measured by the DAI score and histological analysis. These data indicate that L-fucose could be effective as an adjuvant anti-UC approach accompanied with conventional therapy. While an absorption study was not performed to demonstrate the oral bioavailability of L-

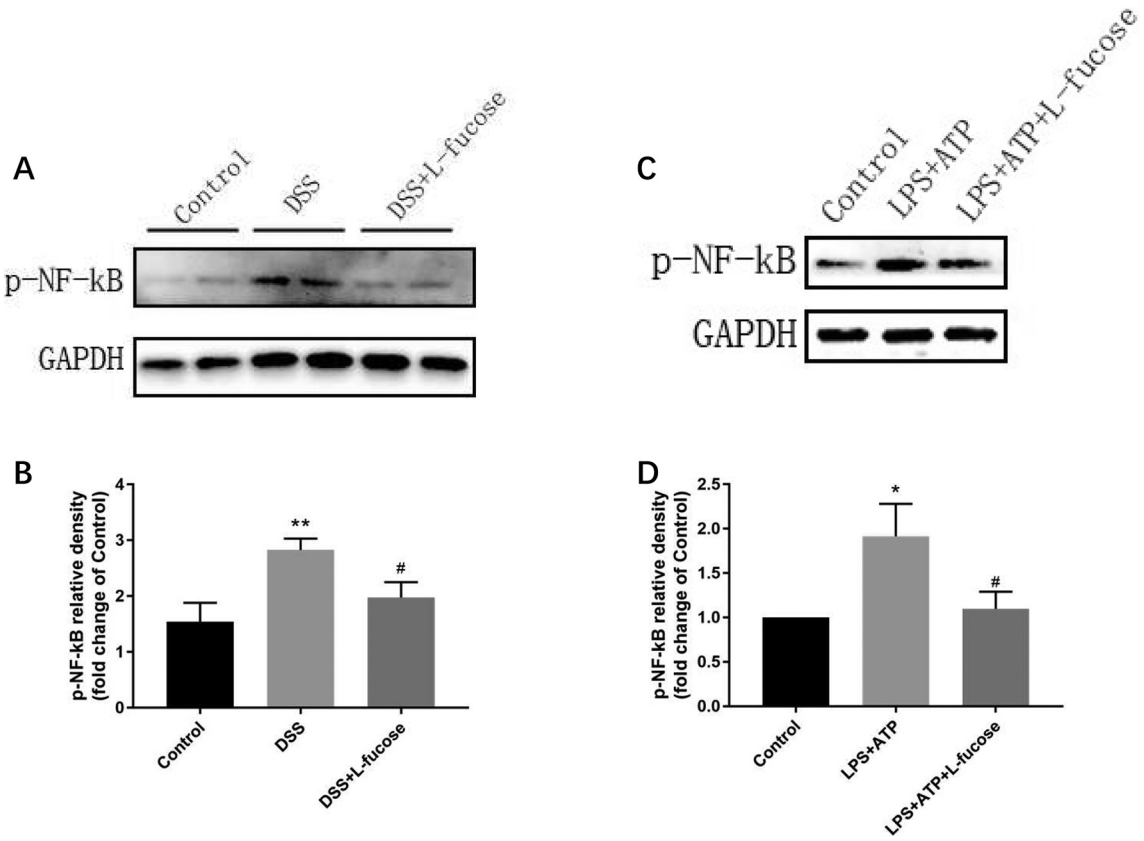

Fig. 7. L-Fucose inhibited the activation of NF-kB *in vitro* and *in vivo*. (A) Representative WB analysis of phospho-NF-kB p65 in colon tissues. (B) Representative WB analysis of p-NF-kB p65 in BMDMs. (C) Histogram of relative expression of p-NF-kB p65 in colon tissues (n = 6 per group). (D) Histogram of relative expression of p-NF-kB p65 in BMDMs (n = 4). The results were analyzed by analysis of variance with the LSD *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the DSS group or LPS + ATP group. The data come from three independent experiments.

fucose in mice, previous study has proven that fucose administrated orally appeared in the blood stream in the rat [29], and oral bioavailability of the compound is strongly supported by the existence of the salvage pathway for L-fucose in humans [30,31]. In addition, oral L-fucose supplementation has led to significant improvement in case studies involving patients with an extremely rare congenital glycosylation disorder known as Leukocyte Adhesion Deficiency Type II [32], suggesting that the L-fucose administration orally would have been absorbed in mice in this study.

The growing studies have shown that the increased levels of proinflammatory cytokines, including TNF-α, IL-1β and IL6 are detected in active IBD and play the key role in the initiation and perpetuation of the intestinal inflammation [15]. TNF-α has been shown to exert various pro-inflammatory functions through inducing hypervascularization and angiogenesis, augmenting pro-inflammatory cytokines production by macrophages and T cells, causing barrier alteration and promoting cell death of intestinal epithelial cells [33] and blockade of tumor necrosis factor (TNF) is now commonly used as a standard therapy for IBD in the clinic [34]. IL-1β is likely to be essential in the early phase of the inflammatory cascade leading to an inflamed colon [35]. There is increased expression and level of IL-1β and IL6 in serum and mucosal biopsies in IBD patients [36,37]. Also, patients' IL-6 serum levels correlate with disease activity [38]. In the present study, the elevated levels of TNF-α, IL-1β and IL6 in local colon tissue of colitis mice were significantly suppressed by L-fucose. Previous studies have showed that free L-fucose can promote the adhesion of beneficial bacterium [39] and inhibit the adhesion and virulence of pathogenic bacterium in intestine [40,41], which lead to the attenuation of local inflammation [42]. In addition, administration of DSS produced an increase in systematic TNF-α, IL-1β and IL6 levels, which were reduced by orally treatment of L-fucose. This fact is probably associated with the molecular changes found in the local inflammatory focus. Our study showed the ability of L-fucose to reduce macrophages infiltration and inhibit the M1 macrophages polarization, which are one of the main sources of cytokines (*i.e.* TNF-α, IL-1β and IL6) [15]. This result suggests an important role for L-fucose as a modulator of the immune system.

It is widely accepted that innate immune system is involved in the development of intestinal inflammation induced by DSS [4]. Macrophages population is prominent in active IBD as compared to healthy donors [43]. Further, percentages of macrophages are increased in DSS-induced colitis [44]. DCs are the most powerful antigen presenting cells and both depletion of both macrophages and DCs before the initiation of DSS-induce colitis resulted in exacerbation of disease [45]. IBD is also linked to an influx of neutrophils and depletion of neutrophils by monoclonal antibodies has been shown to decrease several parameters of DSS-induced colitis [46]. In the present study, we observed increases in DCs, macrophages and neutrophils in the colon of DSS-induced acute colitis mice. L-Fucose treatment attenuated the increases in number of inflammatory cells in intestinal lamina propria, and the change of macrophage population is the most prominent.

Macrophages are tissue sentinels whose activation is pivotal in restoring innate immune responses [47]. M1 macrophages produce inflammatory cytokines and nitric oxide and are considered more inflammatory. In contrast, M2 macrophages are involved in tissue repair and are considered anti-inflammatory [48]. Given macrophage polarization playing key role in colitis, there is a critical need to understand whether L-fucose ameliorates inflammation response by influencing macrophage polarization. In the current study, we discovered that L-fucose inhibited M1 macrophages in DSS-induced acute colitis mice,

while no obvious alteration in M2 macrophages was observed after L-fucose treatment, indicating that L-fucose showed its anti-inflammation effect mainly by inhibiting M1 macrophages polarization rather than promoting M2 macrophages polarization or shifting from M1 to M2 macrophages.

Several reports have indicated that the NLRP3 inflammasome, which is predominantly produced by macrophages, is closely associated with UC [49–51]. It was reported that activation of NLRP3 inflammasome and production of IL1β in macrophages was crucial during the induction of acute colitis [28]. Bauer et al. had proved that NLRP3-deficient mice were significantly protected from DSS induced colitis [50]. Furthermore, Filardy et al. showed that lost control of post-transcriptional NLRP3 contribute to colonic inflammation [52]. In this study, both DSS-induced acute colitis mice and LPS/ATP-induced BMDMs damage had decreased expression of NLRP3, cleaved-caspase-1 and cleaved-IL-1β after L-fucose treatment, suggesting that the suppression of the NLRP3/IL1β pathway in macrophages may be involved in the anti-inflammatory effects of L-fucose on colitis.

The activation of NLRP3 requires two signals, the first signal is the activation of NF-kB mediated by the pathogen-associated molecular pattern (PAMP), and induces the synthesis and accumulation of the precursor proteins including NLRP3 and pro-IL-1β, the second signal is the activation of NLRP3 mediated by the damage-associated molecular pattern (DAMP), recruitment of pro-caspase-1 through the linker protein ASC, which leads to self-catalyzed processing to form activated caspase-1 [16,53]. Therefore, it is becoming increasing clear that NF-kB signaling pathway plays pivotal role in regulating NLRP3 inflammasome. Our experiments demonstrate that L-fucose obviously suppressed NF-kB protein expression both in DSS-induced colitis and BMDMs stimulated with LPS/ATP. L-Fucose has protective effects in colitis, which may be related to inhibition of NF-kB and NLRP3 inflammatory signaling pathway activation.

Amino salicylates, glucocorticoids and immunosuppressive drugs have been mainly used for the treatment and maintenance of remission of UC, but the side effect or toxicity of these drugs represents a major clinical problem [54]. Natural medicine has become an alternative therapy in addition to the conventional therapies that are used to treat UC [55]. L-Fucose is used a food additive without any adverse effect [19]. The anti-inflammatory effect of L-fucose makes it an ideal adjuvant anti-UC approach accompanied with conventional therapy. The dose of L-fucose used in the present study (250 mg/kg) was based on a previous study which evaluated the effects of fucoidan extracts on mice colitis [56]. Future studies are needed to determine whether the efficacy of L-fucose in the colitis model is dose dependent and explore the best dosage.

In conclusion, L-fucose confers protection against DSS-induced acute colitis and LPS/ATP induced BMDMs injury, and its effect may be achieved by downregulating the expression of inflammatory cytokines, suppressing immune cell infiltration, especially through regulating the polarization of macrophages and inhibiting NF-kB signaling and NLRP3 inflammasome activation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.05.013.

Declaration of Competing Interest

All authors declared that they have no conflict of interest.

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