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# The Protect Effects of Sodium Ferulate against Inflammatory Bowel Disease (IBD) Through Repress JAK2/STAT3 Signaling In Young and Aged Mice

**Qin Yang[4], YuPing He[1], Huiting Li[2,3], Hong Yu[1]* and Yang Yu[1,2,3]***

[1]Department of Histology Anatomy and HistoEmbryology, School of Basic Medical Sciences, Southwest Medical University, Luzhou, Sichuan 646000, P.R. China

[2]Key Laboratory of Medical Electrophysiology of Ministry of Education and Medical, Luzhou, Sichuan 646000, P.R. China

[3]Electrophysiological Key Laboratory of Sichuan Province, Institute of Cardiovascular Research, Southwest Medical University, Luzhou, Sichuan 646000, P.R. China

[4]Dazhou Vocational and Technical College, Dazhou, Sichuan 646000, P.R. China

#Keli Yao, Qin Yang contributed equally to this work.

***Corresponding author:**

Yang Yu,
Department of Histology Anatomy and HistoEmbryology, School of Basic Medical Sciences, Southwest Medical University, Luzhou, Sichuan 646000, P.R. China, E-mail: yuyang80@swmu.edu.cn and

Hong Yu,
Department of Histology Anatomy and HistoEmbryology, School of Basic Medical Sciences, Southwest Medical University, Luzhou, Sichuan 646000, P.R. China , Email: 759227112@qq.com

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**Keywords:**

Inflammatory bowel disease (IBD); JAK2/STAT3 signaling Sodium ferulate (SF); Aging

## 1. Abstract

Inflammatory bowel disease (IBD) is a type of immune-mediated intestinal inflammation caused by a variety of environmental, genetic and epigenetic risk factors. Up to now, there has been no effective drug treatment for IBD in elderly patients. Cytokines (such as IL-6) have been reported to activate Janus tyrosine kinase2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling, plays an important pathway associated with IBD. Sodium ferulate (SF), a major component of the traditional Chinese medicine Salvia miltiorrhiza, has been shown to be effective in inhibiting inflammation in many diseases, including IBD by its anti-oxidative stress effects. However, whether it acts via inhibition of JAK2/STAT3 signaling in colitis youth as well as in old age is still unknown. Therefore, the aim of this paper was to investigate the potential anti-inflammatory role of SF in the pathogenesis of a mouse model of experimental colitis in young as well as old adults Our results suggest that SF is highly protective against DSS-induced colitis in both young and elderly mice. Treatment with SF significantly reduced diarrhoea, colonic shortening and histological damage, and significantly inhibited the mRNA expression levels of DDS-induced pro-inflammatory cytokines, including interleukin-1 (IL-1), IL-6, IL-8 and tumour necrosis factor-α (TNF-α) in both young and elderly mice with experimental colitis. Meanwhile, the relative expression levels of phosphoJAK2 and phosphoSTAT3 were significantly reduced in the acute experimental colitis mice after SF administration. In conclusion, this study shows that SF significantly alleviates the symptoms of DSS-induced colitis by inhibiting the phosphorylation of JAK2/STAT3 signalling, and our results may be a promising new therapeutic approach for the treatment of IBD in young as well as in the elderly mice.

## 2. Introduction

Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) is a chronic inflammatory gastrointestinal disease, resulting in compromised intestinal epithelial barrier and chronic mucosal inflammation [1,2]. Studies indicated that patients

with IBD (especially CD) are often diagnosed before 18 years of age, the prevalence of IBD is 20-40 years old, and the incidence of 60-70 years old has become the second peak. In addition, it can cause irreversible damage to the patient's intestinal tract, leading to serious complications [3,4]. Current medication for IBD relies on the use of anti-inflammatory biologics and immunosuppressive drugs, which can cause serious side effects and have limited efficacy [5,6]. Despite this, many people with IBD, especially the elderly, often have comorbidities that affect susceptibility to infection and even increase the risk of malignancy. These differences may closely be related to the changes of the intestinal flora in aged patients [7-10], which make the elderly more prone to complications when receiving immunosuppression and anti-inflammatory drugs [11,12]. The JAK2/STAT3 signaling pathway regulates a variety of diverse physiological and pathological processes, including proliferation, differentiation, apoptosis inflammation, which have been implicated in the pathogenesis of several diseases including inflammatory skin conditions, some cancers, immune deficiency and inflammatory bowel diseases (ulcerative colitis, Crohn's disease) [13-17]. Genome-wide association studies (GWAS) and large cohort studies demonstrated that JAK2, STAT1, STAT3, and STAT4 are associated with susceptibility to IBD [18]. Furthermore, STAT3 is an important transcription factor in the JAK2 pathway and has been shown to be affected in IBD [19]. Previous reports indicated that excessive activation of STAT3 in mice promoted more severe colitis, and inhibited the expression of STAT3 could reduce lamina propria cell apoptosis and alleviate the symptoms of colitis [20,21]. Numerous small, oral administration JAK2/STAT3 signaling inhibitors have been promised therapeutic targets for IBD. However, a higher incidence of serious infections has been observed in elderly patients treated with JAK2 inhibitor placebo [22,23]. Therefore, it's urgent to find efficient therapies and drugs to treat and cure IBD in aged patients. The Traditional Chinese medicines have shown potential therapeutic roles in the treatment of IBD. Sodium ferulate (SF) is a dietary phenolic acid present in whole grains, herbs, and dried fruits, has been reported to have anti-inflammatory action and neuroprotective for the treatment and prevention of many diseases [24]. Previous reports demonstrated that SF exerts therapeutic effects for the treatment of IBD by strengthening intestinal barrier function via the suppression oxidative stress [25]. However, the function of SF underlying its anti-inflammatory effect through repressing JAK2/STAT3 signaling is rarely reported in IBD. In addition, vascular endothelial growth factors (VEGFA) and their receptors 2 (VEGFR-2) have been shown toplay important roles in mediating the pathogenesis of IBD [26,27]. In vitro experiments have shown that VEGFA induces angiogenesis of endothelial cells and the adhesion of neutrophils to the intestinal endothelium [28]. Overexpression of VEGFA worsened their condition and increased mucosal angiogenesis, whereas over-expression of VEGFR had a beneficial effect in mice with DSS-induced colitis [29]. But little is known about the effect of angiogenesis in mice acute colitis models during aging. In our study, we investigated whether SF play an important role in suppressing JAK2/STAT3 and VEGFA/VEGFR2 signaling of DSS-induced acute colitis in mice during age.

## 3. Materials and Methods

### 3.1. Preparation of SF

Sodium ferulic (SF, the sodium salt form of FA, purity >99.9%) was purchased from Dalian Meilunbio Biotechnology Co., Ltd (CAS Registry Number: 24276-84-4, China), dissolved in 0.9% saline solution to a final concentration of 7 mg/ml, and subsequently stored at 4°C.

### 3.2 Animals

To elucidate the inflammation effects of SF, we investigate the anti-inflammatory effect of FA with DSS-induced colitis in young and aged mice. C57BL/6 wild-type adult male mice on 8-11 weeks old (young) and 14-16 months old (aged) were obtained from the Animal Department of Southwest Medical University. They were maintained under standard conditions at our animal facility (12:12 h light-dark cycle), 22°C to 24°C, food and water available.

### 3.3 Induction of Acute Dextran Sulfate (DSS) Colitis

To induce acute colitis, mice were induced by the administration of 2.5% w/v DSS in the drinking water for seven days as previously described [30]. The mice were randomly divided into six groups, each consisting of ten animals, control-young, DSS-young, SF-young, control-aged, DSS-aged, and SF-aged. Mice in the control groups were given normal drinking water followed by intraperitoneal injection with 5μl 0.01MPBS for 7 days. The mice in DSS or SF groups were given drinking water containing 2.5% DDS followed by intraperitoneal injection with 100 mg/kg/d SF or 0.01MPBS for 7 consecutive days.

### 3.4. Evaluation of Disease Activity Index (DAI)

During the model building period, the weight of mice in each group was recorded every day, and the fecal condition and fecal occult blood test were observed. All parameters were examined and scored from day 0 to day 7 during DSS treatment. After 7 days of DSS treatment, mice were sacrificed and the colon was quickly isolated and the colon length was measured between the ileocecal junction to the proximal rectum. Disease activity index (DAI) was determined based on previous reports, which scores body weight loss, stool consistency, and rectal bleeding according to the scoring system [31,32].

### 3.5 Histological Evaluation of the Colon in Mice

For histopathological analysis, the colon tissue samples from different groups were fixed in 4% paraformaldehyde (PFA) overnight and embedded in paraffin, and cut into 5μm sections. The sections were then stained with hematoxylin and eosin (H&E) and photographed using (Motic, BA410E EF-UPR). The criteria used to assess inflammatory cell infiltration, epithelial changes, and mucosal archi-

tecture as categories using a standard histological scoring system as described previously[33], which sections were measured as follows: a) inflammatory cell infiltration distribution in the lamina propria, range 1-3; b) goblet cell loss as a marker of mucin depletion, range 0-2; and c) reactive epithelial hyperplasia/atypia with nuclear changes extension to subjacent layers such as the muscularis mucosa and submucosa, range 0-3; d) the number of intraepithelial lymphocytes in the epithelial crypts, range 0-3; e) abnormal crypt architecture, range 0-3; f) the number of crypt abscesses, range 0-2; g) mucosal erosion to frank ulcerations, range 0-2; for a maximum score of 18.

### 3.6. Quantitative real-time RT-PCR (RT-qPCR)

The total RNA in colon tissues was extracted using Trizol (Invitrogen, USA) and RNA was reverse-transcribed into cDNA using ReverTra Ace qPCR RT Master Mix Kit (FSQ201, TOYOBO, Japan) according to the manufacturer's protocols (800ng total RNA per reaction). Quantitative RT-qPCR was performed with a SYBR1 Green Realtime PCR Master Kit (TOYOBO). The mRNA levels of IL-1alpha, IL-1beta, IL-6, IL-8, and TNF-alpha were examined by RT-PCR using specific primers. The sequences of the primers were as follows (Table 1).

### 3.7. Western Blotting

Western Blotting was performed as described previously [34]. Protein concentrations (n = 5) were determined in the supernatant of colonic tissues by classic BCA protein assay (Beyotime). Equal protein of each sample was fractionated onto sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDSPAGE) and transferred onto polyvinylidene fluoride (PVDF) membrane by a Bio-Rad Western blot apparatus. The membranes were blocked with 5% fat-free milk or 5% bovine serum albumin, and then probed with the following primary antibodies for 24 h at 4◦C: anti-GAPDH (1:5000,14C10, CST); anti-beta-actin (1:5000, Proteintech, 20536-1-AP); anti-JAK2 (1:1000, Immunoway, YT2426); anti-JAK2 (phosphoY1007+Y1008) (1:1000, Abcam, ab32101); anti-STAT3 (1:1000, HuaAn Biotechnology,vET1605-45); anti-STAT3 (phospho Y705) (1:1000, HuaAn Biotechnology, ET1605-45) anti-VEGFA (1:1000, A bcam, ab52917 ) anti-phospho-VEGF Receptor 2 (Tyr1059) (1:1000, CST, 3817). The membranes were then incubated with appropriate horseradish peroxidase-labeled anti-rabbit or anti-mouse IgG (H+L) antibodies (1:2000~1:3000, Abcam, Cambridge, UK) and washed three times with Tween-PBS buffer. The membranes were then developed and visualized by incubation with an enhanced chemiluminescence (ECL) detection reagent (Thermo Fisher Scientific Inc., Waltham, MA, United States). The specific protein bands were visualized and analyzed with a ChemiDoc image analyzer (Bio-Rad, Hercules, CA, United States). Bands were quantified using Image-Pro Plus 5.0 software (Media Cybernetic, Bethesda, MD, USA). All experiments were performed four times in triplicate.

### 3.8. Statistical Analysis

Statistical analysis was performed using the Mann & Whitney test or two-way analysis of variance (ANOVA) when appropriate. Data are presented as means ± S.E.M. All statistical tests were performed in GraphPad Prism 8.0 program. P values $< 0.05$ were considered statistically significant.

## 4. Result

### 4.1. FA Attenuated DDS-Induced Colitis in young and Aged Mice

To investigate the protective effects of SF on experimental colitis in mice, we established the acute experimental colitis model in C57BL/6 mice by 2.5 % DSS in both young and aged groups. The mice in the DSS group in both young and aged groups showed less weight loss compared with the DSS+ SF group mice at 4-7 days ($P<0.05$; Figure 1a,b), significantly shortened the colon lengths compared with the control group ($P<0.01$; Figure 2 a, b), and SF treatment significantly suppressed the shortening of colon length ($P<0.01$; Figure 1a,b). During modeling, no difference in body weight was detected between the DSS+SF group and control group mice (Figure 1a,b). It suggested that SF could significantly promote the recovery from colitis. Then we quantified the disease activity index (DAI) for each group, during modeling, no difference in DAI was detected between each group of mice in the first 3 days, a significant decrease was observed after 4 days in DSS-treated mice compared with the control and SF group. DAI in the DSS group was obviously increased in contrast to the control group ($P< 0.001$, Fig. 2 a, b). Our results demonstrated that intraperitoneal injection SF with 100 mg/kg/d effectively attenuated DAI markedly in young and aged groups with DSS-induced acute colitis ($P< 0.001$, Figure 2a, b). Interestingly, in the aged DSS group, DAI was significantly higher than in their young counterparts at 5-7 days (Figure 2, b), suggesting that aging leads to more severe DAI following DSS treatment than young mice. This result is consistent with previous studies, which reported an increase in the severity of DSS-induced colitis with age [35]. In addition, the shortening of colon length was improved treated with SF both in young and aged groups ($P< 0.001$, Figure 3 a, b, c). In conclusion, FA attenuated DDS-induced colitis in young and aged mice.

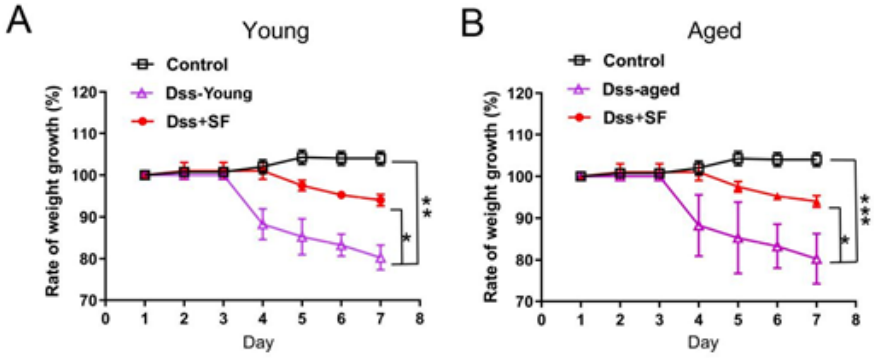


**Figure 1**. Rate of weight growth curves of untreated (control), DSS-treated and DSS+SF mice in young (a) or aged groups (b). Colitis was triggered by the addition of DSS (2.5%) to drinking water for 7 days. Samples were taken before (day 1) and after DSS treatment (day 7). One-way ANOVA followed by Tukey's test was used to determine significance. * $P < 0.05$, ***$P < 0.001$. Data are expressed as mean ± SEM.

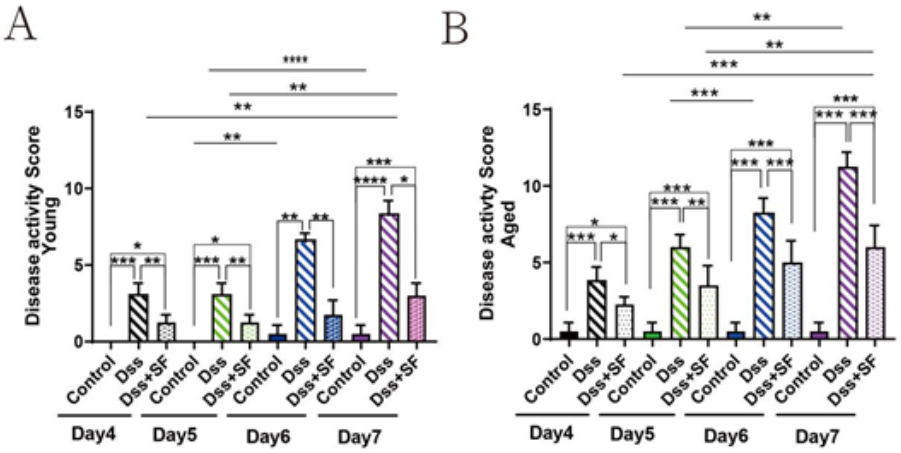


**Figure 2**. Effects of FA on disease activity index (DAI) of mice with DSS-induced colitis. Changes in DAI were evaluated daily. (a) Disease activity index (DAI) scores for DSS mice treated with FA in young groups (n=7 mice per group). Mice were scored according to parameters associated with colitis injury. (b) Disease activity index (DAI) scores for DSS mice treated with FA in aged groups (n=7 mice per group). One-way ANOVA followed by Tukey's test was used to determine significance. * $P < 0.05$, * * $P < 0.01$, ***$P < 0.001$. Data are expressed as mean ± SEM.

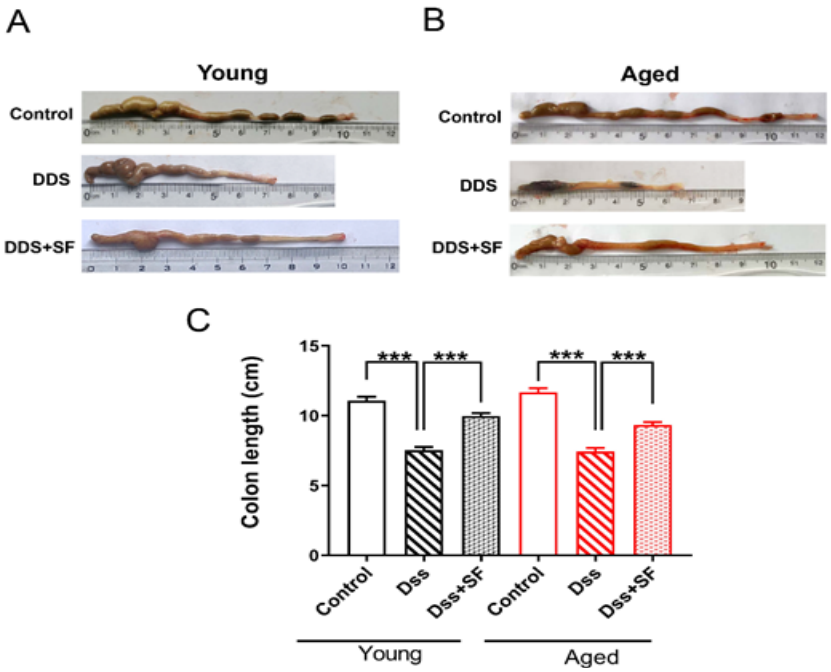


**Figure 3**. FA ameliorated DSS-induced the shortening in the intestine in mice. (a,b) Representative images of mouse colons from each group (n = 6 mice per group). Colons were excised from the cecum (top) to the distal colon (bottom) and washed in ice-cold PBS for two times. (c) Statistics of colon length in each group, colons in each group were obtained after 7 days of DSS administration and their lengths were measured both in young and old mice. The values presented are the mean±S.E.M (n=6 in each group). * $P < 0.05$, * * $P < 0.01$, ***$P < 0.001$.

### 4.2. Histopathological Changes in the Colon of Mice in Each Group after Treated with SF

To further determine the effects of SF on DDS-induced colitis mice during aging, mouse colon samples from each group were histopathologically examined by H&E staining. The colonic mucosal epithelium of mice in the control-young group and the control-aged group was complete, and the colonic glands in the lamina propria were abundant and orderly, and there were a lot of goblet cells in the colonic glands (Figure 3a). In the Dss-young group, the colonic mucosal epithelium was eroded, and the number and structure of the colonic glands were few and incomplete (Figure 3a). There were only a few goblet cells in the colonic glands, and many lymphocytes and neutrophils were infiltrated in the mucosal layer. Dss-aged group showed erosion of colonic mucosal epithelium, no colonic glands were observed in lamina propria, and numerous neutrophils and lymphocytes were infiltrated in the mucosal layer (Figure 3a). Dss-young +SF group and DSS-aged +SF group had a complete colonic mucosal epithelium, with some colonic glands in the lamina propria, some goblet cells in the lamina propria, and a few lymphocytes in the lamina propria (Figure 4a). The histological scores of the colon tissues under a microscope in each group (Figure 4b): the histological scores of the Dss-young and Dss-aged groups were significantly higher than those of the control-young and control-aged groups ($P<0.001$; Figure 4b); similarly, the histological pathological score was much lower in the SF treatment group compared to the DSS treatment, suggesting SF is a surrogate indicator of the treatment effect after chemoradiation therapy intraperitoneal injection of SF alleviated the symptoms of experimental colitis in young as well as aged mice (Figure 4b; $P<0.001$).

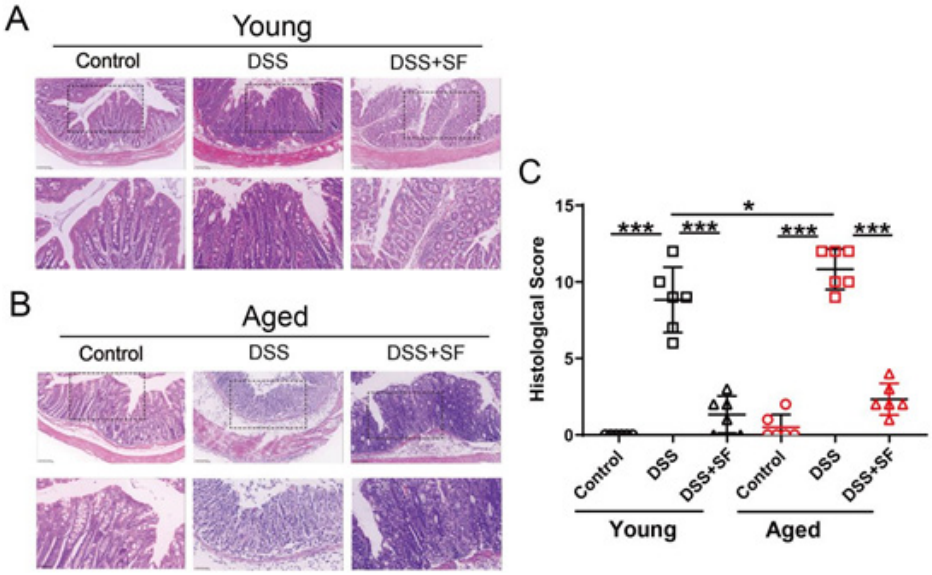


**Figure 4**. Effect of SF on histopathologic changes in colon tissues in DSS-induced mice during aging, (n = 6 mice per group). (a) The HE staining of colon tissue from young groups of mice (control, DSS group and DSS+SF group, (b) The HE staining of colon tissue from aged groups of mice (control, DSS group and DSS+SF). Scale bar: 100μm for A, B scale bar: 50μm; (c) Histological score treated with SF in DSS-induced IBD in young and aged groups. One-way ANOVA followed by Tukey's test was used to determine significance. * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$. Data are expressed as mean ± SEM.

### 4.3 SF Attenuates Inflammatory Cytokines TNF-a, IL-6, IL-1, and IL-8 mRNA Expressions Induced by DSS both in young and old mice

Studies have shown that cytokines play a major role in the development of inflammation and epithelial cell loss in both human colitis and experimental colitis. Previous reports have indicated TNF-α, IL-1 β, IL-6 and IL-8 are crucial pro-inflammatory cytokines have been shown to promote inflammation and epithelial cell necrosis contributes to the immunopathology of IBD patients [36-38]. Therefore, we examined the transcript levels of IL-6, IL-1, and IL-8 and TNF-a in the local lesion tissue of colon through quantitative real-time PCR. In aged control mice, the mRNA expression levels of TNF-α and IL-6 were significantly higher than in young control mice (Figure 5), those results is consistent with the previous report [39]. Beside, the mRNA levels of colon inflammatory cytokines, IL-1(Figure 5a), IL-6 (Figure 5b), IL-8 (Figure 5c), and TGF-a (Figure 5d) were elevated by RT-PCR in each group. In addition, higher levels of colonic IL-6, TNF-α ,and IL-8 were observed in aged Dss-induced mice than in young Dss-induced. Furthermore, pretreatment of SF significantly decreased the elevated expressions of TNF-α and IL-6 induced by acute DSS colitis (Figure 5b, Figure 5d).

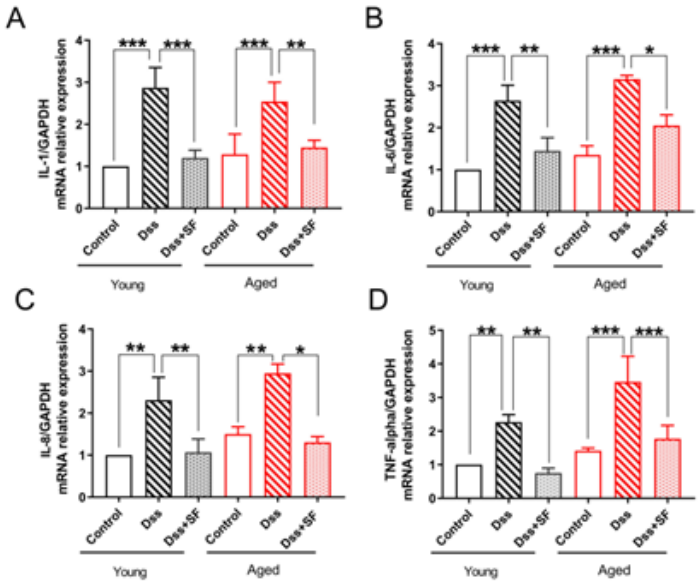


**Figure 5.** Effects of SF on the relative mRNA expression levels of (a) IL6 mRNA ,(b) TNF-α mRNA, (c) lL-1mRNA and (d) IL-8mRNA in colon tissue of each groups induced by DSS compared to control. The values presented are the mean±S.E.M (n=10 in each group). One-way ANOVA followed by Tukey's HSD test was used to determine significance. * $P < 0.05$, * * $P < 0.01$, ***$P < 0.001$. Data are expressed as mean ± SEM.

### 4.4. Effect of SF on JAK2/STAT3 Signaling Pathway in Colitis Induced by DSS in mice

JAK2/STAT3 signaling was associated with the pathogenesis of acute colitis induced by DSS in mice. Our results indicated SF attenuates IL-6 mRNA expressions induced by DSS both in young and old mice. In addition, Dawn et al reported that IL-6 activates the phosphorylation JAK2/STAT3 pathway [40]. Therefore, we speculated that the effect of SF may be related to inhibition of the expressions of phosphorylation of JAK2/STAT3 signaling after DSS-induced coils in the young and aged group. The expression of relative phosphorylation of JAK2/STAT3 protein in acute colitis was examined after being treated with SF during aging by western blot. It can be shown that the ratio of pJAK2/total JAK2 (Figures 6, a–d) was significantly increased, meanwhile, the ratio of JAK2 downregulated molecule pSTAT3 (Y705)/STAT3 was activated after mice with experimental acute colitis treated for 2.5% DDS for 7 days both in the young and aged group (Figures 6, a–b). By contrast, the relative expression ratio of pJak2/Jak2, pStat3 (Y705)/Stat3 proteins were markedly reduced in mouse colon tissues in the DDS-young SF and DDS-aged SF group compared with the DSS young and in the aged DSS-induced mice (Figures 6, a–b). Those results indicated that SF against experiment mice colitis through repressing the phosphorylation of JAK2/STAT3 signaling in both young and aged mice.

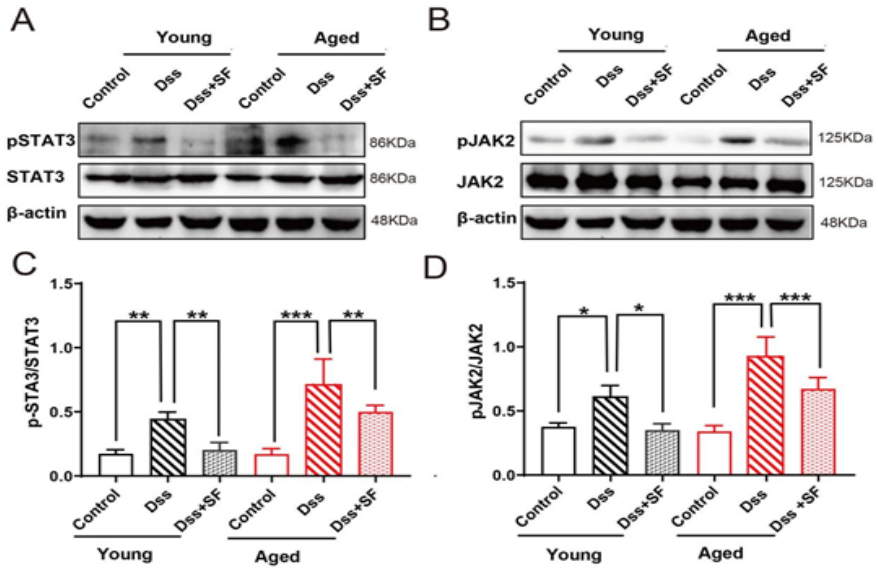


**Figure 6**. SF downregulates the expression of phosphorylation of Jak2/STAT3 signaling protein in colon tissue of mice in each group treated with DSS compared with control. (a, b) Western blot analyses showing the relative levels of the pSTAT3, STAT3, pJak2, and Jak2 proteins. (c, d ) Western blot analysis of the relative expression levels of pSTAT3/STAT3, pJak2/Jak2. These data were normalized to β-actin levels to obtain a relative blot density. Data are shown as mean ± SD (n=4) and analyzed by one-way ANOVA and followed by Tukey's test. *$P$<0.05, **$P$ <0.01 and ***$P$<0.001 versus control group.

### 4.5 SF Reduced the Upregulation of VEGFA in the Young DSS Treated Group but not Aged DSS Treated Group

Angiogenesis has been associated with human inflammatory bowel disease (IBD) and experimental colitis models in mice. To further assess the protective effect of SF through repressing the expression of VEGFA/VEGFR-2 signaling under acute inflammation colitis during aging, we examined the expression of VEGFA and its receptors VEGFR2 within colon samples in each group of DSS-induced acute colitis by Western blotting analysis. Compared with the control-young group and the control-aged group, the expression of VEGFA protein in the colon tissue of mice increased significantly in the DSS-young group but not the DSS-aged group (Figure 6 a, c, $P<0.01$), whereas levels of VEGFR2, was not significantly different in acute mice colitis (Figure 6 a,b), suggesting that young and old colitis may show different biology pathway.

## 5. Discussion

The number of older IBD patients is expected to increase with the accelerated aging of the population, where 25%-35% of patients are aged 60-80 years old [41]. However, it is still not well understood how the pathogenesis differs in older and younger IBD patients. Presumably related to alterations in the intestinal barrier and the gut microbiome. SF, one of the most valuable plants rich in natural ingredients, has anti-inflammatory, antimicrobial, anti-cancer, anti-arrhythmia, and anti-thrombosis, as well as anti-diabetic effects and immune stimulation [42-44]. In chronic or acute inflammation, Ferulic acid (FA) suppresses inflammation through various media such as cytokines, inflammatory media (e.g., ROS, nitric oxide, epoxidase, prostaglandin E2, and TNF-α) [45,46]. Our experiment found that after administration of SF, the DAI score in the DSS-young SF group and DSS-aged SF group significantly raised compared with control in the colonic tissues, the colon length increased significantly, the mouse colonic mucosal epithelium Intact, the lamina propria contains more colon glands, and abundant goblet cells in the mucosa, a few lymphocytes in the lamina propria (Figures 1, 2). Those results indicated SF has a great protective effect in the treatment of experimental colitis modeling in mice during the age, but its protective effect was slightly poor in the aged mice. Studies have shown that blocking the pro-inflammatory cytokines cascade after the onset of colitis reduces the severity of colitis [47]. Our results showed that the mRNA expression levels of pro-inflammatory cytokines TNF-α, IL-6, lL-1β, and IL-8 were higher in aged groups than young groups, as can be seen in Fig.5, levels of TNF-α, IL-6 and IL-1β in colon tissue were elevated markedly after treatment with DSS compared to the control group (Figure. 5a–d). Whereas, the DSS aged group had significantly higher TNF-α and IL-6 mRNA expression than the young group (Figure 5), indicated that weight loss level, histological scores, and expression of pro-inflammatory factors were higher in DSS aged group than in the young DSS-induced mice. The possible reasons might be that the onset characteristics, clinical symptoms, changes in the natural course of the disease and the choice of treatment drugs in aged patients are significantly different from those in young patients [25]. These differences are closely related to the characteristics of the aged, and the immune function and intestinal flora of the aged have changed [48-50]. This makes the elderly more prone to complications when receiving immunosuppressant drugs [51,52]. We demonstrated SF could alleviate DSS-induced body weight loss, diarrhea, fecal bleeding while decreasing the levels of pro-inflammatory cytokines in the colon tissues of mice. In mammals, JAK has 4 family members, and STAT has 7 family members, according to the different ligands and receptors, different combinations of JAK and STAT will be activated with a high degree of specificity and regulate basic biological processes, including a variety of cell types (T cells, B cells, natural killer cells, macrophages, and epithelial cells, etc.) apoptosis, proliferation, migration, development and differentiation [53,54]. Our experiments showed that the protein levels of p-Jak2/Jak2, p-Stat3 (Y705)/Stat3 increased, indicating that IL-6, TNF -α, and other inflammatory factors may initiate the JAK2/STAT3 signaling pathway. In the SF group, the colitis symptoms of the mice were significantly reduced, and the expression of inflammatory factors was reduced. At the same time, the relative expression of p-Jak2/Jak2, p-Stat3 (Y705)/STAT3 was reduced (Figure6), which suggests that SF ameliorated IBD in the colitis model in young/aged mice by inhibiting the phosphorylation level of JAK2/STAT3.

In the present study, there was no change in the protein expression of VEGFR-2 by young and aged DSS treatment group compared to DDS-young SF and DDS-aged SF, but the expression of VEGFA was strongly decreased in the DDS-young SF than in the DSS-young group (Figures 7). In an experimental model of acute ulcerative colitis, the expression of VEGFA maybe earlier than VEGFR2, or, VEGFA is more sensitive to colitis caused by DSS irritation. Previous research has indicated that IL-6 trans-signaling via STAT3 is a critical modulator of LPS-driven proinflammatory responses through cross-talk regulation of the VEGFR2 signaling pathway.

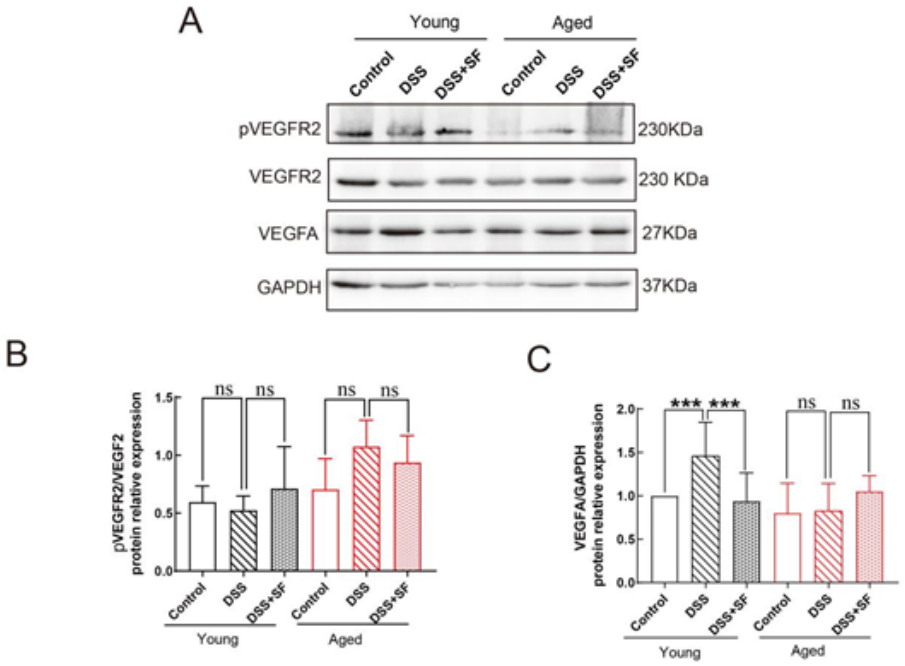


**Figure 7:** Comparison of protein expression of VEGFR-2, VEGFA in colon tissue of mice in each group. (a) Western blot analyses showing the relative levels of VEGFR-2 and VEGFA proteins. (b) Relative expression levels of pVEGFR2/VEGFR2 in Western blot test. (c ) Relative expression levels of VEGFA in Western blot test. These data were normalized to GAPDH levels to obtain a relative blot density. Data are shown as mean ± SD (n=6) and analyzed by one-way ANOVA and followed by Tukey's test. **P*<0.05 and ****P* <0.01 versus control group.n.s means not significant.

## 6. Conclusions

These findings provide a rationale for developing novel strategies to treat acute ulcerative colitis via targeting the JAK2/STAT3/VEGFA pathway. In summary, the present study demonstrated SF exerted beneficial effects on the intestinal epithelium by improving intestinal barrier function, inhibiting inflammation, through JAK2/STAT3/VEGFA signal pathway in DSS-induced colitis mice during age.

## 7. Funding

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