Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Anti-inflammatory Effect of luteolin-7-O-glucoside via the JAK1/STAT6/SOCS1 Pathway in Ulcerative Colitis Treatment

Dajuan Sun[1], Yan Cheng[1], Hua Yan[1], Xiunan Wei[2], Xinqian Dong[3], Lili Chi[1]

[1]Department of Gastroenterology, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Shandong province, China, [2]Shandong University of Traditional Chinese Medicine, [3]Department of Pathology, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Shandong province, China

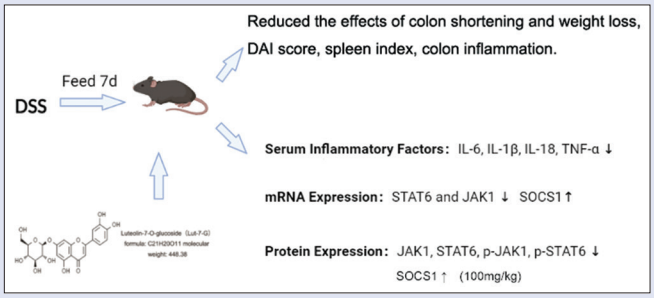
Submitted: 02-Nov-2021 Revised: 03-Jan-2022 Accepted: 27-Apr-2022 Published: 19-Sep-2022

ABSTRACT

Background: Luteolin-7-O-glucoside (Lut-7-G) is an effective compound found in plants, such as *Patrinia and honeysuckle*. It has anti-inflammatory as well as antioxidant properties; however, its anti-inflammatory effect on ulcerative colitis (UC) is hardly understood. **Objectives:** We evaluated the effects of Lut-7-G in dextran sodium sulfate (DSS)-induced UC in mice models, and then explore the underlying mechanism by studying the JAK1/STAT6/SOCS1 signaling pathway. **Materials and Methods:** Induction of acute colitis in mice was achieved by feeding 2.5% DSS for 7 days. Bodyweight loss, colon length, disease activity index (DAI) score, and spleen index were determined and hematoxylin-eosin and Periodic Acid-Schiff staining were performed to study the pathological changes in mouse colons. The inflammatory factor levels were determined by ELISA, JAK1, STAT6, and SOCS1 expression in colon tissues by RT-qPCR, and signaling pathway proteins by Western blotting. **Results:** It was found that treatment with Lut-7-G reduced the effects of colon shortening and weight loss, DAI score, spleen index, as well as colon inflammation. In addition, it significantly decreased DSS-induced overexpression of IL-6, IL-1β, IL-18, as well as TNF-α, and considerably reduced mRNA expression of JAK1 and STAT6 but upregulated the SOCS1 expression. Furthermore, Lut-7-G treatment dose-dependently decreased JAK1 and STAT6 protein expression, and only DSS + Lut-7-G (100 mg/kg) could downregulate p-JAK1 and p-STAT6 expression and upregulate SOCS1 protein expression. Moreover, Lut-7-G (100 mg/kg) was as effective as mesalazine. **Conclusion:** Lut-7-G may regulate the secretion of inflammatory factors and inhibit inflammatory responses through the JAK1/STAT6/SOCS1 pathway, as a determinant in the treatment of UC.
Key words: Dextran sodium sulfate, inflammation, JAK1/STAT6/SOCS1, luteolin-7-O-glucoside, ulcerative colitis

SUMMARY

- Luteolin-7-O-glucoside (Lut-7-G) is an effective compound found in plants, such as Patrinia and honeysuckle. It is known to have anti-inflammatory and antioxidant properties; however, its anti-inflammatory effect in UC is hardly understood.
- In this study, treatment with Lut-7-G improved weight loss and colon shortening in mice and decreased the DAI score, spleen index, and histopathological score. Under the microscope, the colon tissue structure improved and the degree of infiltration of inflammatory cells decreased. We also found that Lut-7-G treatment decreased DSS-induced excessive expression of IL-6, IL-1β, IL-18, and TNF-α, indicating that Lut-7-G could inhibit the expression of pro-inflammatory cytokines and control the occurrence of inflammation. Lut-7-G intervened in the progression of UC through the JAK1/STAT6/SOCS1 signaling pathway.



Abbreviations used: Lut-7-G: Luteolin-7-O-glucoside; UC: ulcerative colitis; DSS: dextran sodium sulfate; DAI: disease activity index; RT-qPCR: reverse transcription–real - time fluorescence quantification polymerase chain reaction; HE: hematoxylin-eosin; PAS: Periodic Acid-Schiff; PBS: phosphate buffered saline; BCA: bicinchoninic acid; CAC: colitis-associated colon cancer; TLR2: toll-like receptor 2; SOCS1: suppressors of cytokine signaling 1.

Correspondence:
Dr. Lili Chi,
Department of Gastroenterology, Afliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong Province, China.
E-mail: chililiyl@126.com
DOI: 10.4103/pm.pm_506_21

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Ulcerative colitis (UC) is a bowel disease that is associated with chronic non-specific inflammation, where in the lesions affect the rectum and colon and invade the intestinal mucosa and submucosa. Globally, clinical incidences of UC have rapidly increased.[1,2] Currently, UC is a worldwide public health challenge that has been recognized as a lifelong disease by the evidence-based consensus of the European Organization for Ulcerative Colitis and Crohn's Disease.[3] The path mechanisms of UC has not been fully established; nevertheless, relevant studies have shown that it is affected by multiple factors such as genetic environment, immune infection, and other factors, resulting in impaired local barrier functions of the intestinal mucosa, translocation of intestinal flora, and triggering or aggravation of the local inflammatory response of the intestinal mucosa.[4,5] The occurrence of UC is closely associated with inflammatory factors. TNF-α, CRP, IL-6, and other inflammatory mediators can be used as evaluation indicators for the severity of UC and the effects of treatment and prevention.[6-9]

Cite this article as: Sun D, Cheng Y,Yan H, Wei X, Dong X,Chi L. Anti-inflammatory effect of luteolin-7-O-glucoside via the JAK1/STAT6/SOCS1 pathway in ulcerative colitis treatment. Phcog Mag 2022;18:627-34.

Aminosalicylic acid and glucocorticoid preparations, which show anti-inflammatory effects to control the symptoms of UC, are widely regarded as drugs for the treatment of UC. However, there are many issues, such as easy recurrence after drug withdrawal, several side effects, and a long medication course. The targeted therapy for UC using new small-molecule drugs or biological agents has made encouraging progress, but it may increase the risk of cancer and infection. Therefore, the search for safe and effective drugs has become a research hotspot.

Recently, due to their safety and relatively high efficacy, there has been increasing interest in the use of traditional Chinese medicines. Certain traditional Chinese medicines, such as *honeysuckle*[10] and *Patrinia*,[11,12] have unique advantages in the clinical treatment of UC. Furthermore, luteolin-7-O-glucoside (Lut-7-G), a natural flavonoid, is an effective component of *Patrinia*.[13] Recent studies have shown the protective effects of Lut-7-G against inflammatory diseases.[14-16] Studies have shown that Lut-7-G can inhibit the inflammatory response by inhibiting the STAT pathway.[17] Other studies have shown that some natural flavonoids such as curcumin can treat experimental colitis through JAK/STAT/suppressors of cytokine signaling (SOCS) signaling pathway.[18] In the present study, we investigated the therapeutic effect of Lut-7-G in mice suffering from UC, and explored whether Lut-7-G could have anti-inflammatory activity via the JAK/STAT/SOCS signaling pathway.

MATERIALS AND METHODS

Reagents

Lut-7-G [$C_{21}H_{20}O_{11}$, HPLC ≥98%, CAS No. 5373-11-5, Figure 1a] was provided by Beijing Solebo Technology Co., Ltd. (Beijing, China). DSS (MW36000-50000) was purchased from MP Biomedicals Inc. (Solon, OH, USA). Mesalazine sustained-release granules (#151191) were provided by Shanghai Aidifa Pharmaceutical Co., Ltd. (Shanghai, China). Enzyme-linked immunosorbent assay (ELISA) kits for mouse IL-6 (#E-EL-M0044c), TNF-α (#E-eEL-M0049c), IL-18 (#E-EL-M0730c), and IL-1β (#E-MSEL-M0003) were purchased from Elabscience (Wuhan, China). The p-STAT6 antibody (#ab263947) was provided by Abcam (Cambridge, UK). The p-JAK1 antibody (Tyr1022/try1023, #AF2012) was provided by Affinity Biosciences Ltd. (Jiangsu, China). Suppressors of cytokine signaling 1 (SOCS1) rabbit polyclonal antibodies (#A7754) were provided by ABclonal (Wuhan, China). GAPDH (#af1186) was obtained from Beyotime Biotechnology Institute (Shanghai, China). STAT6 rabbit polyclonal antibody (# 51073-1-AP) and JAK1 mouse monoclonal antibody (#66466-1-lg) were purchased from Proteintech (Wuhan, China). Reverse transcription–real-time fluorescence quantification polymerase chain reaction (RT-qPCR) primers for JAK1 and STAT6 were procured from Baori Medicine Technology Co., Ltd. (Beijing, China). Hematoxylin-eosin (HE) staining kits (#C0105) were purchased from Shanghai Biyuntian Biological Company (Shanghai, China) and the Periodic Acid-Schiff (PAS) staining kit (#G1281) was procured from Beijing Solebo Technology Co., Ltd. (Beijing, China).

Animals and ethical statement

Animal ethics were followed in strict accordance with the related operation and experiment of experimental animals, the animal welfare ethical review guide (GB/t 35892-2018), the principle of which was with the approvement by the Animal Ethics Committee of The Affiliated Hospital of Shandong University of Traditional Chinese Medicine (AWE-2021-41). Fifty C57BL/6 male mice (20–22 g, 8-week-old) were obtained from the Jinan Pengyue Experimental Animal Breeding Co., Ltd., animal Qualification Certificate No. SCXK (Lu) 20190003. The animals were raised in the Animal Experimental Center of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine. The laboratory conditions were as follows: temperature 22–26°C, light exposure for 12 h, and light protection for 12 h alternated as well as the relative humidity of 40–70%. Before the experiments, animal acclimatization was performed for 7 d.

DSS-induced colitis and treatments

The experimental mice were randomly assigned into five treatment groups (n = 10): normal, DSS (negative control), DSS + Lut-7-G (50 mg/kg), DSS + Lut-7-G (100 mg/kg), as well as the DSS + mesalazine (52 mg/kg, positive control) groups. DSS was used to establish UC models.[19] Except for normal mice, all mice were free to drink 2.5% DSS water from day 1 to day 7 of the experiment, and normal drinking water was used for the following 3 days. Referring to related the literature and dose-conversion methods between humans and animals,[15,20-22] the doses were set as Lut-7-G (50 mg/kg), Lut-7-G (100 mg/kg), and mesalazine (52 mg/kg). Once a day, the mice were intragastrically administered with 0.2 mL of the medicine, beginning on the third day and lasting for 8 d. Normal saline at the same volume was administered to mice in normal and DSS groups for 10 d.

Serum and tissue collection

After all the mice were administered medicine or normal saline for 8 d in a row, they were only given water for 24 h The mice were anesthetized using 2% isoflurane anesthesia, and blood was obtained from orbital veins. After centrifugation of the venous blood at 26°C at 1,000 × g for 5 min, serum was obtained and kept at −80°C. Then, the mice were sacrificed via cervical dislocation, their colons were collected, and their lengths determined. The colons from mice were then divided into two parts for further experiments. The mice abdominal cavities were quickly sliced open along anterior midlines and the spleen was separated. After washing with phosphate-buffered saline (PBS) and drying, the spleens were weighed using an electronic balance to calculate the spleen index.

Body weights and disease activity index (DAI) scores of mice

Body weights, fecal traits, as well as fecal blood of mice were observed daily according to Cooper's method,[23] and DAI was evaluated as follows: DAI = (loss score of body mass + stool blood score + stool trait score)/3. The higher the score, the more severe the inflammation. The DAI score criteria are presented in Table 1.

Colon histologic grading and HE staining

Colon tissue was fixed with 4% paraformaldehyde for dehydration and paraffin embedding, cut into sections, and stained with HE.

Table 1: Disease activity index score

Score	Weight loss (%)	Stool consistency	Hematochezia
0	0	Normal	Negative
1	1-5		
2	6-10	Loose stool	Hemoccult positive
3	11-15		
4	>21	Liquid stool	Gross blood

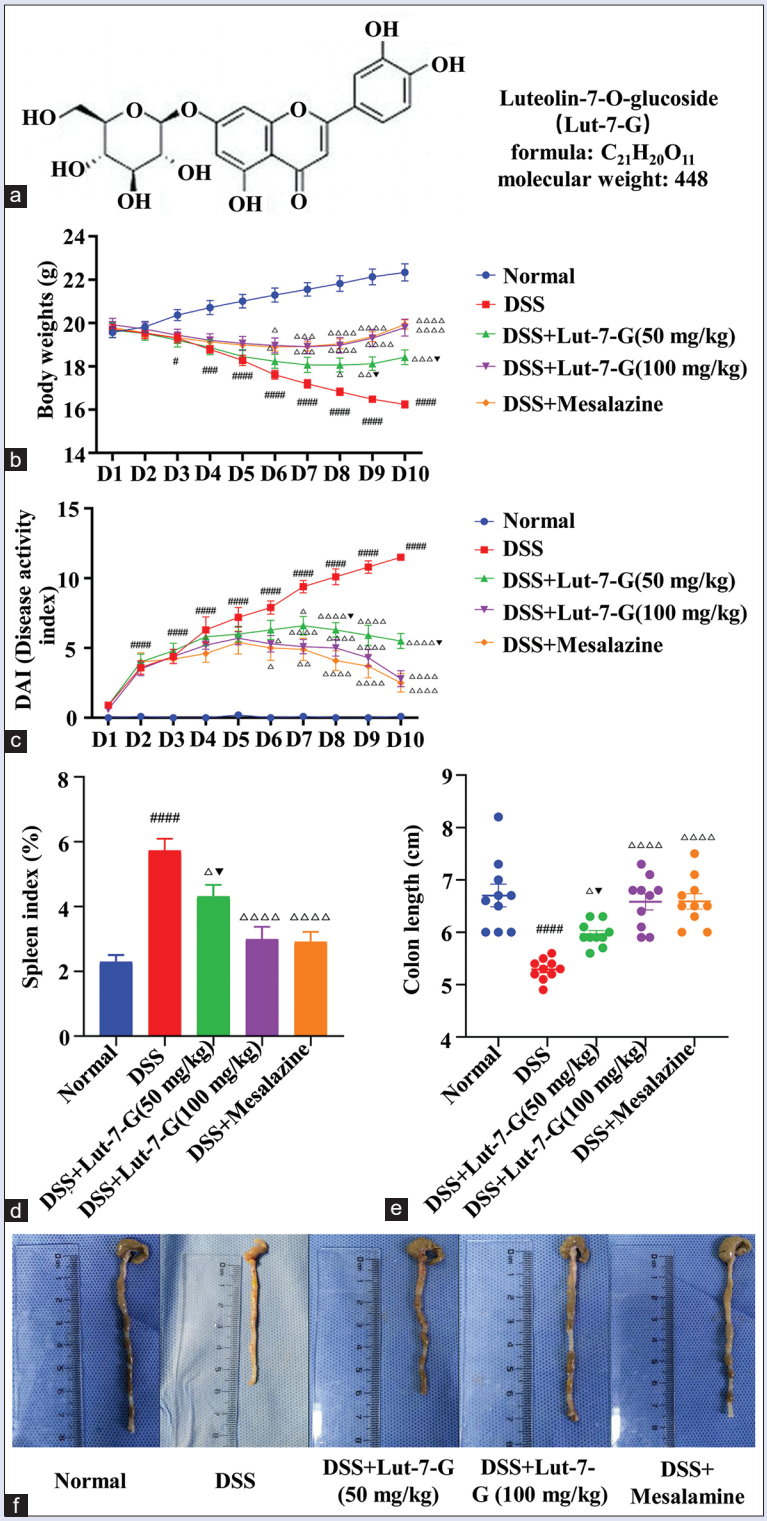


Figure 1: Lut-7-G alleviates acute colitis induced by DSS. (a) Chemical structure of Lut-7-G. (b) Changes in the body weight in each group. (c) Changes in DAI in each group. (d) Spleen index in every group. (e and f) Colon length in each group. Data are expressed as mean ± SEM ($n = 10$). ps: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ vs. normal. $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$, $^{\triangle\triangle\triangle}p < 0.001$, $^{\triangle\triangle\triangle\triangle}p < 0.0001$ vs. DSS. $^{\blacktriangledown}p < 0.05$, $^{\blacktriangledown\blacktriangledown}p < 0.01$, $^{\blacktriangledown\blacktriangledown\blacktriangledown}p < 0.001$, $^{\blacktriangledown\blacktriangledown\blacktriangledown\blacktriangledown}p < 0.0001$ vs. DSS + mesalazine. DSS, dextran sodium sulfate; Lut-7-G, luteolin-7-O-glucoside.

According to Geboes scoring criteria,[24] pathological sections of each sample were scored histologically, and each section was randomly selected with more than three high-power fields (×200). The average blind scores were determined by two experienced pathologists.

PAS staining

To clarify the severity of goblet cell loss, PAS staining was performed on the colon tissues. The experimental procedure was conducted as instructed by the manufacturer of the staining kit.

ELISA of inflammatory factors

The venous blood was removed from the posterior orbital vein. After centrifugation at 4°C with a radius of 15 cm and a radius of 2,000 r/min for 5 min, the blood supernatant was collected. Then, IL-6, IL-1β, IL-18, and TNF-α levels were determined using ELISA kits. The absorbance of the samples was determined at 450 nm using a microplate reader and determined using a standard curve.

RT-qPCR

An appropriate amount of mouse colon tissue was removed and ground with liquid nitrogen. After RNA was extracted using an RNA extraction kit, RNA concentrations of samples were determined using a multifunctional microplate reader. The RNA was reverse-transcribed into cDNA, and RT-qPCR was conducted with β-actin as the internal standard. The results were calculated via the $2^{-\Delta\Delta Ct}$ strategy. Table 2 shows the primers used in this assay.

Western blotting

After the colon tissues were extracted, the protein lysate was added for homogenization. The supernatant was discarded after centrifugation at 1,000 × *g* for 15 min. The protein concentrations were evaluated by the bicinchoninic acid (BCA) assay and adjusted to 4 μg/μL. After denaturation, the protein was stored at −80°C. After gel preparation, we performed 10 μL of sample loading, electrophoresis, wet transfer, blocking with 5% skim milk powder, overnight incubation with primary antibody at 4°C, followed by incubation in the presence of a secondary antibody for 1 h. ECL chemiluminescence solution was added to the membrane and protein chemiluminescence was used to obtain images. With GAPDH as an internal parameter, the ImageJ software (version 1.8.0, National Institutes of Health, Bethesda, Maryland, USA) was used to calculate the gray value.

Statistical analysis

Data are shown as mean ± S.D and were analyzed by GraphPad Prism 8.0. For indicators meeting homogeneity of variance, data were analyzed using ordinary one-way ANOVA, and for inter-group comparisons, the Tukey's multiple index test. For indicators that did not meet the homogeneity of variance, data were analyzed by Welch's ANOVA method, and for between-group comparisons, the Games-Howell's multiple order test.

RESULTS

Lut-7-G alleviates acute colitis

A mouse model of acute UC was established by administering the mice with 2.5% DSS (w/v) in their drinking water for a week. The results revealed that, compared with normal mice, DSS mice had hematochezia and diarrhea accompanied by significant weight loss. In addition, the DAI and histological scores of the DSS group were increased, indicating that the UC model was successfully developed. Compared with DSS-alone treatment (DSS group), 8 d of treatment with Lut-7-G (100 mg/kg), Lut-7-G (50 mg/kg), or mesalazine (52 mg/kg) considerably mitigated the bodyweight loss and significantly decreased the DAI score. The colon length and spleen index were also reduced to varying degrees [Figure 1b-f]. The DSS + Lut-7-G (100 mg/kg) and DSS + mesalazine groups had the most significant effect, however, differences were not marked ($p > 0.05$).

Table 2: Sequences of primers used for RT-qPCR

Primer name	Primer sequence
Mus-JAK1-F	5'-CTCTCTGTCACAACCTCTTCGC-3'
Mus-JAK1-R	5'-TTGGTAAAGTAGAACCTCATGCG-3'
Mus-STAT6-F	5'-ATCCACTCATTTCAGAGCCTAGAAG-3'
Mus-STAT6-R	5'-GGGCATGGTTATCTGGCTCA-3'
Mus-SOCS1-F	5'-CTCCTCGTCCTCGTCTTCGT-3'
Mus-SOCS1-R	5'-GAAGGTGCGGAAGTGAGTGTC-3'
Mus-β-actin-F	5'-CATCCGTAAAGACCTCTATGCCAAC-3'
Mus-β-actin-R	5'-ATGGAGCCACCGATCCACA-3'

Lut-7-G attenuates histological inflammatory injury

Acute UC leads to histological inflammatory damage, including inflammatory cell infiltration, mucosal remodeling, epithelial defects, and goblet cell loss.[25] HE staining was conducted to assess colonic inflammation severity, and PAS staining was conducted to evaluate goblet cell loss. HE and PAS staining of the colon of mice in the DSS group showed the following: first, the colonic mucosa was incomplete with the appearance of crypt inflammation and crypt abscess and the disappearance of the local crypt. Second, the lamina propria was infiltrated by many lymphocytes. Third, the goblet cell numbers were markedly reduced relative to the DSS group. HE and PAS staining results of colonic mucosa of mice in the DSS + Lut-7-G (100 mg/kg) group and the DSS + mesalazine group showed the following: first, colonic mucosa tended to be normal without crypt structural reconstruction. Second, the lamina propria was infiltrated by a few inflammatory cells. Third, the amounts of epithelial goblet cells in the colon mucosa increased. As for the DSS + Lut-7-G (50 mg/kg) group, HE and PAS staining showed that the severity of mucosal inflammation was low relative to that of the DSS group, but not as low as that of the DSS + Lut-7-G (100 mg/kg) and DSS + mesalazine groups [Figure 2 a-c].

Effects of Lut-7-G on inflammatory factors

To assess the inhibitory effects of Lut-7-G on colon inflammation in the mice, ELISA was used to detect the levels of IL-6, IL-1β, TNF-α, as well as IL-18 in the serum of mice. The serum levels of these cytokines were elevated in the DSS group, compared to the normal group ($p < 0.0001$), suggesting that DSS induced marked inflammatory responses consistent with the severity of its clinical symptoms. The expressions of IL-6, IL-1β, TNF-α, as well as IL-18 in the serum of DSS + Lut-7-G (100 mg/kg), DSS+ Lut-7-G (50 mg/kg), as well as DSS + mesalazine groups were decreased ($p < 0.001$, $P < 0.0001$), relative to the DSS group. Both DSS+ Lut-7-G (100 mg/kg) and DSS + mesalazine groups showed significant effects. The DSS + mesalazine group was slightly superior to the DSS+ Lut-7-G (100 mg/kg) group in terms of the expression of IL -6, TNF-α, and IL -1β, with statistically significant differences ($p < 0.01$, $P < 0.001$, $P < 0.0001$). However, there were no marked differences in the IL-18 levels between the groups ($p > 0.05$). As shown in Figure 3, these results indicate that Lut-7-G has an anti-inflammatory activity.

Effects of Lut-7-G on the JAK1/STAT6/SOCS1 signaling pathway

The JAK/STAT signaling pathway is a remarkable pathway in the non-controllable inflammatory response of UC. It participates in the inflammatory response of UC, including cell proliferation, differentiation, and apoptosis, and serves in many important biological processes, such as immune regulation.[26] Among them, SOCS1 is a powerful inhibitor of JAK/STAT.[27] Durham *et al.*[28] showed that targeting the SOCS protein could inhibit the transduction of the JAK/STAT signaling pathway in diseases. On this basis, we attempted to find out whether the anti-inflammatory effects of Lut-7-G were associated with the regulation of JAK1/STAT6/SOCS1 signaling. As shown in Figure 4a, the mRNA expressions of JAK1 and STAT6 in colon tissues obtained from DSS mice increased ($p < 0.0001$), while the mRNA expression of SOCS1 decreased ($p < 0.0001$) compared to those of the

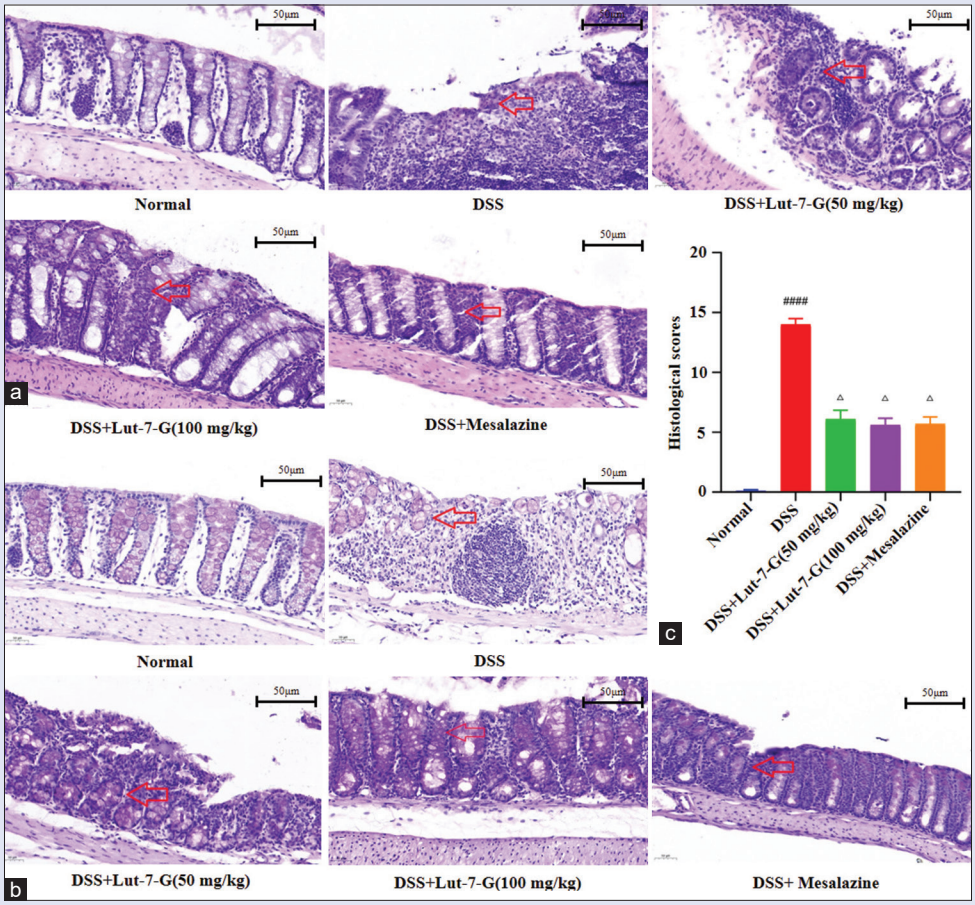


Figure 2: Lut-7-G alleviates DSS-induced colon damage in mice. (a) Microscopic images of HE staining (×200). (b) Microscopic images of PAS staining (×200). (c) Histological scores in each group. Bar = 50 μm. Data are expressed as mean ± SEM (n = 10). ps: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ vs. normal. $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$, $^{\triangle\triangle\triangle}p < 0.001$, $^{\triangle\triangle\triangle\triangle}p < 0.0001$ vs. DSS. $^{\blacktriangledown}p < 0.05$, $^{\blacktriangledown\blacktriangledown}p < 0.01$, $^{\blacktriangledown\blacktriangledown\blacktriangledown}p < 0.001$, $^{\blacktriangledown\blacktriangledown\blacktriangledown\blacktriangledown}p < 0.0001$ vs. DSS + mesalazine. DSS, dextran sodium sulfate; Lut-7-G, luteolin-7-O-glucoside.

normal mice. The Lut-7-G treatment notably decreased the mRNA expressions of STAT6 and JAK1 ($p < 0.0001$) and elevated the mRNA expressions of SOCS1 ($p < 0.0001$). Only the DSS + Lut-7-G (100 mg/kg) group exhibited comparable effects to those of the DSS + mesalazine group in terms of mRNA expressions of SOCS1 and JAK1, but not for STAT6, without any statistical difference ($p > 0.05$).

The protein levels of JAK1, STAT6, p-JAK1, as well as p-STAT6 in the colonic mucosa of DSS mice were increased, relative to those in normal mice ($p < 0.05$, $P < 0.001$, $P < 0.0001$). Lut-7-G dose-dependently suppressed JAK1 and STAT6 protein levels in colonic tissues ($p < 0.05$, $P < 0.001$); only the DSS + Lut-7-G (100 mg/kg) group markedly blocked DSS-mediated JAK1 and STAT6 protein phosphorylation ($p < 0.05$, $P < 0.001$) and increased SOCS1 protein expression ($p < 0.05$). Moreover, the DSS + mesalazine group was superior to the DSS + Lut-7-G (100 mg/kg) group, however, the difference was insignificant ($p > 0.05$). As shown in Figure 4b–d, the JAK1/STAT6/SOCS1 signaling pathway is important for the protective effects of Lut-7-G on acute colitis in mice.

DISCUSSION

UC has a long disease course and tends to recur. Some UC develop into colitis-associated colon cancer (CAC).[29,30] Therefore, studies have aimed at evaluating effective treatments for UC. Recent studies have found that many natural compounds in medicinal plants have a positive effect on UC treatment.[31–33]According to literature reports, Lut-7-G has anti-inflammatory, antioxidant, and allergic effects. For example, luteolin and Lut-7-G inhibit lipopolysaccharide-induced 264.7 cell inflammation by regulating NF-κB/AP-1/PI3K-Akt signaling,[14] and Lut-7-G protects the uterus from *Staphylococcus aureus*-mediated injury and inflammation by downregulating nuclear factor κB (NF-κB) signaling and toll-like receptor 2 (TLR2).[16] However, there is no clinical or experimental study of Lut-7-G in the treatment of UC that has been reported up till now. In this study, treatment with Lut-7-G improved weight loss and colon shortening in mice and decreased the DAI score, spleen index, as well as histopathological score. Under the microscope, the colon tissue structure improved and the degree of infiltration of inflammatory cells decreased. Therefore, Lut-7-G can improve colon inflammation and injury to a certain extent and relieve the symptoms of UC.

Two important characteristics of UC are inflammatory cytokine secretion and production of neutrophils, which are often used to evaluate UC disease activity.[34] The secretion of pro-inflammatory cytokines, such as IL-6, TNF-α, as well as IL-1β, is important for the development of UC.[35] IL-1β stimulates the secretion of inflammatory transmitters and cytokines. IL-6, a cytokine produced by T cells and fibroblasts, serves as a major mediator of inflammatory responses. TNF-α is mainly secreted by NK cells, macrophages, and T cells, which have immunomodulatory functions and participate in the inflammatory response. TNF-α and IL-1β can induce the production of IL-6 inflammatory cytokines and

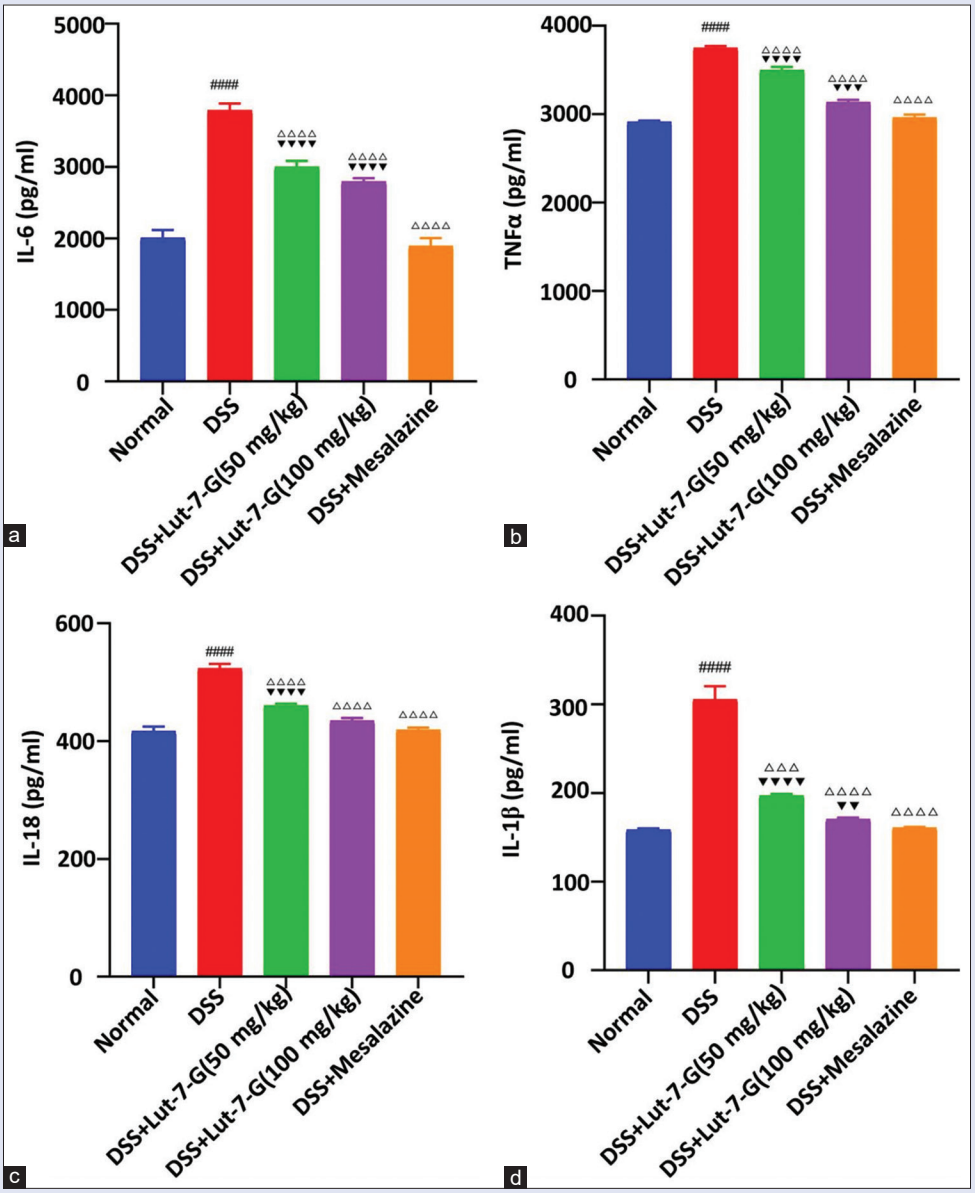


Figure 3: Serum IL-6, IL-1β, TNF-α, and IL-18 levels in mice. Data are expressed as mean ± SEM; $n = 10$). ps: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ vs. normal. $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$, $^{\triangle\triangle\triangle}p < 0.001$, $^{\triangle\triangle\triangle\triangle}p < 0.0001$ vs. DSS. $^{\blacktriangledown}p < 0.05$, $^{\blacktriangledown\blacktriangledown}p < 0.01$, $^{\blacktriangledown\blacktriangledown\blacktriangledown}p < 0.001$, $^{\blacktriangledown\blacktriangledown\blacktriangledown\blacktriangledown}p < 0.0001$ vs. DSS + mesalazine. DSS, dextran sodium sulfate; Lut-7-G, luteolin-7-O-glucoside.

induce a cascade of inflammatory amplifications. Here, we measured the expression of cytokines secreted by mouse macrophages, which include IL-6, IL-1β, IL-18, and TNF-α. Because mesalazine is a traditional anti-inflammatory drug for UC treatment, the DSS + mesalazine group was used as a positive control to evaluate the protective effect of Lut-7-G on UC. In this study, we found that Lut-7-G treatment decreased DSS-induced excessive IL-6, IL-18, IL-1β, and TNF-α expressions, implying that Lut-7-G could inhibit pro-inflammatory cytokine levels and the development of inflammation.

Moreover, these inflammatory mediators interact with multiple signaling pathways to form a complex network that is involved in UC development. The JAK-STAT signaling pathway serves the purpose of an inflammatory response in inflammatory bowel disease and is currently considered a potential therapeutic target.[36-38] STAT6 activation was noted in laminar propria leukocytes and IECs from UC patients.[39,40] Activated Jak phosphorylates STAT6 after which STAT6 homodimers are translocated to the nucleus and bind to DNA sequences of genes that play various roles in proliferation, apoptosis, and immune responses.[41] Oliveira *et al.*[42] documented that STAT6-deficient mice were highly vulnerable to mucosal damage during DSS-induced colitis. Moreover, the mice exhibited a heightened intestinal epithelial cell apoptosis, inflammatory responses, and tissue injury. Therefore, inhibition of the JAK/STAT signaling pathway may result in the downregulation of many inflammatory cytokines, slowing tissue damage and promoting intestinal mucosal healing. The SOCS family of proteins are negative regulators of the JAK/STAT pathway. SOCS1 represses the activation of JAK1/3 and STAT6.[43] Moreover, SOCS1 is involved in UC pathogenesis.[44] The upregulation of SOCS1 inhibits the production of pro-inflammatory mediators, including TNF-α, IL-1β, and IL-6, thus inhibiting the inflammatory process.[45,46] In our present study, Lut-7-G intervened in the progression of UC through the JAK1/STAT6/SOCS1 signaling pathway.

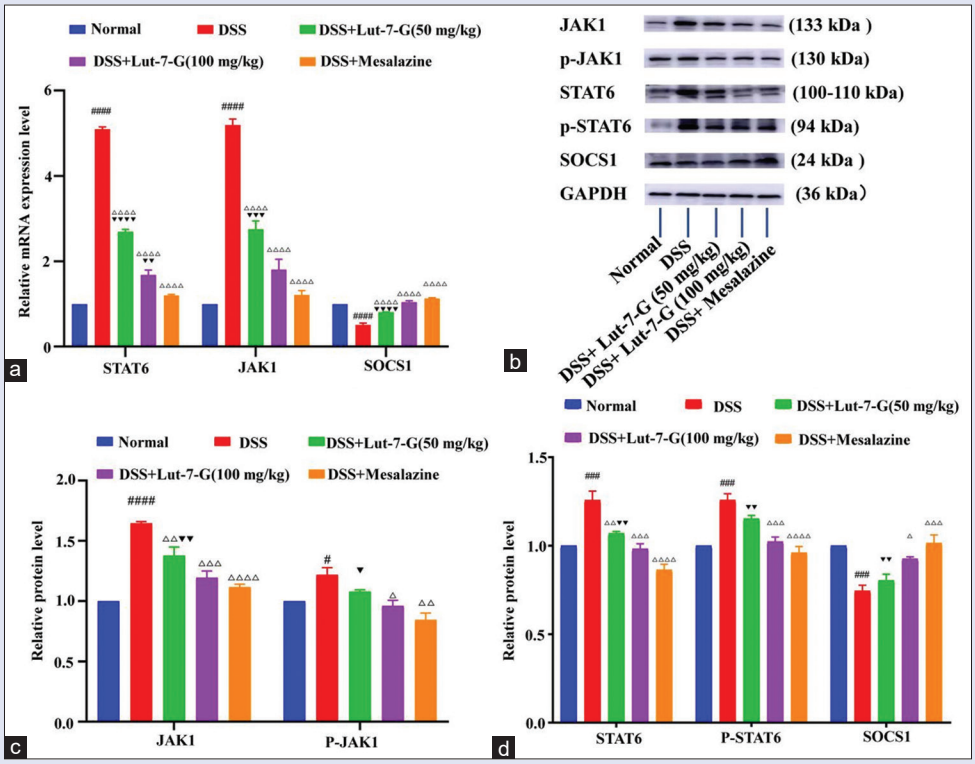


Figure 4: Relative mRNA and protein expression levels of JAK1, STAT6, and SOCS1. (a) Relative mRNA expression levels relative to those of β-actin in colon tissues of mice ($n = 10$). (b-d) Protein expression levels of JAK1, STAT6, p-JAK1, p-STAT6, and SOCS1 relative to those of GAPDH in colon tissues of mice ($n = 3$). Data are shown as mean ± SEM. ps: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ vs. normal. $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$, $^{\triangle\triangle\triangle}p < 0.001$, $^{\triangle\triangle\triangle\triangle}p < 0.0001$ vs. DSS. $^{\blacktriangledown}p < 0.05$, $^{\blacktriangledown\blacktriangledown}p < 0.01$, $^{\blacktriangledown\blacktriangledown\blacktriangledown}p < 0.001$, $^{\blacktriangledown\blacktriangledown\blacktriangledown\blacktriangledown}p < 0.0001$ vs. DSS + mesalazine. DSS, dextran sodium sulfate; Lut-7-G, luteolin-7-O-glucoside.

CONCLUSION

Lut-7-G may be an effective option for UC treatment. According to the results of different dosing groups in our experiment, Lut-7-G at a high dose (100 mg/kg) performed better than at a low dose (50 mg/kg). Further research regarding the appropriate dose is needed. The underlying therapeutic mechanism may be that Lut-7-G regulates the secretion of inflammatory mediators and inhibits the inflammatory response through the JAK1/STAT6/SOCS1 pathway, thus, making a difference in UC therapy. In this study, only an experimental UC mouse model was used. In the future, *in vitro* studies and clinical tests will be conducted to further explore the targets and key mechanisms of Lut-7-G in UC.

Financial support and sponsorship

This study was supported by the Scientific Research Fund of the National Natural Science Foundation of China [grant number 81673969].

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, *et al.* Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: A systematic review of population-based studies. Lancet 2017;390:2769-78.
2. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology 2011;140:1785-94.
3. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, *et al.* European Crohn's and Colitis Organization [ECCO]. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. J Crohns Colitis 2017;11:649-70.
4. Kobayashi T, Siegmund B, Le Berre C, Wei SC, Ferrante M, Shen B, *et al.* Ulcerative colitis. Nat Rev Dis Primers 2020;6:74.
5. Macpherson AJ, Yilmaz B, Limenitakis JP, Ganal-Vonarburg SC. IgA function in relation to the intestinal microbiota. Annu Rev Immunol 2018;36:359-81.
6. Zhang H, Gong C, Qu L, Ding X, Cao W, Chen H, *et al.* Therapeutic effects of triptolide via the inhibition of IL-1beta expression in a mouse model of ulcerative colitis. Exp Ther Med 2016;12:1279-86.
7. Tatiya-Aphiradee N, Chatuphonprasert W, Jarukamjorn K. Immune response and inflammatory pathway of ulcerative colitis. J Basic Clin Physiol Pharmacol 2018;30:1-10.
8. Wine E, Mack DR, Hyams J, Otley AR, Markowitz J, Crandall WV, *et al.* Interleukin-6 is associated with steroid resistance and reflects disease activity in severe pediatric ulcerative colitis. J Crohns Colitis 2013;7:916-22.
9. Solem CA, Loftus EV Jr, Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. Inflam Bowel Dis 2005;11:707-12.
10. Liu D, Yu X, Sun H, Zhang W, Liu G, Zhu L. Flos lonicerae flavonoids attenuate experimental ulcerative colitis in rats via suppression of NF-κB signaling pathway. Naunyn Schmiedebergs Arch Pharmacol 2020;393:2481-94.
11. Cho EJ, Shin JS, Noh YS, Cho YW, Hong SJ, Park JH, *et al.* Anti-inflammatory effects of methanol extract of Patrinia scabiosaefolia in mice with ulcerative colitis. J Ethnopharmacol 2011;136:428-35.
12. Gong L, Zou W, Zheng K, Shi B, Liu M. The Herba Patriniae (Caprifoliaceae): A review on traditional uses, phytochemistry, pharmacology and quality control. J Ethnopharmacol 2021;265:113264.
13. Wen-Lan L, Xue Z, Xin-Xin Y, Shuai W, Lin Z, Huan-Jun Z, *et al.* Species classification and bioactive ingredients accumulation of BaiJiangCao based on characteristic inorganic elements analysis by inductively coupled plasma-mass spectrometry and multivariate analysis. Pharmacogn Mag 2015;11:756-63.

14. Park CM, Song YS. Luteolin and luteolin-7-O-glucoside inhibit lipopolysaccharide-induced inflammatory responses through modulation of NF-κB/AP-1/PI3K-Akt signaling cascades in RAW 264.7 cells. Nutr Res Pract 2013;7:423-9.
15. Park CM, Song YS. Luteolin and luteolin-7-O-glucoside protect against acute liver injury through regulation of inflammatory mediators and antioxidative enzymes in GalN/LPS-induced hepatitic ICR mice. Nutr Res Pract 2019;13:473-9.
16. Wang X, Yuan T, Yin N, Ma X, Zhang Z, Zhu Z, *et al.* Luteoloside protects the uterus from *Staphylococcus aureus*-induced inflammation, apoptosis, and injury. Inflammation 2018;41:1702-16.
17. De Stefano A, Caporali S, Di Daniele N, Rovella V, Cardillo C, Schinzari F, *et al.* Anti-inflammatory and proliferative properties of luteolin-7-O-glucoside. Int J Mol Sci 2021;22. doi: 10.3390/ijms22031321.
18. Zhao HM, Xu R, Huang XY, Cheng SM, Huang MF, Yue HY, *et al.* Curcumin Suppressed Activation of Dendritic Cells via JAK/STAT/SOCS Signal in Mice with Experimental Colitis. Front Pharmacol 2016;7:455.
19. Tran CD, Ball JM, Sundar S, Coyle P, Howarth GS. The role of zinc and metallothionein in the dextran sulfate sodium-induced colitis mouse model. Dig Dis Sci 2007;52:2113-21.
20. Li Q, Tian Z, Wang M, Kou J, Wang C, Rong X, *et al.* Luteoloside attenuates neuroinflammation in focal cerebral ischemia in rats via regulation of the PPARγ/Nrf2/NF-κB signaling pathway. Int Immunopharmacol 2019;66:309-16.
21. Wang X, Yuan T, Yin N, Ma X, Zhang Z, Zhu Z, *et al.* Luteoloside Protects the Uterus from *Staphylococcus aureus*-Induced Inflammation, Apoptosis, and Injury. Inflammation 2018;41:1702-16.
22. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008;22:659-61.
23. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 1993;69:238-49.
24. Landy J, Ronde E, English N, Clark SK, Hart AL, Knight SC, *et al.* Tight junctions in inflammatory bowel diseases and inflammatory bowel disease-associated colorectal cancer. World J Gastroenterol 2016;22:3117-26.
25. Luissint AC, Parkos CA, Nusrat A. Inflammation and the intestinal barrier: Leukocyte-epithelial cell interactions, cell junction remodeling, and mucosal repair. Gastroenterology 2016;151:616-32.
26. Hermanns HM, Wohlfahrt J, Mais C, Hergovits S, Jahn D, Geier A. Endocytosis of pro-inflammatory cytokine receptors and its relevance for signal transduction. Biol Chem 2016;397:695-708.
27. Liau NPD, Laktyushin A, Lucet IS, Murphy JM, Yao S, Whitlock E, *et al.* The molecular basis of JAK/STAT inhibition by SOCS1. Nat Commun 2018;9:1558.
28. Durham GA, Williams JJL, Nasim MT, Palmer TM. Targeting SOCS proteinS to control JAK-STAT signaling in disease. Trends Pharmacol Sci 2019;40:298-308.
29. Choi CR, Al Bakir I, Ding NJ, Lee GH, Askari A, Warusavitarne J, *et al.* Cumulative burden of inflammation predicts colorectal neoplasia risk in ulcerative colitis: A large single-center study. Gut 2019;68:414-22.
30. Rogler G. Chronic ulcerative colitis and colorectal cancre. Cancer Lett 2014;345:235-41.
31. Yuan J, Cheng W, Zhang G, Ma Q, Li X, Zhang B, *et al.* Protective effects of iridoid glycosides on acute colitis via inhibition of the inflammatory response mediated by the STAT3/NF-κB pathway. Int Immunopharmacol 2020;81:106240.
32. Xie F, Zhang H, Zheng C, Shen XF. Costunolide improved dextran sulfate sodium-induced acute ulcerative colitis in mice through NF-κB, STAT1/3, and Akt signaling pathways. Int Immunopharmacol 2020;84:106567.
33. Zhu L, Gu P, Shen H. Protective effects of berberine hydrochloride on DSS-induced ulcerative colitis in rats. Int Immunopharmacol 2019;68:242-51.
34. Fu X, Sun F, Wang F, Zhang J, Zheng B, Zhong J, *et al.* Aloperine protects mice against DSS-induced colitis by PP2A-mediated PI3K/Akt/mTOR signaling suppression. Mediators Inflamm 2017;2017:5706152. doi: 10.1155/2017/5706152.
35. Chen Q, Duan X, Fan H, Xu M, Tang Q, Zhang L, *et al.* Oxymatrine protects against DSS-induced colitis via inhibiting the PI3K/AKT signaling pathway. Int Immunopharmacol 2017;53:149-57.
36. Cordes F, Foell D, Ding JN, Varga G, Bettenworth D. Differential regulation of JAK/STAT-signaling in patients with ulcerative colitis and Crohn's disease. World J Gastroenterol 2020;26:4055-75.
37. Cordes F, Lenker E, Weinhage T, Spille LJ, Bettenworth D, Varga G, *et al.* Impaired IFN-γ-dependent STAT3 activation is associated with dysregulation of regulatory and inflammatory signaling in monocytes of ulcerative colitis patients. Inflam Bowel Dis 2021;27:887-901.
38. Li JA, Wang YD, Wang K, Wang ZL, Jia DY, Yang BY, *et al.* Downregulation of miR-214-3p may contribute to pathogenesis of ulcerative colitis via targeting STAT6. BioMed Res Int 2017;2017:8524972. doi: 10.1155/2017/8524972.
39. Wick EC, LeBlanc RE, Ortega G, Robinson C, Platz E, Pardoll DM, *et al.* Shift from pStat6 to pStat3 predominance is associated with inflammatory bowel disease-associated dysplasia. Inflam Bowel Dis 2012;18:1267-74.
40. Rosen MJ, Frey MR, Washington MK, Chaturvedi R, Kuhnhein LA, Matta P, *et al.* STAT6 activation in ulcerative colitis: A new target for prevention of IL-13-induced colon epithelial cell dysfunction. Inflam Bowel Dis 2011;17:2224-34.
41. Danese S, Argollo M, Le Berre C, Peyrin-Biroulet L. JAK selectivity for inflammatory bowel disease treatment: Does it clinically matter? Gut 2019;68:1893-9.
42. De Oliveira T, Ramakrishnan M, Diamanti MA, Ziegler PK, Brombacher F, Greten FR. Loss of Stat6 affects chromatin condensation in intestinal epithelial cells causing diverse outcome in murine models of inflammation-associated and sporadic colon carcinogenesis. Oncogene 2019;38:1787-801.
43. Dickensheets H, Vazquez N, Sheikh F, Gingras S, Murray PJ, Ryan JJ, *et al.* Suppressor of cytokine signaling-1 is an IL-4-inducible gene in macrophages and feedback inhibits IL-4 signaling. Genes Immun 2007;8:21-7.
44. Chinen T, Komai K, Muto G, Morita R, Inoue N, Yoshida H, *et al.* Prostaglandin E2 and SOCS1 have a role in intestinal immune tolerance. Nat Commun 2011;2:190. doi: 10.1038/ncomms1181.
45. Hu J, Wang W, Hao Q, Zhang T, Yin H, Wang M, *et al.* Suppressors of cytokine signaling (SOCS)-1 inhibits neuroinflammation by regulating ROS and TLR4 in BV2 cells. Inflamm Res 2020;69:27-39.
46. Christophi GP, Rong R, Holtzapple PG, Massa PT, Landas SK. Immune markers and differential signaling networks in ulcerative colitis and Crohn's disease. Inflam Bowel Dis 2012;18:2342-56.