doi: 10.4149/gpb_2021044

Leonurine exerts a protective effect in dextran sodium sulfate-induced experimental inflammatory bowel disease mice model

Le Qi[1], Xi Chen[1], Ying Pan[2], Zhiyi Zha[3], Min Tang[2], Change Shi[2], Bingbing Yang[2] and Hongqian Wang[4]

[1] *Department of Gastroenterology, The First Affiliation Hospital of AnHui Medical University, Hefei City, Anhui Province, China*
[2] *Department of Gastroenterology, The Fourth Affiliated Hospital of Nanjing Medical University, Nanjing City, Jiangsu Province, China*
[3] *Department of Gastroenterology, Changzhou Fourth People's Hospital, Changzhou City, Jiangsu Province, China*
[4] *Department of Gastroenterology, AnHui Medical University, Hefei City, Anhui Province, China*

Abstract. Inflammatory bowel disease (IBD) is a common chronic inflammatory gastrointestinal disease**.** The therapeutic strategies of IBD are limited. IBD mouse models were established by administering 4% dextran sodium sulfate (DSS), which were further treated with Leonurine (7.5, 15, 30 mg/kg). The disease phenotypes, cell apoptosis, inflammation factors and oxidative stress related chemicals were evaluated. In addition, the potential related mechanism was also explored. Consequently, Leonurine ameliorated IBD-associated disease phenotypes and increase colon lengths and inhibited intestinal cell apoptosis in DSS-induced IBD mice. In addition, Leonurine reduced the expression of inflammation factors and oxidative stress level in DSS-induced IBD mice. Finally, Leonurine inhibited TLR4/NF-κB signaling pathway and activated of Nrf2/HO-1 signaling pathway. Leonurine can ameliorate IBD-induced apoptosis, inflammation response and oxidative stress *via* the activation of Nrf2/HO-1 signaling pathway and suppression of TLR4/NF-κB pathway.

Key words: Leonurine — Inflammatory bowel disease — Apoptosis — Oxidative stress — Nrf2/HO-1 signaling pathway — TLR4/NF-κB pathway

Introduction

Inflammatory bowel disease (IBD) is a common chronic inflammatory gastrointestinal disease including Crohn's disease and ulcerative colitis (Arkuran et al. 2000). IBD is characteristic by chronic diarrhea, abdominal pain, rectal bleeding, weight loss, and a shortened colon. Although the etiology of IBD remains uncertain, the pathogenesis of IBD is known to be related to irregular immune response, intestinal microflora, and genetic and environmental factors (Baumgart et al. 2007; de Souza et al. 2016). For the treatment of IBD, currently commonly used drugs include amino salicylate, corticosteroids and immunosuppressants, but these drugs have therapeutic limitations and cause side effects (Mowat et al. 2011). Vitamin D has recently been recognized as an immunoregulator, and vitamin D can regulate gastrointestinal inflammation and affect the gut microbiome (Kakodkar et al. 2017). Previous study has shown that vitamin D supplementation is available and can be a therapeutic option for IBD (Tabatabaeizadeh et al. 2018; Arihiro et al. 2019). In addition, the therapeutic effect of targeted drugs on single target of IBD (such as IL-7, CCR9 receptor, etc.) is also

Correspondence to: Xi Chen, Department of Gastroenterology, The First Affiliation Hospital of AnHui Medical University, No. 218, Jixi Road, Shushan District, Hefei City, Anhui Province, China
E-mail: chenxi12258@163.com

not obvious (Alimohammadi et al. 2020). Therefore, there is an urgent need to develop a multi-target drug to treat IBD.

Traditional Chinese medicine monomer has multi-target characteristics and has been used for immunoregulation in many therapeutic fields (Chen et al. 2018). Leonurine, the active ingredient of *Herba Leonurine*, has been shown to have a variety of biological activities, including heart protection, antioxidant, anti-apoptosis and anti-inflammatory properties (Hu et al. 2019; Xu et al. 2020). Leonurine has been shown to regulate multiple signaling pathways. For example, Leonurine improves the inflammatory response to LPS-induced endometritis by inhibiting TLR4 and NF-κB expression (Wu et al. 2018). Furthermore, Leonurine can activate Nrf2 signaling pathway and improve D-galactose-induced aging in mice (Chen P et al. 2019), and it can also relieve inflammatory symptoms and antioxidant stress in mice with ischemic stroke (Xie et al. 2019). In addition, motherwort alkaloid also has anti-apoptotic effect. It has been reported that Leonurine inhibits NF-κB and MAPK signaling and inhibits chondrocyte apoptosis, thereby improving adverse symptoms of arthritis (Hu et al. 2019). Leonurine also inhibits osteoclast production and prevents osteoporosis associated with estrogen deficiency by inhibiting the NF-κB and PI3K/Akt signaling pathways (Yuan et al. 2015). However, there are few reports about the effects of Leonurine on the IBD model, and the mechanism is not clear. Therefore, in this study, we aim to clarify the role of Leonurine in the IBD mice. We also primarily explore the underlying regulatory mechanism of Leonurine in the IBD mice.

Materials and Methods

Animals and experiments

A total of 30 ($n = 5$) 20-week-old female C57BL/6 mice (Jackson Laboratory, Bar Harbor, USA) weighing at least 26 g were used in this study. Each cage has 6 mice, with the light/dark cycle 12:12 h (lights turn on at 7 o'clock), and food and water are provided freely. All mice were acclimatized to the feed group and water bottle for a week before treatment began. All procedures were approved by the First Affiliation Hospital of AnHui Medical University. Dextran sodium sulfate (DSS; molecular weight 40,000; Sigma-Aldrich, USA) was mixed in their drinking water (4% v/v) for 7 days, followed by 7 days of recovery with distilled water for consumption. Mice were grouped into 5 groups with 6 mice in each group: sham, DSS model, DSS + Leonurine (7.5 mg/kg), DSS + Leonurine (15 mg/kg), and DSS + Leonurine (30 mg/kg). The Sham group animals were administered distilled water. The mice were treated with different dose of Leonurine by gavage. The mice's survival and weight were monitored daily.

Assessment of disease activity index

To assess the severity of IBD, IBD was scored based on body weight, stool consistency, and blood content in the stool. Weight score 0, none; Score 1, 5%; Score 2, 5–10%; Score 3, 10−20%; Score 4, >20%. Diarrhea score: 0, normal; 2 points, loose stool; 4 points, watery diarrhea. Blood in stool score: 0, normal; 2 points, mild bleeding; 4 points, massive bleeding. The disease activity index (DAI) score was derived from the above score and used to assess the severity of IBD.

Tissue collection

The mice were euthanized by carbon dioxide (CO_2) asphyxia. The mice were dissected, the colon tissue was separated, washed in PBS solution, and the excess water was removed. The full lengths of the colon were measured and then tissues were weighed. Colon segments for histology analysis were placed into tissue embedding cassettes for 12 h and fixed in 10% neutral buffered formalin. The segments for other analyses were snap frozen in liquid nitrogen and then stored at −80°C.

Tissue processing

Colon samples were sectioned at a thickness of 5 μm and collected on the slides in a ratio of 1:10. At least two slides were collected from each mouse, with each containing four or more sections. Finally, staining was performed with hematoxylin and eosin (H&E), followed by pathological evaluation with Alcian blue solution. The stained slides were imaged at 40× magnification using an automatic imager (Leica, model SL801).

TUNEL

The TUNEL staining was used to detect the level of apoptosis in the colon tissue according to the manufacturer's instructions (Roche, Shanghai, China). Briefly, the samples were fixed by 4% PFA in PBS (phosphate buffered saline) for 30 min, incubated with H_2O_2 for 10 min, and then incubated with PBS containing 0.3% Triton X-100 for 2 min, followed by the TUNEL reaction at 37°C for 1 h in the dark. Finally, DAPI was applied to the reaction for 5 min and an *in situ* cell death detection kit was used to detect apoptosis. The images were obtained with a fluorescence microscope.

Western blotting

Colon tissue was ground into powder in liquid nitrogen, and then RIPA buffer containing protease inhibitor was added immediately, and the protein concentration of samples in each group was determined. 40 μg proteins from each sample

were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane activated with methanol. The protein was sealed with 5% skimmed milk for 1 h at room temperature. Then the primary antibodies were incubated at 4°C overnight, the antibodies incubated mainly include anti-BAX (1:5000, ab32503, Abcam), anti-Cleaved Caspase-3 (1:5000, ab214430, Abcam), anti-BCL-2 (1:5000, ab182858, Abcam), anti-TLR4 (1:500, ab217274, Abcam), anti-p-NF-κB (phosphor S536,1:5000, ab239882, Abcam), anti-NF-κB (1:2000, ab16502, Abcam), anti-Nrf2 (1:1000, ab92946, Abcam), anti-Heme Oxygenase 1 (HO-1;1:2000, ab52947, Abcam) and anti-β-actin (1:5000, ab8227, Abcam). The second day, the membranes were washed with PBST and incubated with the goat anti-rabbit immunoglobulin G (IgG) for 1 h at 37°C, and then target proteins were visualized by chemiluminescence (Millipore, USA), and imaged using imaging software (GE Healthcare, Life Sciences, USA).

RT-qPCR

The total RNA in the colon tissues was extracted with TRIzol Reagent (Gibco, USA) according to the manufacturer's instructions, and then cDNAs were synthesized by the Transcriptor first-strand cDNA synthesis kit (Roche Diagnostics, Mannheim, Germany). The mRNA expressions were determined using RT-qPCR and β-actin was used as an internal reference. The primers were listed as following: IL-6, F: AAGTCCGGAGAGGAGACTTC; R: TGGATGGTCTTGGTCCTTAG. TNF-α, F: AACTCCAGGCGGTGCCTATG; R: TCCAGCTGCTCCTCCACTTG; IL-1β, F: AGCTTCAGGCAGGCAGTATC; R: TCATCTCGGAGCCTGTAGTG. β-actin, F :5′- TCACCCACATGTGCCCATCTACGA -3′; R: 5' -CAGCGGAACCGCTCATTGCCAATGG-3'.

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of TNF-α, IL-6 and lL-1β in the tissue suspension were determined using commercial ELISA kit including Mouse IL-6 ELISA Kit (ab222503, Abcam, Cambridge, MA, USA), Mouse TNF-α ELISA Kit (ab208348, Abcam) and Mouse lL-1β ELISA Kit (ab197742, Abcam) following the manufacturer's instructions.

Measurement of malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione (GSH) activity in IBD mice

Colon tissue was ground into powder in liquid nitrogen, and kept at 4°C. MDA, SOD and GSH activities in colon tissues were determined using relevant assay kits (#A001-3-2, #A003-1-2, #A006-2-1, Nanjing Jiancheng Biological Engineering Research Institute Co. Ltd, Najing, China), respectively.

Statistical analysis

All quantitative analyses and graphical representations in the study were performed using Graphpad Prism (Prism 6.0 for Windows). For comparisons among groups, analyses of variance (ANOVAs) followed by *post hoc* analyses using Tukey tests were performed. Specifically, body weight data were analyzed using mixed Factorial ANOVAs with time. All graphs are shown as group means ± standard error of the mean (SEM). ** $p < 0.01$ compared with sham group; $^{\#\#}$ $p < 0.01$ compared with DSS group.

Results

Leonurine ameliorated IBD-associated disease phenotypes and increase colon lengths

Administration of DSS solution consistently induced significant body weight loss of mice compared with the sham group (Fig. 1A), and Leonurine significantly reversed the body weight loss caused by DSS in a dose-dependent manner (Fig. 2A). In addition, the DAI was scored in the 5 group mice, and the results showed that the DAI from pow to high ranked as the following: Sham group, DSS + Leonurine (30 mg/kg), DSS + Leonurine (15 mg/kg), DSS + Leonurine (7.5 mg/kg) and DSS group (Fig. 2B). The represent sample of each group was shown in Figure 2C, and the length was measured. The results showed that the sham group had the normal length of colon, while the DSS group had the shortest colon, and Leonurine can reversed the shortened colon caused by DSS in a dose-dependent manner (Fig. 2D). Colon samples from mice were stained with H&E and Alcian blue and pathological changes were shown in Figure 2E. The results indicated that inflammatory cells infiltration, mucosal necrosis, ulcerations and cryptal cell loss were observed in the colon in DSS group, while the disease phenotypes were improved in Leonurine group. These results indicated that Leonurine can alleviate the disease phenotypes induced by DSS in mice.

Leonurine inhibited intestinal cell apoptosis in DSS-induced IBD mice

As depicted in Figure 2A, the cryptal cells were almost destroyed and the TUNEL positive cells (red) were enhanced in DSS group compared with the Sham group, which suggested that DSS induced severe apoptosis in intestinal cell. Furthermore, the apoptosis induced by DSS can be ameliorated by Leonurine treatment in a dose-dependent manner.

We assessed the expressions of apoptosis-related proteins. BAX and cleaved caspase-3 were upregulated in DSS group and decreased in Leonurine treatment group in a dose-dependent manner, while BCL-2 had contrary result. The results indicated that Leonurine can alleviate the apoptosis induced by DSS in mice.

Leonurine reduced the inflammation factors level in DSS-induced IBD mice

As shown in Figure 3A, ELISA results showed that the serum inflammation factors including TNF-α, IL-6 and L-1β were elevated in DSS group and decreased in Leonurine group in a dose-dependent manner. The expressions of inflammation factors in intestinal tissue were detected by RT-qPCR, and the results had the similar trend with the serum inflammation factor protein levels (Fig. 3B). These results indicated that Leonurine can alleviate the inflammation response in DSS-induced IBD mice.

Leonurine alleviated oxidative stress in DSS-induced IBD mice

The oxidative stress index in intestinal tissue was detected by commercial kit, and the results showed that the MDA

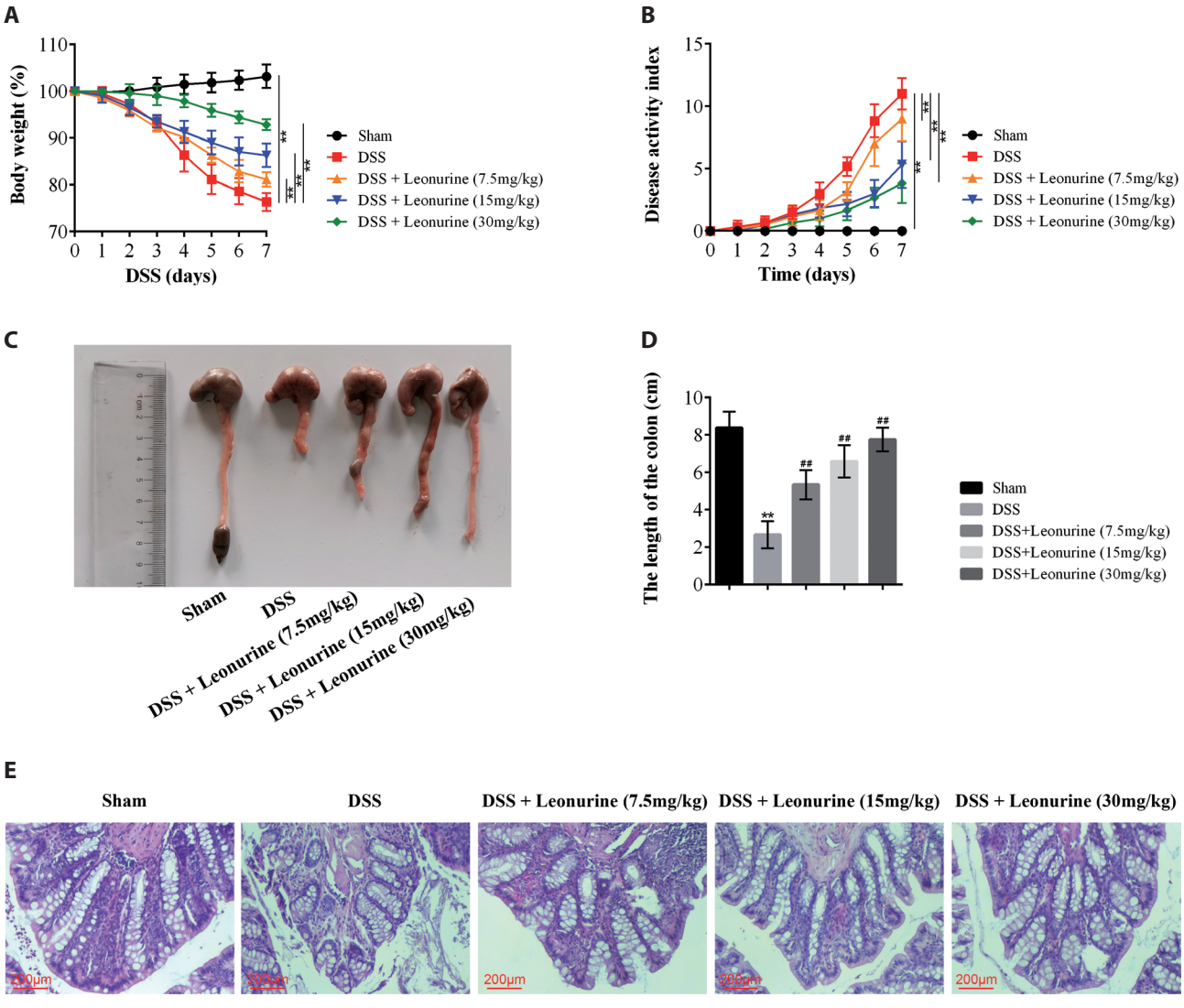

Figure 1. Leonurine ameliorated IBD-associated disease phenotypes and increased colon lengths. **A.** Time-dependent increasing of body weight of IBD mice and dose-dependent positive effect of Leonurine. **B.** The clinic disease activity index (DAI) of mice. **C.** The colon morphology of mice. **D.** Colon length statistical analysis. **E.** H&E staining results of colon in mice. Magnification ×40. ** $p < 0.01$ *vs.* Sham group; $^{\#\#}$ $p < 0.01$ *vs.* DSS group. IBD, inflammatory bowel disease; DSS, IBD mouse model established by administering 4% dextran sodium sulfate.

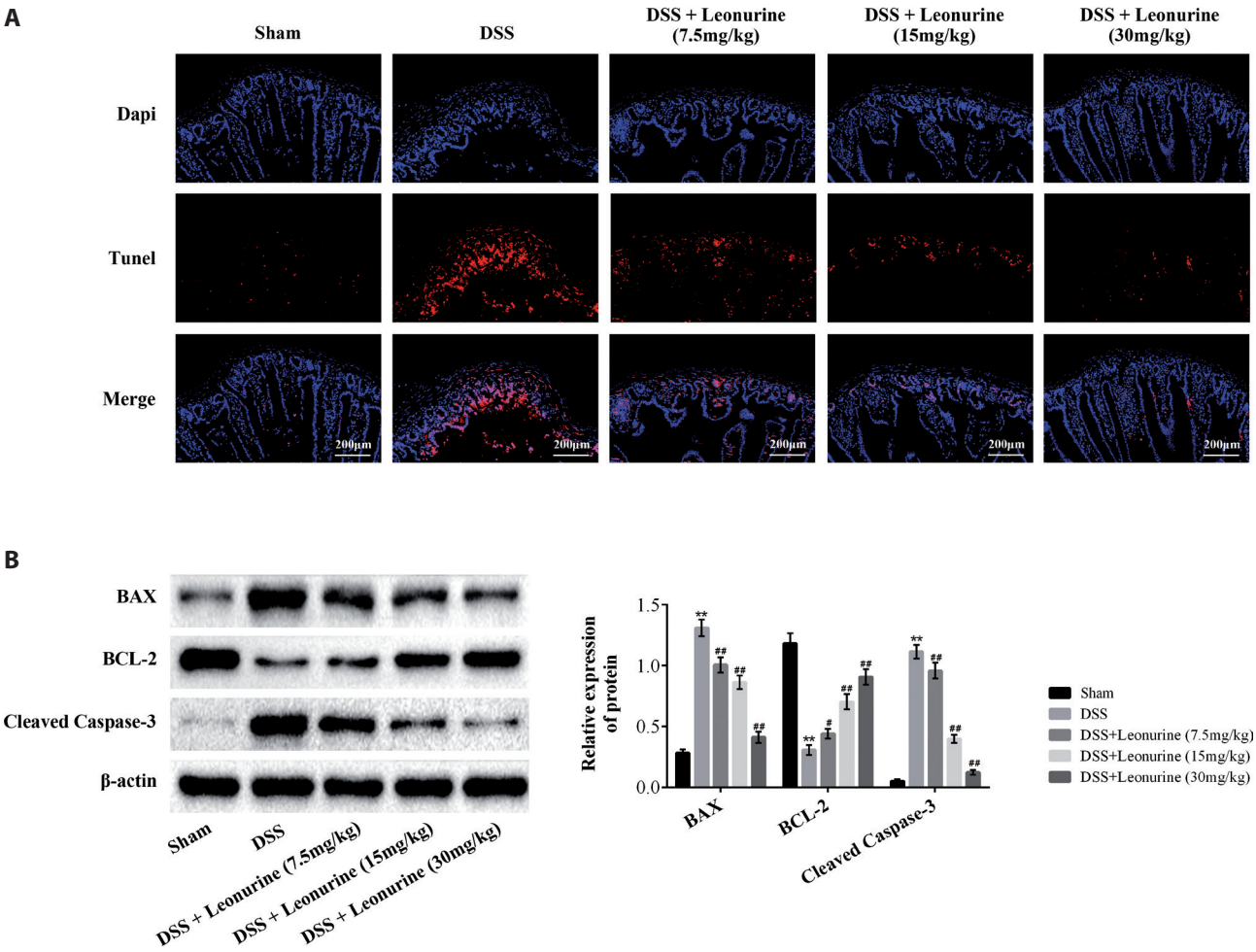

Figure 2. Leonurine inhibited intestinal cell apoptosis in DSS-induced IBD mice. **A.** TUNEL staining of colon tissue in mice. **B.** Western blot result of BAX, BCL-2 and cleaved caspase-3 in the colon tissues. ** $p < 0.01$ *vs.* Sham group; ## $p < 0.01$ *vs.* DSS group. For abbreviations, see Fig. 1.

level was elevated in DSS group and decreased in Leonurine group in a dose-dependent manner (Fig. 4). The SOD level and GSH level were reduced in DSS group and increased in Leonurine group in a dose-dependent manner. These results indicated that Leonurine can increased the levels of ROS scavengers including SOD and GSH, and reduce oxidative stress product MDA, suggesting that Leonurine alleviated the oxidative stress in DSS-induced IBD mice.

Leonurine inhibited TLR4/NF-κB signaling pathway and activated of Nrf2/HO-1 signaling pathway

To detect the inflammation associated pathway, the protein members of TLR4/NF-κB signaling pathway were detected by Western blot. As shown in Figure 5A, the protein expression of TLR4 and p-NF-κB was upregulated in DSS group and downregulated in Leonurine group in a dose-dependent manner. In addition, the oxidative stress associated Nrf2/HO-1 signaling pathway was also investigated. The protein expressions of Nrf2 and HO-1 were downregulated in DSS group and gradually upregulated in Leonurine group in a dose-dependent manner (Fig. 5B). These results suggested that Leonurine inhibited TLR4/NF-κB signaling pathway and activated of Nrf2/HO-1 signaling pathway, which might be the protective mechanism of Leonurine in IBD mice.

Discussion

The DSS-induced colitis model is commonly used to study IBD in mice, because DSS is a sulfated polysaccharide especially toxic to the colonic epithelium. In current study, we established the IBD mouse model *via* giving 4% DSS

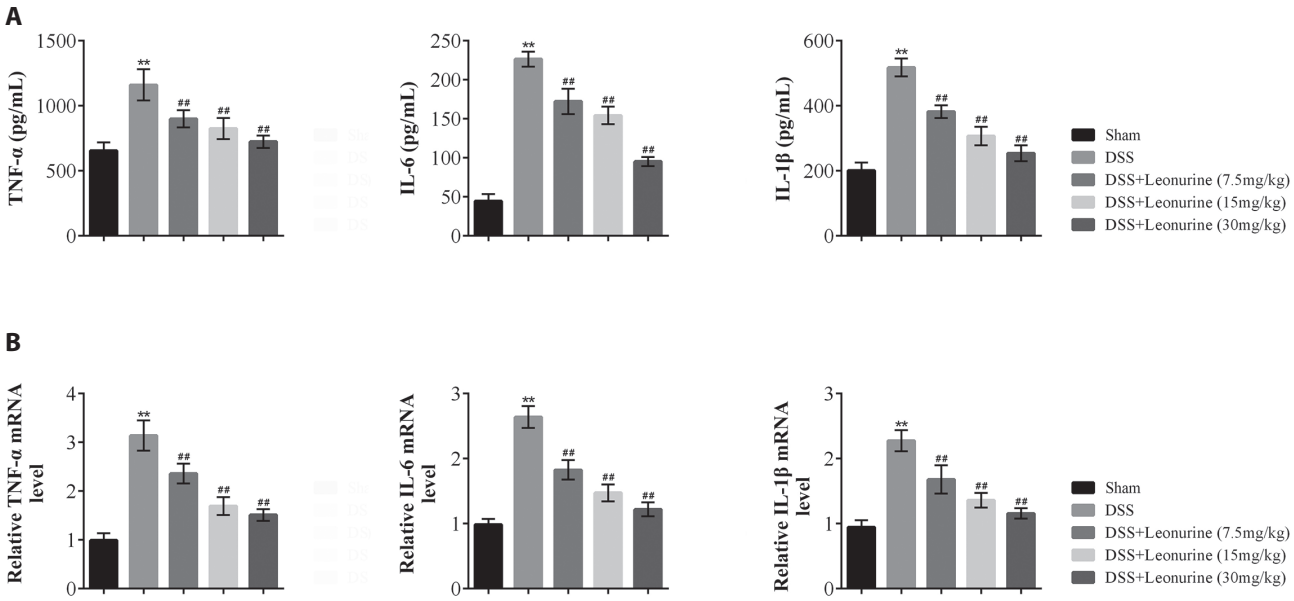

Figure 3. Leonurine reduced the inflammation factors level in DSS-induced IBD mice. **A.** ELISA results of the concentrations of TNF-α, IL-6 and lL-1β in the serum of mice. **B.** RT-qPCR results of the expressions of TNF-α, IL-6 and lL-1β in colonic tissues. ** $p < 0.01$ *vs.* Sham group; ## $p < 0.01$ *vs.* DSS group. For abbreviations, see Fig. 1.

for 7 consecutive days. The IBD model was successfully established evidenced by the shortened colon length, inflammatory cells infiltration, mucosal necrosis, ulcerations and cryptal cells loss.

Recent study has demonstrated that apoptosis suppression can attenuate the experimental IBD in rats (Arab et al. 2021). In current study, we found that DSS induced obvious cell apoptosis in intestinal epithelial cells, while Leonurine contributed to apoptosis suppression, which suggest that Leonurine might attenuate the experimental IBD in mice *via* inhibiting apoptosis.

Inflammation is the pathological feature of IBD. TLR4 was identified as a signal receptor for lipopolysaccharide in the outer membrane of Gram-negative bacteria (Poltorak et al. 1998). Mechanically, TLR4 binds to LPS to activate NF-κB, subsequently, effector cells are stimulated to secrete TNF-α and other cytokines, resulting in the induction of inflammatory response (Wu et al. 2019). Thus, TLR4 is also required for activating pro-inflammatory cell signaling pathways in response to endogenous molecules (Schröder et al. 2005). A lot of articles have shown that activation/inhibition of TLR4 is associated with inflammation presence. In IBD, inhibition of TLR4 can significantly inhibit the occurrence of inflammatory response and ultimately play a role in intestinal protection (Matsunaga et al. 2011). In addition, high expression of TLR4 was significantly associated with IBD risk (Liu et al. 2019). TLR4/NF-κB pathway suppression has been demonstrated to suppress IBD in a variety articles (Chen X et al. 2019; Liu et al. 2019; Dejban et al. 2020). Our experiments showed that

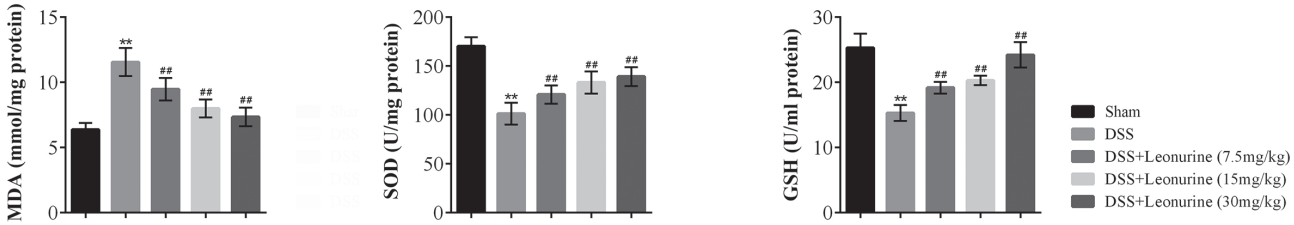

Figure 4. Leonurine alleviated oxidative stress in DSS-induced IBD mice. The level of malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione (GSH) activity in IBD mice. ** $p < 0.01$ *vs.* Sham group; ## $p < 0.01$ *vs.* DSS group. For abbreviations, see Fig. 1.

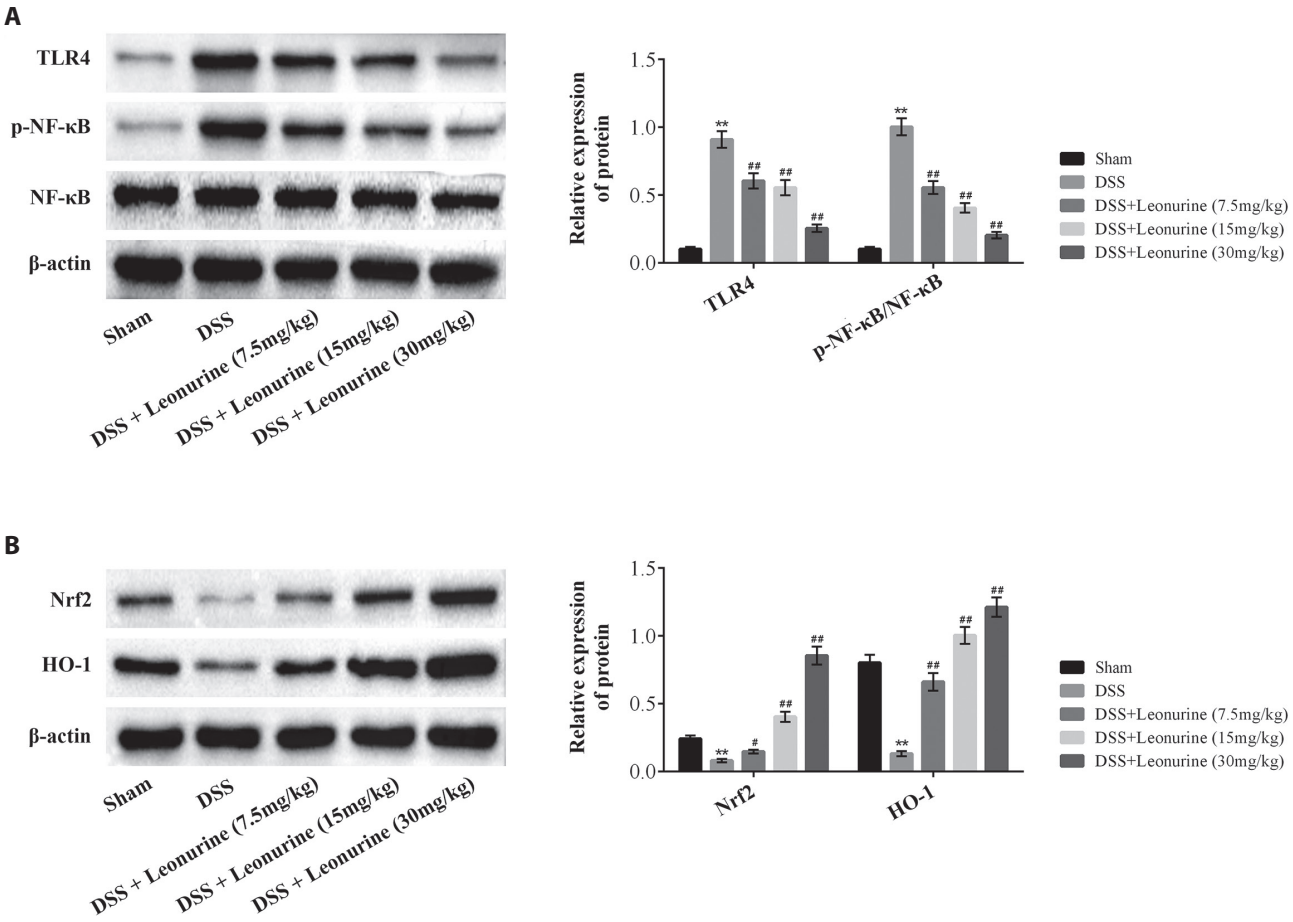

Figure 5. Leonurine inhibited TLR4/NF-κB signaling pathway and activated Nrf2/HO-1 signaling pathway. **A.** Western blot result of TLR4, p-NF-κB and NF-κB in the colon tissues. **B.** Western blot result of Nrf2 and HO-1 in the colon tissues. ** $p < 0.01$ *vs.* Sham group; ## $p < 0.01$ *vs.* DSS group. For abbreviations, see Fig. 1.

Leonurine inhibited TLR4/NF-κB pathway and Leonurine might inhibit inflammation in the experimental IBD in mice *via* inhibiting TLR4/NF-κB pathway.

Oxidative stress has recently been implicated in the pathogenesis and procession in IBD (Tian et al. 2017). Nrf2 is an important antioxidant regulator, which can combine with antioxidant reaction elements to regulate the expression of HO-1 and NQO1 (Alimohammadi et al. 2020). Nrf2 is involved in IBD (Piotrowska et al. 2021). Several reports have shown that activation of Nrf2 can reduce inflammatory response and oxidative stress in IBD, thus protecting intestinal tissues (Wang et al. 2018; Pompili et al. 2019). Khor et al. (2006) used a colitis model induced by experimental DSS and found that mice with the Nrf2 gene (Nrf2-/-) knocked out were more likely to acquire histological features of colitis (such as short colonic and rectal bleeding). Furthermore, a lot of drugs were believed to be potential benefit for IBD due to the Nrf2/HO-1 signaling pathway activation (Zhu et al. 2018; Mei et al. 2019; Tang et al. 2020; Zhang et al. 2020; Arab et al. 2021). We found that the Nrf2/HO-1 signaling pathway was activated after treating with Leonurine in mice, and Leonurine might ameliorate oxidative stress in IBD *via* activating Nrf2/HO-1 signaling pathway, which is consistent with the above literatures.

Conclusion

In summary, our study demonstrated that Leonurine can ameliorate IBD in experimental mouse by inhibiting apoptosis, inflammation response and oxidative stress. The underlying mechanism might be associated with the activation of Nrf2/HO-1 signaling pathway and suppression of TLR4/NF-κB pathway.

Funding. This work was supported by the Foundation of Anhui Medical University. (Grant No. 2020xkj038).

Conflict of interests. The authors state that there are no conflicts of interest to disclose.

Ethics approval. Ethical approval was obtained from the Ethics Committee of the First Affiliation Hospital of AnHui Medical University for the use of animals and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines (Approval No. 2019-0432).

Statement of human and animal rights. All procedures in this study were conducted in accordance with the Animal Ethics Committee of the First Affiliation Hospital of AnHui Medical University approved protocols.

Authors' contributions. LQ and XC designed the study, supervised the data collection; YP and ZZ analyzed the data, interpreted the data; MT, CS, BY, and HW prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

References

Alimohammadi N, Koosha F, Rafeian-Kopaei M (2020): Current, new and future therapeutic targets in inflammatory bowel disease: A systematic review. Curr. Pharm. Des. **26,** 2668-2675 https://doi.org/10.2174/1381612826666200406081920

Arab HH, Al-Shorbagy MY, Saad MA (2021): Activation of autophagy and suppression of apoptosis by dapagliflozin attenuates experimental inflammatory bowel disease in rats: Targeting AMPK/mTOR, HMGB1/RAGE and Nrf2/HO-1 pathways. Chem. Biol. Interact. **335,** 109368 https://doi.org/10.1016/j.cbi.2021.109368

Arihiro S, Nakashima A, Matsuoka M, Suto S, Uchiyama K, Kato T, Mitobe J, Komoike N, Itagaki M, Miyakawa Y, et al. (2019): Randomized trial of Vitamin D supplementation to prevent seasonal influenza and upper respiratory infection in patients with inflammatory bowel disease. Inflamm. Bowel Dis. **25,** 1088-1095 https://doi.org/10.1093/ibd/izy346

Arkuran C, McComb P (2000): Crohn's disease and tubal infertility: the effect of adhesion formation. Clin. Obstet. Gynecol. **27,** 12-13

Baumgart DC, Carding SR (2007): Inflammatory bowel disease: cause and immunobiology. Lancet **369,** 1627-1640 https://doi.org/10.1016/S0140-6736(07)60750-8

Chen P, Chen F, Zhou BH (2019): Leonurine ameliorates D-galactose-induced aging in mice through activation of the Nrf2 signalling pathway. Aging **11,** 7339-7356 https://doi.org/10.18632/aging.101733

Chen S, Xu MB, Zhou XL, Rong PQ, Jin TY, Zheng GQ (2018): Chinese herbal medicine for Myasthenia Gravis: A systematic review and meta-analysis. Front. Pharmacol. **9,** 969 https://doi.org/10.3389/fphar.2018.00969

Chen X, Liu G, Yuan Y, Wu G, Wang S, Yuan L (2019): NEK7 interacts with NLRP3 to modulate the pyroptosis in inflammatory bowel disease via NF-κB signaling. Cell Death Dis. **10,** 906 https://doi.org/10.1038/s41419-019-2157-1

de Souza HS, Fiocchi C (2016): Immunopathogenesis of IBD: current state of the art. Nat. Rev. Gastroenterol. Hepatol. **13,** 13-27 https://doi.org/10.1038/nrgastro.2015.186

Dejban P, Nikravangolsefid N, Chamanara M, Dehpour A, Rashidian A (2020): The role of medicinal products in the treatment of inflammatory bowel diseases (IBD) through inhibition of TLR4/NF-kappaB pathway. Phytother. Res. **35,** 835-845 https://doi.org/10.1002/ptr.6866

Hu PF, Sun FF, Qian J (2019): Leonurine exerts anti-catabolic and anti-apoptotic effects via nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways in chondrocytes. Med. Sci. Monit. **25,** 6271-6280 https://doi.org/10.12659/MSM.916039

Kakodkar S, Mutlu EA (2017): Diet as a therapeutic option for adult inflammatory bowel disease. Gastroenterol. Clin. North Am. **46,** 745-767 https://doi.org/10.1016/j.gtc.2017.08.016

Khor TO, Huang MT, Kwon KH, Chan JY, Reddy BS, Kong AN (2006): Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. Cancer Res. **66,** 11580-11584 https://doi.org/10.1158/0008-5472.CAN-06-3562

Liu Y, Zhao J, Zhao Y, Zong S, Tian Y, Chen S, Li M, Liu H, Zhang Q, Jing X, et al. (2019): Therapeutic effects of lentinan on inflammatory bowel disease and colitis-associated cancer. J. Cell. Mol. Med. **23,** 750-760 https://doi.org/10.1111/jcmm.13897

Matsunaga N, Tsuchimori N, Matsumoto T, Ii M (2011): TAK-242 (resatorvid), a small-molecule inhibitor of Toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. Mol. Pharmacol. **79,** 34-41 https://doi.org/10.1124/mol.110.068064

Mei Y, Wang Z, Zhang Y, Wan T, Xue J, He W, Luo Y, Xu Y, Bai X, Wang Q, Huang Y (2019): FA-97, a new synthetic caffeic acid phenethyl ester derivative, ameliorates DSS-induced colitis against oxidative stress by activating Nrf2/HO-1 pathway. Front. Immunol. **10,** 2969 https://doi.org/10.3389/fimmu.2019.02969

Mowat C, Cole A, Windsor A, Ahmad T, Arnott I, Driscoll R, Mitton S, Orchard T, Rutter M, Younge L, et al. (2011): Guidelines for the management of inflammatory bowel disease in adults. Gut **60,** 571-607 https://doi.org/10.1136/gut.2010.224154

Piotrowska M, Swierczynski M, Fichna J, Piechota-Polanczyk A (2021): The Nrf2 in the pathophysiology of the intestine: Molecular mechanisms and therapeutic implications for inflammatory bowel diseases. Pharmacol. Res. **163,** 105243 https://doi.org/10.1016/j.phrs.2020.105243

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, et al. (1998): Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science **282,** 2085-2088 https://doi.org/10.1126/science.282.5396.2085

Pompili S, Sferra R, Gaudio E, Viscido A, Frieri G, Vetuschi A, Latella G (2019): Can Nrf2 modulate the development of intestinal fibrosis and cancer in inflammatory bowel disease? Int. J. Mol. Sci. **20,** 4061

https://doi.org/10.3390/ijms20164061
Schröder NW, Schumann RR (2005): Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. Lancet Infect. Dis. **5,** 156-164 https://doi.org/10.1016/S1473-3099(05)01308-3
Tabatabaeizadeh SA, Tafazoli N, Ferns GA, Avan A, Ghayour-Mobarhan M (2018): Vitamin D, the gut microbiome and inflammatory bowel disease. J. Res. Med. Sci. **23,** 75 https://doi.org/10.4103/jrms.JRMS_606_17
Tang B, Zhu J, Zhang B, Wu F, Wang Y, Weng Q, Fang S, Zheng L, Yang Y, Qiu R, et al. (2020): Therapeutic potential of triptolide as an anti-inflammatory agent in dextran sulfate sodium-induced murine experimental colitis. Front. Immunol. **11,** 592084 https://doi.org/10.3389/fimmu.2020.592084
Tian T, Wang Z, Zhang J (2017): Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. Oxid. Med. Cell. Longev. **2017,** 4535194 https://doi.org/10.1155/2017/4535194
Wang K, Lv Q, Miao YM, Qiao SM, Dai Y, Wei ZF (2018): Cardamonin, a natural flavone, alleviates inflammatory bowel disease by the inhibition of NLRP3 inflammasome activation via an AhR/Nrf2/NQO1 pathway. Biochem. Pharmacol. **155,** 494-509 https://doi.org/10.1016/j.bcp.2018.07.039
Wu CP, Bi YJ, Liu DM, Wang LY (2019): Hsa-miR-375 promotes the progression of inflammatory bowel disease by upregulating TLR4. Eur. Rev. Med. Pharmacol. Sci. **23,** 7543-7549
Wu H, Dai A, Chen X, Yang X, Li X, Huang C, Jiang K, Deng G (2018): Leonurine ameliorates the inflammatory responses in lipopolysaccharide-induced endometritis. Int. Immunopharmacol. **61,** 156-161 https://doi.org/10.1016/j.intimp.2018.06.002
Xie YZ, Zhang XJ, Zhang C, Yang Y, He JN, Chen YX (2019): Protective effects of leonurine against ischemic stroke in mice by activating nuclear factor erythroid 2-related factor 2 pathway. CNS Neurosci. Ther. **25,** 1006-1017 https://doi.org/10.1111/cns.13146
Xu W, Cui J, Zhou F, Bai M, Deng R, Wang W (2020): Leonurine protects against dexamethasone-induced cytotoxicity in pancreatic β-cells via PI3K/Akt signaling pathway. Biochem. Biophys. Res. Commun. **529,** 652-658 https://doi.org/10.1016/j.bbrc.2020.05.184
Yuan FL, Xu RS, Jiang DL, He XL, Su Q, Jin C, Li X (2015): Leonurine hydrochloride inhibits osteoclastogenesis and prevents osteoporosis associated with estrogen deficiency by inhibiting the NF-κB and PI3K/Akt signaling pathways. Bone **75,** 128-137 https://doi.org/10.1016/j.bone.2015.02.017
Zhang Y, Yan T, Sun D, Xie C, Wang T, Liu X, Wang J, Wang Q, Luo Y, Wang P, et al. (2020): Rutaecarpine inhibits KEAP1-NRF2 interaction to activate NRF2 and ameliorate dextran sulfate sodium-induced colitis. Free Radic. Biol. Med. **148,** 33-41 https://doi.org/10.1016/j.freeradbiomed.2019.12.012
Zhu S, Fu W, Zhang L, Fu K, Hu J, Jia W, Liu G (2018): LINC00473 antagonizes the tumour suppressor miR-195 to mediate the pathogenesis of Wilms tumour via IKKalpha. Cell Prolif. **51,** e12416 https://doi.org/10.1111/cpr.12416

Received: September 1, 2021
Final version accepted: October 26, 2021