

Original Article

Deoxyschizandrin treats mice with ulcerative colitis possibly via the TLR4/NF- κ B signaling pathway

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Abstract: Objective: This study aimed to investigate the effects and mechanisms of deoxyschizandrin (DSD) on treatment of ulcerative colitis (UC). Methods: The models of mice with UC were established through dextran sulfate sodium (DSS) administration, and the successful models were treated with DSD. The therapeutic effects of DSD on UC mice were evaluated and its behind mechanisms were analyzed. Results: After DSS induction, the mice showed increased body weight and colon length, worse disease activity index (DAI) and body inflammation, oxidative stress injury and increased apoptosis of colonic epithelial cells, which were remarkably relieved after DSD intervention. Besides, the levels of TLR4, MyD88 and NF- κ B in the colon tissues were elevated in UC mouse models, while DSD treatment reduced the levels of these markers. Conclusion: DSD can alleviate the symptoms of mice with DSS-induced UC via inhibiting body inflammation, improving oxidative stress and reducing the apoptosis of colonic epithelial cells, which may be attributed to DSD inhibition of the TLR4/NF- κ B signaling pathway.

Keywords: Deoxyschizandrin, ulcerative colitis, TLR4/NF- κ B, mice

Introduction

As an inflammatory disease of the large intestine, ulcerative colitis (UC) is characterized by abdominal pain, bloody diarrhea and fecal urgency [1]. Compared with general population, patients with the disease are more likely to undergo colectomy and suffer from colorectal cancer [2]. At present, there is no radical cure for UC, so drugs are mainly used to relieve symptoms and to prevent recurrence in clinical practice [3]. There are many drugs such as 5-aminosalicylic acids, glucocorticoids and immunosuppressants, all of which have certain side effects that would limit their clinical application [4]. Therefore, it is of significant clinical relevance to find potential therapeutic drugs that are safer and more effective.

Deoxyschizandrin (DSD) is a bioactive lignan that is mainly derived from the dried and mature fruits of Magnoliaceae, *Schisandra chinensis*. In addition to many positive medicinal values, such as anti-inflammatory, anti-oxidative and anti-tumor effects [5, 6], DSD has also

shown certain therapeutic effects on UC mice [7], but its potential mechanism in UC treatment has not been explored. TLR4/NF- κ B, a classical signaling pathway, is known to play a pivotal function during the pathogenesis of various inflammatory diseases [8, 9]. For instance, through inhibition of the TLR4/NF- κ B pathway, baicalin may protect people from developing colitis [10], while lycium barbarum polysaccharide is able to reduce lipopolysaccharide-induced inflammation [11]. Therefore, we speculate that the therapeutic effects of DSD on UC mice may also be correlated with inhibition of the TLR4/NF- κ B signal transduction.

Material and methods

Animal sources

After purchased from Hunan Slack Jingda Experimental Animal Co., Ltd., male Sprague Dawley (SD) mice free to food and water were cultured in an animal house with a room temperature of 21-26°C, a relative humidity of 51-57% and natural light. The animal experi-

Table 1. DAI scores

Scores	Weight loss (%)	Fecal consistency	Occult blood
0	No	No	No
1	1-5	Pasty and semi-formed	No
2	5-10	Pasty and semi-formed	Occult blood positive
3	10-15	Diarrhea	Occult blood positive
4	15-20	Diarrhea	Lots of blood

ments have been approved by the Ethics Committee of our hospital.

Mice grouping and treatment

Grouping and modeling: Fifty mice were evenly divided into a normal group, a model group, a low dose group, a middle dose group and a high dose group based on random number tables. The first group was given normal drinking water, while the other groups were given natural drinking water that contained 3% dextran sulfate sodium (DSS) every day. The modeling was considered successful if the mice had loose stools and mucopurulent bloody stools. Drug treatment: At the same time of modeling, the last three groups were respectively given 20, 40 and 80 mg/kg of DSD daily for 14 days, while the first two groups were intragastrically administered with the same amount of distilled water.

Physical conditions of mice

The body mass, stool property and hematochezia of the mice were recorded daily, so as to calculate the scores of the Disease Activity Index (DAI) (**Table 1**).

Collecting materials and observing pathological changes of colon tissues

At 14 days after the experiments, the mice were injected intraperitoneally with 1% pentobarbital sodium (20 mg/kg) for anesthesia, and then dissected for collecting the whole colon and measuring its length. After the colon tissues were put on ice, the severity of the damage to the colonic mucosa was observed with naked eyes and then marked. Next, a section of the colon close to the anus was clipped, and then fixed with 4% formaldehyde, embedded in paraffin, frozen section and subjected to HE staining. Finally, the pathological changes of the colon tissues such as inflammatory cell infiltration and tissue damage were observed under the microscope. The remaining part of

the colon was placed in freezing tubes and then stored at a refrigerator (-80°C) for later testing.

Inflammatory cytokines and their detection

The colon tissues were taken out, prepared in 10% tissue homogenate with a PBS solution,

and finally centrifuged at 12000×g (10 min, 4°C) to obtain the supernatant. One part of the supernatant was used for detection of TNF- α , IL-1 β and IL-6 (Abcam, USA), while the other part was used for measurement of CAT, SOD and MDA (Nanjing Jiancheng Bioengineering Institute), according to protocols as described in respective kits.

Western blot

After culture, the cells were cleaved with RIPA lysis buffer (Thermo Fisher Scientific, USA), with BCA protein assay kits (Thermo Fisher Scientific, USA) used for determining protein concentration. The loading quantity of sample (40 μ g) was taken out for 10% polyacrylamide gel electrophoresis (100 V) and PVDF membrane transfer. After sealed in 5% skimmed milk powder for 2 hours, the membrane was cleaned with TBST buffer solution, and then added with caspase-3, Bax, Bcl-2, TLR4, MyD88, NF- κ B and β -catenin (1:1000; Abcam, USA) for sealing all night at 4°C. After washed to remove the antibodies, it was added with goat anti-mouse IgG (1:4000; Sigma, USA), incubated at 37°C for 1 hour, and finally rinsed with PBS over 5 min for 3 times. After unnecessary liquid on the membrane was removed, ECL (Millipore, USA) was used for luminescence and developing. The protein bands were scanned to analyze their gray values in the software Quantity One.

Statistical analysis

In this study, SPSS21.0 (IBM Corp, Armonk, NY, USA) was used to statistically analyze the collected data. GraphPad 7 was used to plot the required figures. The comparison of measurement data between two groups was conducted by an independent samples t test, and the comparison between multiple groups was conducted by one-way analysis of variance,

Effects of schizandrin A on ulcerative colitis in mice

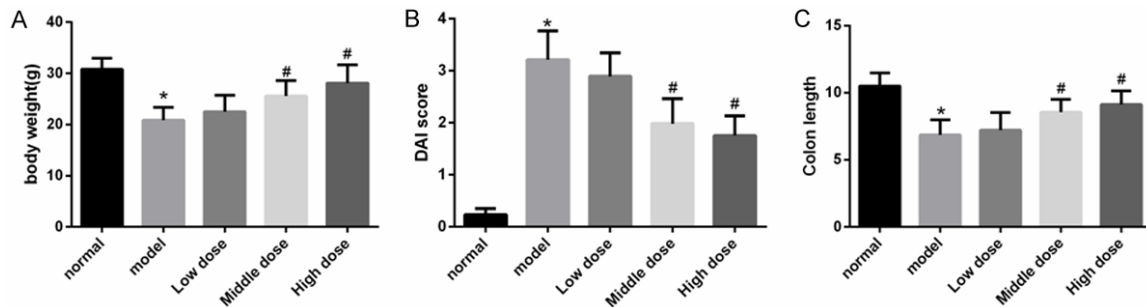


Figure 1. DSD could relieve clinical symptoms of UC mice. A: The mice in the model group had weight loss compared with the normal group, but those in the treatment groups had increased body weight compared with the model group. B: The mice in the model group had increased DAI scores compared with the normal group, but those in the treatment groups had decreased DAI scores compared with the model group. C: The mice in the model group had shortened colon length compared with the normal group, but those in the treatment groups had increased colon length compared with the model group. Note: * indicates $P < 0.05$ compared with the normal group. # indicates $P < 0.05$ compared with the model group.

with Tukey HSD method used for verification. When $P < 0.05$, the difference was statistically significant.

Results

DSD could relieve clinical symptoms of UC mice

Changes in the physical conditions and colon morphology of the mice were observed and compared. Compared with those in the normal group, the mice in the model group suffered from weight loss, shortened colon length and increased DAI scores. Compared with those in the model group, the mice in the treatment groups showed increased body weight and colon length and decreased DAI scores, suggesting that DSD can relieve the clinical symptoms of UC mice (**Figure 1**).

DSD could improve pathological morphology of UC mice

It could be visualized that mice in the normal group showed unthickened intestinal wall, clear texture of the wrinkled wall, no adhesion in the intestine, smooth mucosa, intact epithelium, no inflammatory cell infiltration, and no congestion, edema or ulcer in each layer. Meanwhile, those mice in the model group showed obvious ulcers, adhesions in the intestinal wall, thickened intestine, necrosis of the submucosal tissue or loss of the mucosal layer, with a large number of inflammatory cells infiltrated (including neutropenia and the loss of glands) and occasional intestinal tympanites. Those in the low and middle dose groups showed certain

necrotic substances, ulcers and lymph nodes in the colon, but were obviously better compared with those in the model group. Whereas those in the high dose group showed no obvious ulcers, obviously repaired intestinal mucosa, with occasional inflammatory cell infiltration (**Figure 2**).

DSD could reduce body inflammation in UC mice

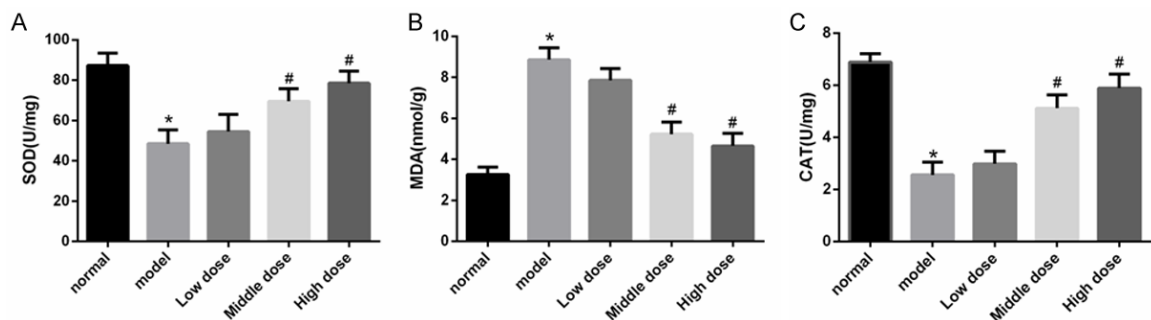
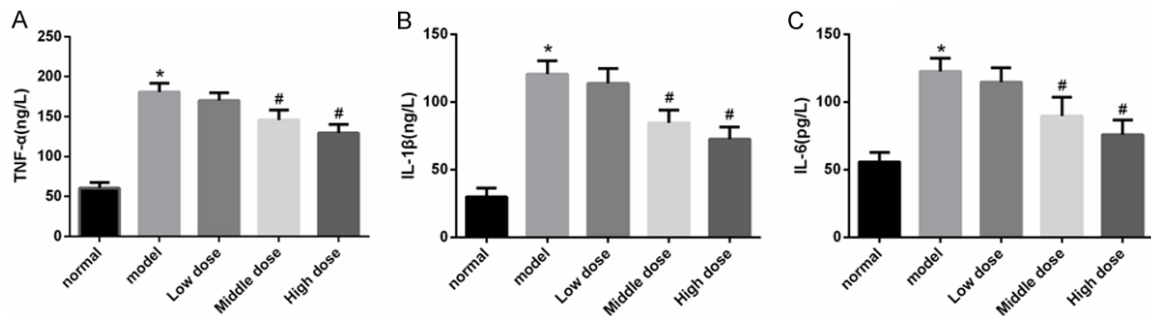
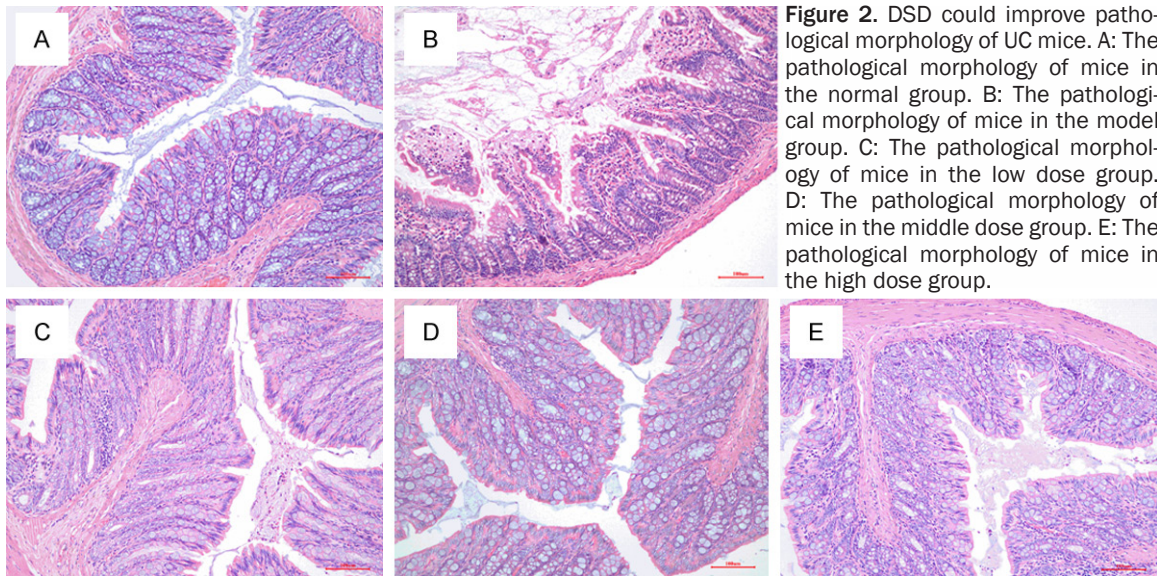
UC is an inflammatory disease and its major therapeutic pathways include reducing body inflammation, so we analyzed its anti-inflammatory effect in this part. The levels of TNF- α , IL-1 β and IL-6 in the serum of the mice were detected and compared. The levels of these factors increased in the model group compared with the normal group, but reduced in the treatment groups compared with the model group (**Figure 3**).

DSD could reduce oxidative stress injury in UC mice

The levels of CAT, SOD and MDA in the serum of the mice were examined and compared. Compared with those in the normal group, MDA levels increased but SOD and CAT levels decreased in the model group. Compared with those in the model group, MDA levels decreased but SOD and CAT levels increased in the treatment groups (**Figure 4**).

DSD could reduce apoptosis of colonic epithelial cells in UC mice

Apoptosis marker proteins in the colonic epithelial cells and tissues of the mice were



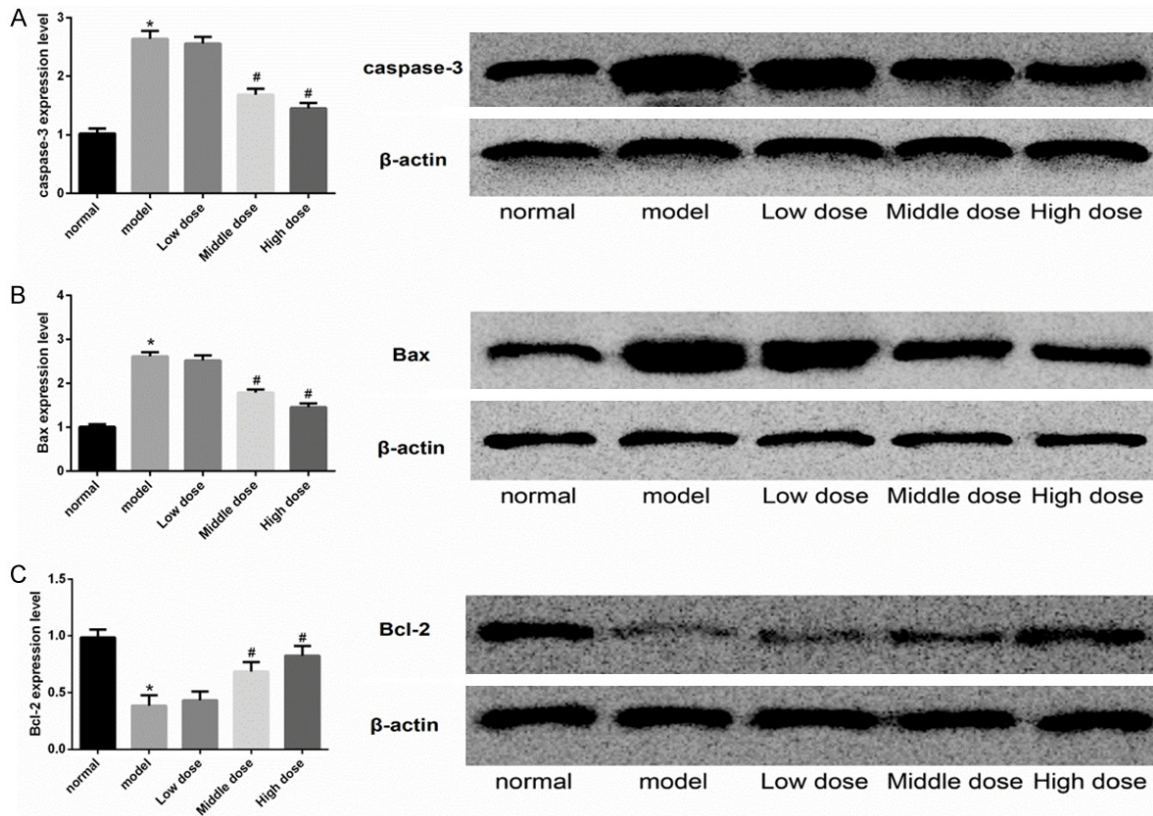


Figure 5. DSD could reduce apoptosis of colonic epithelial cells in UC mice. A: Caspase-3 levels increased in the model group compared with the normal group, but decreased in the treatment groups compared with the model group. B: Bax levels increased in the model group compared with the normal group, but decreased in the treatment groups compared with the model group. C: Bcl-2 levels decreased in the model group compared with the normal group, but increased in the treatment groups compared with the model group. Note: * indicates P<0.05 compared with the normal group. # indicates P<0.05 compared with the model group.

detected. After DSS induction, caspase-3 and Bax contents rose while Bcl-2 contents reduced in the colonic epithelial tissues. After DSD treatment, levels of caspase-3 and Bax decreased while Bcl-2 contents increased in the tissues examined (**Figure 5**).

DSD could reduce levels of TLR4, MyD88 and NF- κ B in colon tissues of UC mice

The TLR4/NF- κ B signaling pathway is activated in inflammatory diseases and considered as an important participant in their pathogenesis. To investigate whether this pathway plays a role in DSD treated UC mice, we analyzed the changes of TLR4, MyD88 and NF- κ B levels in the colon tissues of UC mice after DSD intervention. The results showed that the levels increased in the model group compared with the normal group, but reduced in the treatment groups compared with the model group (**Figure 6**).

Discussion

It is currently believed that the causes involving UC pathogenesis are complex, but are mostly related to the interaction of genetic, immune, microbiological and environmental factors [12]. There are a variety of drugs that can be used for UC patients, but many of them have low efficiency and side effects [13]. Therefore, UC is difficult to cure in clinical practice. Given its high risk of getting colon cancer, it is of significant clinical relevance to find new therapeutic drugs for UC.

The major cause of UC recurring and worsening is uncontrolled inflammation, so the effective control of inflammation is the main strategy of treating the disease [14]. It has been confirmed that DSD has an excellent anti-inflammatory effect [15, 16]. IL-1 β , TNF- α and IL-6 are pro-inflammatory cytokines, which play an impor-

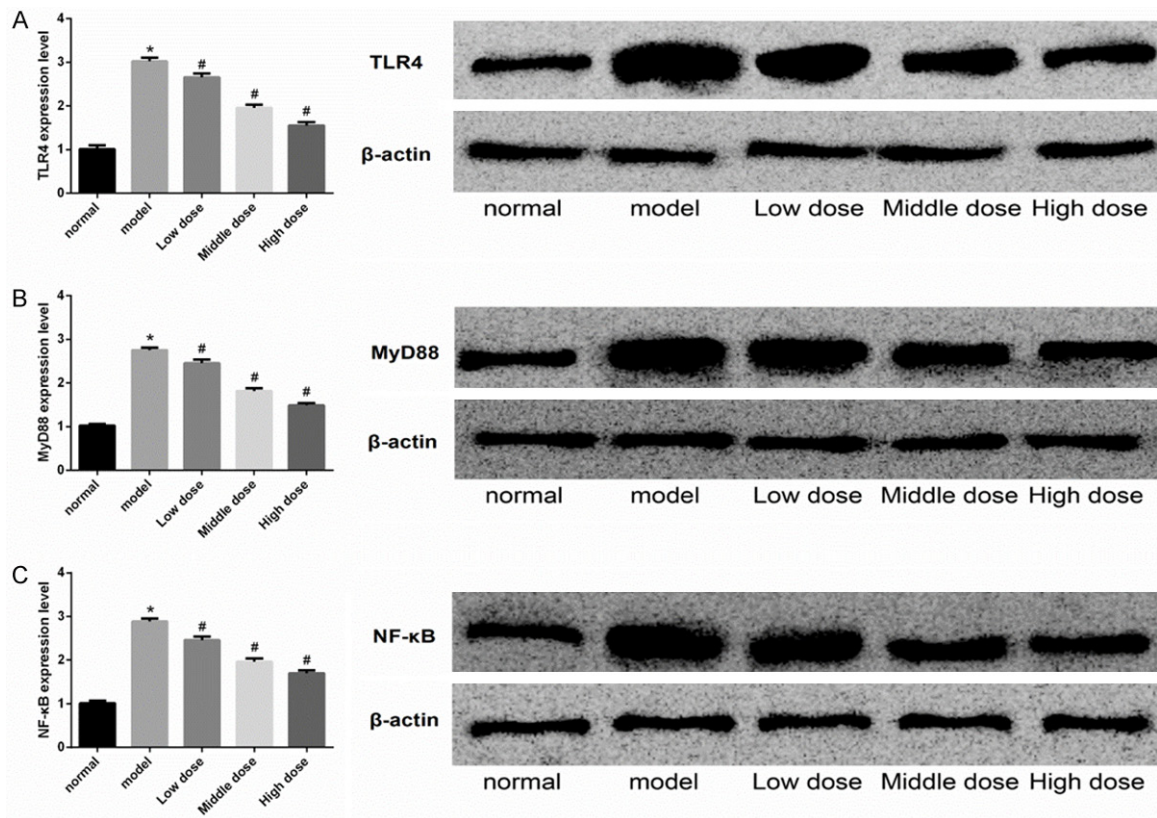


Figure 6. DSD could reduce levels of TLR4, MyD88 and NF-κB in colon tissues of UC mice. A: TLR4 levels increased in the model group compared with the normal group, but decreased in the treatment groups compared with the model group. B: MyD88 levels increased in the model group compared with the normal group, but decreased in the treatment groups compared with the model group. C: NF-κB levels increased in the model group compared with the normal group, but decreased in the treatment groups compared with the model group. Note: * indicates $P < 0.05$ compared with the normal group. # indicates $P < 0.05$ compared with the model group.

tant role in the development and progression of UC and other inflammatory diseases [17, 18]. In our study, the three cytokines increased in the colon tissues of the mice with DSS-induced UC, but the increase could be inhibited by DSD. This suggests that this drug can relieve body inflammation via inhibition of the excessive release of inflammatory cytokines. Oxidative stress is another important factor for the pathogenesis of UC, as it produces a large number of oxygen free radicals that would cause certain damage to human tissues [19]. As an end product of lipid peroxidation, MDA results in the polymerization and crosslinking of membrane components, and finally causes serious cell damage [20]. SOD, a main endogenous antioxidant enzyme, may neutralize free radicals and protect tissues from the harmful effects of the free radicals and other reactive oxygen species [21]. As an important component of the enzyme system in the antioxidant system, CAT usually exists in cells' peroxisome, and mainly removes

H_2O_2 and protects the body from damage [22]. According to previous studies, DSD can reduce the oxidative stress-induced damage in some tissues and cells [23, 24]. In this study, after DSS induction, MDA levels increased but SOD and CAT levels decreased in mouse serum, while the effects of DSS on the three levels could be reduced by DSD. This indicates that DSD may inhibit the oxidative stress of mice with DSS-induced UC and thus protect their colon tissues.

It has been reported that the excessive apoptosis of colonic epithelial cells is part of the causes of UC colon injury [25, 26], so we analyzed the effects of DSD on the cell apoptosis. Bcl-2 homodimers can inhibit cell apoptosis, and caspase-3 and Bax are important participants in promoting this behavior, so the three proteins are considered as effective markers of apoptosis [27]. In our study, levels of caspase-3 and Bax increased while level of Bcl-2

decreased in the colonic epithelial tissues after DSS induction, while DSD administration could prevent the changes. This demonstrates that this drug can enhance the anti-apoptotic ability of colonic epithelial cells. This result is similar to a previous research which showed that DSD can inhibit cell apoptosis in the colon tissues of mice with DSS-induced UC [7].

Our study confirmed that DSD has a certain therapeutic value for UC, but the exact molecular mechanism involved was not fully understood. TLR4/NF- κ B is a common inflammatory signaling pathway. TLR4 activates the downstream nuclear transcription factor NF- κ B; the activated NF- κ B causes the abnormal expression of relevant inflammatory cytokines and then imbalance of pro-/anti-inflammatory cytokines, thus resulting in a variety of damaged biological effects [28, 29]. More evidence has confirmed that the TLR4/NF- κ B signaling pathway is activated in various inflammatory diseases (including UC) and involved in their pathogenesis [30, 31]. Therefore, inhibiting the over-activation of this pathway is considered as an important direction of treating these inflammatory diseases. In our study, we analyzed the correlation of DSD with this pathway, and found that TLR4, MyD88 and NF- κ B rose in the colon tissues of mice with DSS-induced UC, while DSD could inhibit the increase. This suggests an involvement of the TLR4/NF- κ B signaling pathway in DSD treated UC mice.

In summary, DSD can alleviate the symptoms of mice with DSS-induced UC by inhibiting body inflammation, decreasing oxidative stress and reducing the apoptosis of colonic epithelial cells, possibly via inhibition of the TLR4/NF- κ B signaling pathway.

Disclosure of conflict of interest

None.

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References

[1] Loftus EV Jr, Colombel JF, Feagan BG, Vermeire S, Sandborn WJ, Sands BE, Danese S, D'Haens GR, Kaser A, Panaccione R, Rubin DT, Shafran

I, McAuliffe M, Kaviya A, Sankoh S, Mody R, Abhyankar B and Smyth M. Long-term efficacy of vedolizumab for ulcerative colitis. *J Crohns Colitis* 2017; 11: 400-411.

[2] Castano-Milla C, Chaparro M and Gisbert JP. Systematic review with meta-analysis: the declining risk of colorectal cancer in ulcerative colitis. *Aliment Pharmacol Ther* 2014; 39: 645-659.

[3] Feuerstein JD and Cheifetz AS. Ulcerative colitis: epidemiology, diagnosis, and management. *Mayo Clin Proc* 2014; 89: 1553-1563.

[4] Tripathi K and Feuerstein JD. New developments in ulcerative colitis: latest evidence on management, treatment, and maintenance. *Drugs Context* 2019; 8: 212572.

[5] Xu M, Wang G, Xie H, Wang R, Wang W, Li X, Li H, Zhu D and Yue L. Determination of schizandrin in rat plasma by high-performance liquid chromatography-mass spectrometry and its application in rat pharmacokinetic studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005; 828: 55-61.

[6] Opletal L, Sovova H and Bartlova M. Dibenzo[a,c]cyclooctadiene lignans of the genus Schisandra: importance, isolation and determination. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004; 812: 357-371.

[7] Zhang WF, Yang Y, Su X, Xu DY, Yan YL, Gao Q and Duan MH. Deoxyschizandrin suppresses dss-induced ulcerative colitis in mice. *Saudi J Gastroenterol* 2016; 22: 448-455.

[8] Tang ZH, Peng J, Ren Z, Yang J, Li TT, Li TH, Wang Z, Wei DH, Liu LS, Zheng XL and Jiang ZS. New role of PCSK9 in atherosclerotic inflammation promotion involving the TLR4/NF- κ B pathway. *Atherosclerosis* 2017; 262: 113-122.

[9] Ni H, Jin W, Zhu T, Wang J, Yuan B, Jiang J, Liang W and Ma Z. Curcumin modulates TLR4/NF- κ B inflammatory signaling pathway following traumatic spinal cord injury in rats. *J Spinal Cord Med* 2015; 38: 199-206.

[10] Cui L, Feng L, Zhang ZH and Jia XB. The anti-inflammation effect of baicalin on experimental colitis through inhibiting TLR4/NF- κ B pathway activation. *Int Immunopharmacol* 2014; 23: 294-303.

[11] Peng Q, Liu H, Shi S and Li M. Lycium ruthenicum polysaccharide attenuates inflammation through inhibiting TLR4/NF- κ B signaling pathway. *Int J Biol Macromol* 2014; 67: 330-335.

[12] Sun M, Du B, Shi Y, Lu Y, Zhou Y and Liu B. Combined signature of the fecal microbiome and plasma metabolome in patients with ulcerative colitis. *Med Sci Monit* 2019; 25: 3303-3315.

[13] Sandborn WJ, Su C and Panes J. Tofacitinib as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2017; 377: 496-497.

- [14] de Lange KM, Moutsianas L, Lee JC, Lamb CA, Luo Y, Kennedy NA, Jostins L, Rice DL, Gutierrez-Achury J, Ji SG, Heap G, Nimmo ER, Edwards C, Henderson P, Mowat C, Sanderson J, Satsangi J, Simmons A, Wilson DC, Tremelling M, Hart A, Mathew CG, Newman WG, Parkes M, Lees CW, Uhlig H, Hawkey C, Prescott NJ, Ahmad T, Mansfield JC, Anderson CA and Barrett JC. Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. *Nat Genet* 2017; 49: 256-261.
- [15] Zhong S, Nie YC, Gan ZY, Liu XD, Fang ZF, Zhong BN, Tian J, Huang CQ, Lai KF and Zhong NS. Effects of Schisandra chinensis extracts on cough and pulmonary inflammation in a cough hypersensitivity guinea pig model induced by cigarette smoke exposure. *J Ethnopharmacol* 2015; 165: 73-82.
- [16] Kwon DH, Cha HJ, Choi EO, Leem SH, Kim GY, Moon SK, Chang YC, Yun SJ, Hwang HJ, Kim BW, Kim WJ and Choi YH. Schisandrin A suppresses lipopolysaccharide-induced inflammation and oxidative stress in RAW 264.7 macrophages by suppressing the NF-kappaB, MAPKs and PI3K/Akt pathways and activating Nrf2/HO-1 signaling. *Int J Mol Med* 2018; 41: 264-274.
- [17] Kim Y, Lim HJ, Jang HJ, Lee S, Jung K, Lee SW, Lee SJ and Rho MC. Portulaca oleracea extracts and their active compounds ameliorate inflammatory bowel diseases in vitro and in vivo by modulating TNF-alpha, IL-6 and IL-1beta signalling. *Food Res Int* 2018; 106: 335-343.
- [18] Qiao C, Wan J, Zhang L, Luo B, Liu P, Di A, Gao H, Sun X and Zhao G. Astragaloside II alleviates the symptoms of experimental ulcerative colitis in vitro and in vivo. *Am J Transl Res* 2019; 11: 7074-7083.
- [19] Deng QJ, Deng DJ, Che J, Zhao HR, Yu JJ and Lu YY. Hypothalamic paraventricular nucleus stimulation reduces intestinal injury in rats with ulcerative colitis. *World J Gastroenterol* 2016; 22: 3769-3776.
- [20] Chang R, Li Y, Yang X, Yue Y, Dou L, Wang Y, Zhang W and Li X. Protective role of deoxyschizandrin and schisantherin A against myocardial ischemia-reperfusion injury in rats. *PLoS One* 2013; 8: e61590.
- [21] Ballal SA, Veiga P, Fenn K, Michaud M, Kim JH, Gallini CA, Glickman JN, Quere G, Garault P, Beal C, Derrien M, Courtin P, Kulakauskas S, Chapot-Chartier MP, van Hylckama Vlieg J and Garrett WS. Host lysozyme-mediated lysis of Lactococcus lactis facilitates delivery of colitis-attenuating superoxide dismutase to inflamed colons. *Proc Natl Acad Sci U S A* 2015; 112: 7803-7808.
- [22] Krajka-Kuzniak V, Paluszczak J and Baer-Dubowska W. The Nrf2-ARE signaling pathway: an update on its regulation and possible role in cancer prevention and treatment. *Pharmacol Rep* 2017; 69: 393-402.
- [23] Chen Q, Zhan Q, Li Y, Sun S, Zhao L, Zhang H and Zhang G. Schisandra lignan extract protects against carbon tetrachloride-induced liver injury in mice by inhibiting oxidative stress and regulating the NF-kappaB and JNK signaling pathways. *Evid Based Complement Alternat Med* 2017; 2017: 5140297.
- [24] Choi YH. Schisandrin A prevents oxidative stress-induced DNA damage and apoptosis by attenuating ROS generation in C2C12 cells. *Biomed Pharmacother* 2018; 106: 902-909.
- [25] Qiu W, Wu B, Wang X, Buchanan ME, Regueiro MD, Hartman DJ, Schoen RE, Yu J and Zhang L. PUMA-mediated intestinal epithelial apoptosis contributes to ulcerative colitis in humans and mice. *J Clin Invest* 2011; 121: 1722-1732.
- [26] Zhao QJ, Yu YB, Zuo XL, Dong YY and Li YQ. Milk fat globule-epidermal growth factor 8 is decreased in intestinal epithelium of ulcerative colitis patients and thereby causes increased apoptosis and impaired wound healing. *Mol Med* 2012; 18: 497-506.
- [27] Yang B, Johnson TS, Thomas GL, Watson PF, Wagner B, Furness PN and El Nahas AM. A shift in the Bax/Bcl-2 balance may activate caspase-3 and modulate apoptosis in experimental glomerulonephritis. *Kidney Int* 2002; 62: 1301-1313.
- [28] Feng Y, Cui Y, Gao JL, Li MH, Li R, Jiang XH, Tian YX, Wang KJ, Cui CM and Cui JZ. Resveratrol attenuates neuronal autophagy and inflammatory injury by inhibiting the TLR4/NF-kappaB signaling pathway in experimental traumatic brain injury. *Int J Mol Med* 2016; 37: 921-930.
- [29] Zhang P, Guo ZF, Xu YM, Li YS and Song JG. N-Butylphthalide (NBP) ameliorated cerebral ischemia reperfusion-induced brain injury via HGF-regulated TLR4/NF-kappaB signaling pathway. *Biomed Pharmacother* 2016; 83: 658-666.
- [30] Rashidian A, Mohammadnejad A, Dehpour AR, Mehr SE, Akhavan MM, Shirkoobi R, Chamanara M, Mousavi SE and Rezayat SM. Atorvastatin attenuates TNBS-induced rat colitis: the involvement of the TLR4/NF-kB signaling pathway. *Inflammopharmacology* 2016; 24: 109-118.
- [31] Fu H, Hu Z, Di X, Zhang Q, Zhou R and Du H. Tenuigenin exhibits protective effects against LPS-induced acute kidney injury via inhibiting TLR4/NF-kappaB signaling pathway. *Eur J Pharmacol* 2016; 791: 229-234.