Original Article
Cent Asian J Med Sci. 2019 Dec;5(4):300-307. https://doi.org/10.24079/CAJMS.2019.12.007
Central Asian Journal of Medical Sciences
CAJMS

Buteelch-5, A Traditional Formula Ameliorates Dextran Sulfate Sodium-Induced Colitis in Mice

Hasiqiqige[1], Fanfan Zhang[2], Tserendagva Dalkh[1], Bold Sharav[1], Molor-Erdene Perenlei[1]

[1]International School of Mongolian Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia; [2]Wuxi Yilin Biomedical Service Center, Jiangshu Province, China

Submitted: October 17, 2019
Revised: November 1, 2019
Accepted: December 2, 2019

Corresponding Author
Hasiqiqige, MD
Department of Internal Medicine, International School of Mongolian Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar 14210, Mongolia.
Tel: +976-89895853
E-mail: 1091116725@qq.com

Objectives: To examine the therapeutic effects of buteelch-5, a traditional formula on DSS-induced colitis in C57/BL6 mice and its possible mechanisms. **Methods:** Colitis in mice was induced by oral administration of 5% dextran sulfate sodium (DSS) for seven days. On the eighth day after administration of DSS, buteelch-5 (500 mg/kg, twice a day) was given orally to mice for five consecutive days. Ciprofloxacin (50 mg/kg, once a day for 5 days) was given to mice as a comparison. Two hours after the last administration of buteelch-5 and ciprofloxacin, mice were euthanized, and colon tissues were removed. Protein and mRNA levels of occludin, claudin-1 and zonula occludens (ZO)-1 in colon tissues were determined by western blot and real time-qPCR respectively. Histopathological analysis of colon tissues was performed. **Results:** Histological analysis revealed successful establishments of colitis models. Treatment with buteelch-5 markedly inhibited DSS-induced colon injury. Furthermore, buteelch-5 increased (2.14-2.67 fold) the occludin, claudin and ZO-1 protein and mRNA levels in colon tissues of mice administered with DSS. Significant increase was observed in occludin mRNA levels after buteelch-5 treatment ($p<.05$). **Conclusion:** Buteelch-5 improves microscopic inflammation and increases tight junction protein expressions such as occludin, claudin, and ZO-1 in mice with DSS-induced colitis.

Keywords: Mongolian Traditional Medicine, Colitis, Tight Junction Proteins

Introduction

Ulcerative colitis (UC), one of the most common chronic inflammatory bowel diseases, is characterized by dysfunction of the innate and adaptive immunity, resulting in colonic mucosal injuries and histological changes in intestines manifested by body weight loss, altered stool consistency, bloody feces, and colonic shortening [1].

Inflammatory bowel diseases are characterized by inflammation that compromises the integrity of the epithelial barrier. Tight junction proteins including claudins, occludins and zonula occludens (ZO) are critical in the maintenance of epithelial barrier function and control of paracellular permeability. Decrease in tight junction protein expression could result in the alteration or disruption of the intestinal barrier, which contributes to infection, diarrhea, pyemia, and sepsis [2].

Buteelch-5, a traditional formula, has been used for treatment of phlegm and impure blood disorders in Mongolian traditional medicine. It is composed of chong.zhi (calcite), a.ru.ra (*Terminalia chebula Retz.*), hong.len [*Lagotis integrifolia*

(Willd.) Schischk.], re.skon (*Salvia miltiorrhiza* Bunge) and mumio (dried droplets of Trogopterus xanthipes) [3].

Clinical manifestations of brown phlegm disorder of the colon are similar to that of ulcerative colitis. Brown phlegm disorders are caused by imbalances of multiple factors such as wind, phlegm, bile, and impure blood [4]. In traditional medicine, buteelch-5 is used to treat external and internal disorders as it enhances the balance of these factors [3].

Therapeutic effects of buteelch-5 on inflammatory bowel diseases have not been studied so far. *T. chebula*, one ingredient of buteelch-5, has been shown to improve acetic acid-induced colonic damage and weight loss [5]. Gautam et al [6] demonstrated that dried fruit pulp extract of *T. chebula* healed trinitrobenzene sulfonic acid-induced colitis by promoting antioxidant status and decreasing intestinal bacterial load, free radical, and myeloperoxidase (MPO) levels. Moreover, KM1608, a traditional formula composed of T. chebula, *Aucklandia lappa* DC and *Zingiber officinale* Roscoe has been shown to improve DSS-induced colitis symptoms, such as disease activity index, colon length, and colon weight, as well as suppress the expression of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and MPO in the dextran sulfate sodium (DSS)-induced colitis model [7]. Wen et al. [8] demonstrated that S. miltiorrhiza extract inhibited colon injury, shortening of the colon and weight reduction. Salvianolic acid A, an active phenolic acid derived from S. miltiorrhiza has been shown to protect DSS-induced damage by increasing tight junction-related gene expression such as ZO-1 and occludins [9]. Soyolt T et al. [10] showed that traditionally processed calcite exhibited healing effects against acetic acid-induced gastric ulcer in rats within 21 days. Based on these results we speculate that buteelch-5 may possess protective potential against colitis.

To the best of our knowledge, there is only one study showing the effects of a Mongolian multicomponent compound composed of 2 components namely garidisan on experimental colitis induced by a mixture containing equal volumes of 2,4,6-trinitrobenzene sulfonic and 50% ethanol. Garidisan promoted tissue maturation of regenerated tissues by regulating the immune balance and improved functional maturity of regenerated tissues by reducing collagen formation, promoting maturation of new blood vessels, and increasing expression of growth factors and their receptors [11].

There are several studies showing the anti-inflammatory potential of various Chinese multicomponent formulas in the colitis model. However, few studies showed the effect of herbal medicines on the tight junction protein expressions including occludin, claudin-1, and ZO-1 in the colitis model. Qing Hua Chang Yin (QHCY), a traditional Chinese formula significantly ameliorated the clinical manifestations of colitis including the disease activity index, colon shortening, and the histological evidence of colitis in DSS-induced acute colitis in mice. The administration of the formula profoundly reduced the DSS-induced increase of TNF-α levels in both colonic tissue and serum and MPO expression in colonic tissue, along with a decrease in serum amyloid A levels. Furthermore, QHCY significantly reversed the DSS-induced down regulation of ZO-1, occludin, and claudin-1 inversely and reduced the DSS-induced increase in the phosphorylation of Elk-1 [12]. Zou et al. [13] showed that Huangqin-tang (HQT), a traditional Chinese medicine significantly ameliorated phenotypic, histopathologic and inflammatory manifestations of DSS-induced acute and chronic colitis model. DSS-induced nuclear factor-κB signaling was inhibited by HQT. Moreover, HQT-treated mice demonstrated significant changes in cell apoptosis, expression of apoptosis-associated genes such as caspase-3, bax, bcl-2, and intestinal permeability. HQT also increased occludin and ZO-1, inhibited cell proliferation (Ki67), and increased regulatory T cells numbers, protein expression of Foxp3 and IL-10 in the colonic tissue. In vitro, HQT down-regulated production of pro-inflammatory cytokines and suppressed the nuclear factor-κB signaling pathway in lipopolysaccharide-induced RAW 264.7 macrophages.

In the present study, we examined the effect of a Mongolian multicomponent compound named buteelch-5 on DSS-induced colitis in mice. Since, tight junction proteins play important roles in intestinal defense, we examined the effect of buteelch-5 on protein and mRNA expression levels of tight junction proteins such as occludin, claudin-1, and ZO-1 in mice administered with DSS. Further study is needed to examine immunomodulatory effects of buteelch-5 on the colitis model.

Materials and Methods

Reagents and Experimental Animals

Six to eight-week-old male C57/BL6 mice were purchased from the Key laboratory of Parasite and Vector Control, Wuxi, Jiangsu province, China. Mice were kept in a specific pathogen

free environment at a temperature of 22±°C, and a light/dark cycle of 12/12 h. Free access to food and water was provided for two days before starting experiments. Animal experiments were carried out in the laboratory of Wuxi Yilin Biomedical Service Center, Jiangshu province China.

Buteelch-5 was purchased from Hospital of Tibetan Medicine, Beijing, China. DSS was purchased from MP Biomedicals, China. Buteelch-5 granules were dissolved in water and given to mice via gastric tube.

Horseradish peroxidase secondary antibody was obtained from Vector Laboratories, USA. Antibodies against claudin-1, occludin, and ZO-1 were purchased from Abcam, Germany. RIPA buffer, Trizol reagent and reverse transcription kit were purchased from Sangon Biotech Company Ltd, Shanghai, China. Fast SYBR Green PCR kit was provided by Applied Biosystems, USA.

Research Design

This study was performed by using experimental design. Forty C57/BL6 mice weighing 19-21 grams were divided into 4 groups with 3 (western blot analysis) or 5 (real-time qPCR) animals in each group called: control (CT), DSS (DSS), buteelch-5 treatment (DSS+B5) and ciprofloxacin treatment (DSS+CP) group. Mice of DSS, buteelch-5, and ciprofloxacin groups were given with 5% DSS instead of water for seven days to induce colitis [14]. Control group received distilled water instead of DSS. On the eighth day, buteelch-5 treatment group was administered orally buteelch-5 at 500 mg/kg body weight twice a day for 5 days. Ciprofloxacin was given orally at dose of 50 mg/kg body weight to ciprofloxacin treatment group. Simultaneously, the control and DSS groups were given distilled water. Two hours after the last administration of buteelch-5 and ciprofloxacin, mice were euthanized, and colonic tissues were collected for western blot, real time qPCR, and histological analysis.

Histological Analysis

Colonic tissue was collected from mice and sectioned for hematoxylin-eosin stain. Briefly, colonic tissues were fixed in 10% formalin and embedded in paraffin. Colon specimens were cut and stained with haematoxylin-eosin and mounted on glass slides. All sections were viewed under the microscope.

Western Blot

About 5 mg piece of colon tissue was homogenized with 300 µl RIPA lysis buffer by using an electric homogenizer. Then the lysate was centrifuged for 20 min at 12000 rpm at 4°C in a micro centrifuge and the supernatants were collected. The protein concentrations in each lysate was measured. Next, the lysate was mixed with 5x SDS-PAGE sample buffer. Equal amounts of proteins were separated by a standard 10% SDS-PAGE gel and then transferred to a PVDF western blot membrane. After blocking in a 5% bovine serum albumin buffer for 1 h, membranes were incubated with respective primary antibodies at 4°C overnight. The following primary antibodies were used for protein detection: anti-occludin (dilution of 1:1000) anti-claudin-1 (dilution of 1:200), and anti-ZO-1 (dilution of 1:1000). After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Protein-antibody complexes were detected by Clarity Western Enhanced Chemiluminescent Substrate. The relative density of the band for the protein of interest was compared with the band for the house keeping protein β actin in each group.

Real-Time qPCR

To analyze the mRNA expression levels of genes, total RNA in mouse colon tissue was extracted with Trizol reagent according to the manufacturer's instructions. RNA concentration in each sample was determined by spectrophotometer (NanoDrop 2000) at 260nm. The cDNA was synthesized from 1 µg RNA using a reverse transcription kit according to the supplier's protocol. cDNA obtained by reverse transcription was used to determine mRNA expression levels of occludin, claudin-1, and ZO-1 by using specific primers. The nucleotide sequences of the primers were as follows: occludin sense (5'-TGGCTATGGAGGCGGCTATGG-3'), occludin antisense (5'-AAGGAAGCGATGAAGCAGAAGGC-3'), claudin-1 sense (5'-GGTGCCTGGAAGATGATGAGGTG-3'), claudin antisense (5'-GCCACTAATGTCGCCAGACCTG-3'), ZO-1 sense (5'-AGCTGCCTCGAACCTCTACTCTAC-3'), ZO-1 antisense (5'-GCCTGGTGGTGGAACTTGCTC-3'), β-actin sense (5'-TGCTGTCCCTGTATGCCTCT-3'), and β-actin antisense (5'-TTGATGTCACGCACGATTTC-3'). The reactions were run on an ABI 7700 real time PCR system. β-actin house keeping gene expression was used as calibrator after verification of its stability under the experimental conditions.

Statistical Analysis

Data was presented as the mean ± SD from five samples of one representative experiment. The difference among four groups was evaluated by using Kruskal-Wallis test. Pairwise comparisons were performed. Significant values have been adjusted by the

Bonferroni correction for multiple tests. Differences between the groups were considered significant at $p<0.05$. Statistical analysis was performed using SPSS version 23.0.

Ethical Statements

All experiments were approved by the Ethical Committee of Mongolian National University of Medical Sciences and followed the guidelines of animal experimentation of Wuxi Yilin Biomedical Service Center, Jiangshu province, China.

Results

Histopathological Analysis of DSS-Induced Acute Colitis

Histological examination of the colon tissue of DSS-treated mice revealed marked inflammatory cellular infiltration predominantly with lymphocytes in the mucosa and submucosa and epithelial damage including ulceration. While healthy mice showed no signs of colon damage. Mice treated with buteelch-5 exhibited repaired epithelial structure and no inflammatory cell infiltration. In the ciprofloxacin-treated group, a similar pattern to buteelch-5 was observed. Data is shown in Figure 1.

Effect of Buteelch-5 on Colonic Protein Levels of Occludin, Claudin-1 and ZO-1 in Mice with DSS-Induced Colitis

DSS markedly decreased expression of occludin (p=.002), claudin-1 (p=.002) and ZO-1 compared to control (p=.002). In the buteelch-5 treatment group, occludin level increased 2.32 fold (p=.087), claudin-1 level increased 2.51 fold (p=.068), and ZO-1 level increased 2.67 fold (p=.087) compared to the DSS group. Similar to the buteelch-5 treated group, a 2.08 fold increase in occludin level (p=.171), a 2.25 fold increase in claudin-1 level (p=.21) and a 2.26 fold increase in ZO-1 level were observed (p=.171) in ciprofloxacin-treated group. Data are shown in Table 1 and Figure 2.

Colonic protein levels of occludin (A), claudin-1 (B), and ZO-1 (C) in the control group (CT), model group (DSS), buteelch-5 treated group (DSS+B5), and ciprofloxacin treated group (DSS+CP) were determined by western blot. Quantitation values are shown as means (fold change) ± SD from 3 animals in each group. Representative blots of occludin, claudin-1, ZO-1 proteins expression are shown (D); $^{*}p<.01$ vs control; SD, standard deviation.

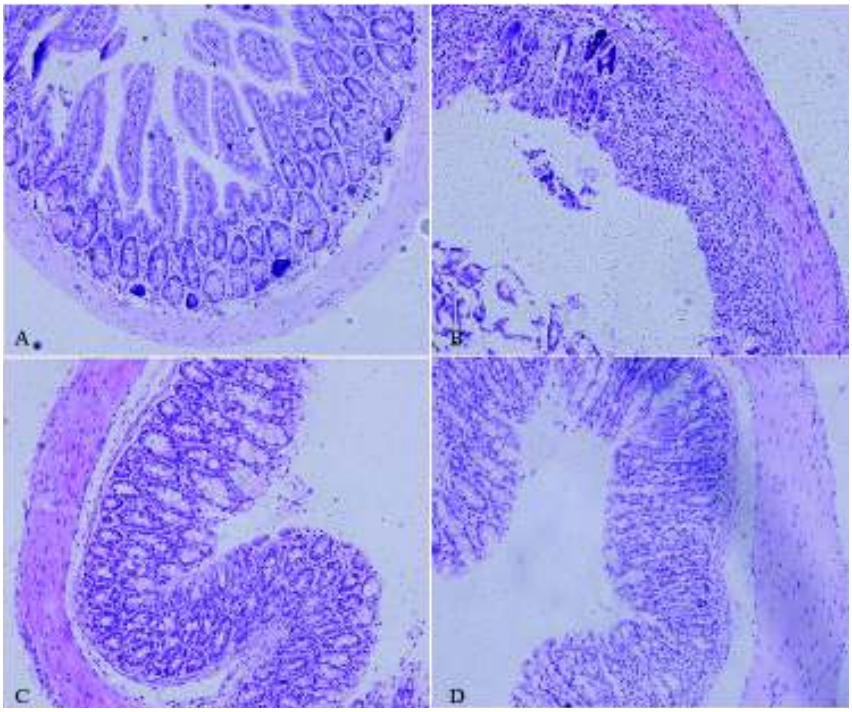

Figure 1. Histological section of mice colon.

Colon tissues were stained with H&E (x200) as: (A) control group (CT), (B) model group (DSS), (C) buteelch-5 treated group (DSS+B5), and (D) ciprofloxacin treated group (DSS+CP). Representative images of the colonic tissues are displayed.

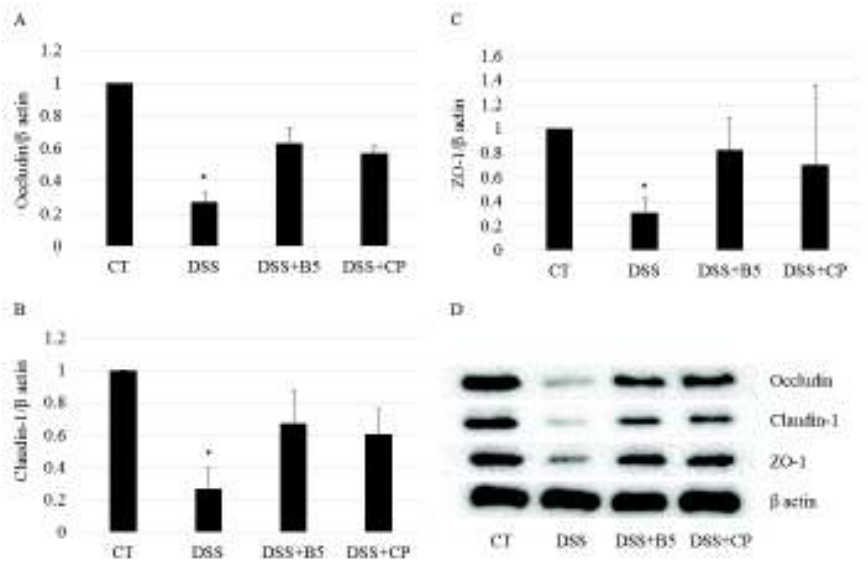

Figure 2. Effect of buteelch-5 on colonic protein levels of occludin, claudin-1, and ZO-1 in mice with DSS-induced colitis.

Effect of Buteelch-5 on Colonic mRNA Levels of Occludin, Claudin-1, and ZO-1 in Mice with DSS-Induced Colitis

Further we examined the mRNA levels of occludin, claudin-1, and ZO-1 by real time PCR. As shown in Table 2 and Fig. 3, the mRNA expressions of occludin (p=.001), claudin-1 (p=.001), and ZO-1 (p=.003) were significantly decreased in colon tissue of mice administered with DSS compared to the control. In the buteelch-5 treatment group, occludin mRNA level was increased 2.28 fold (p=.042), claudin-1 mRNA level was increased 2.14 fold (p=.069), and ZO-1 mRNA level was increased 2.59 fold (p=.069) compared to the DSS group. The mRNA levels of occludin (p=.012), claudin-1 (p=.019) and ZO-1 (p=.033) were significantly increased by ciprofloxacin treatment (Fig. 3).

Colonic mRNA levels of occludin (A), claudin-1 (B), and ZO-1 (C) in control group (CT), model group (DSS), buteelch-5 treated group (DSS+B5), and the ciprofloxacin treated group (DSS+CP) were assessed by RT-qPCR. Values are means (fold change) ± SD from 5 animals in each group. *p<.01 vs control; **p<.05 vs DSS. RT-qPCR, real-time quantitative polymerase chain reaction; SD, standard deviation.

Discussions

Ulcerative colitis is a recurrent and prolonged inflammatory disease affecting the colon, and its incidence is rising worldwide. Pathological manifestations of ulcerative colitis include body weight loss, altered stool consistency, bloody feces, and colonic shortening [1]. Buteelch-5, a traditional formula, has been used for treatment of phlegm and impure blood disorders in Mongolian traditional medicine. Buteelch-5 is composed of chong.zhi (calcite), a.ru.ra (*T. chebula*), hong.len (*Lagotis integrifolia*), re.skon (*S. miltiorrhiza*) and mumio (dried droplets of Trogopterus xanthipes) [3]. The ingredients of buteelch-5 possess humor balancing, heat dispelling, impure blood clearing, anti-diarrheal, and pain relieving potencies [4]. Therapeutic effects of buteelch-5 on inflammatory bowel diseases have

Table 1. Effect of buteelch-5 on colonic protein levels of occludin, claudin-1 and ZO-1 in mice with DSS-induced colitis.

Groups	Occludin	Claudin-1	ZO-1
Control (n=3)	1	1	1
DSS (n=3)	0.271±0.05*	0.267±0.12*	0.309±0.12*
DSS+B5 (n=3)	0.629±0.1	0.671±0.2	0.826±0.26
DSS+CP (n=3)	0.569±0.04	0.603±0.15	0.705±0.16

*p<.01vs control

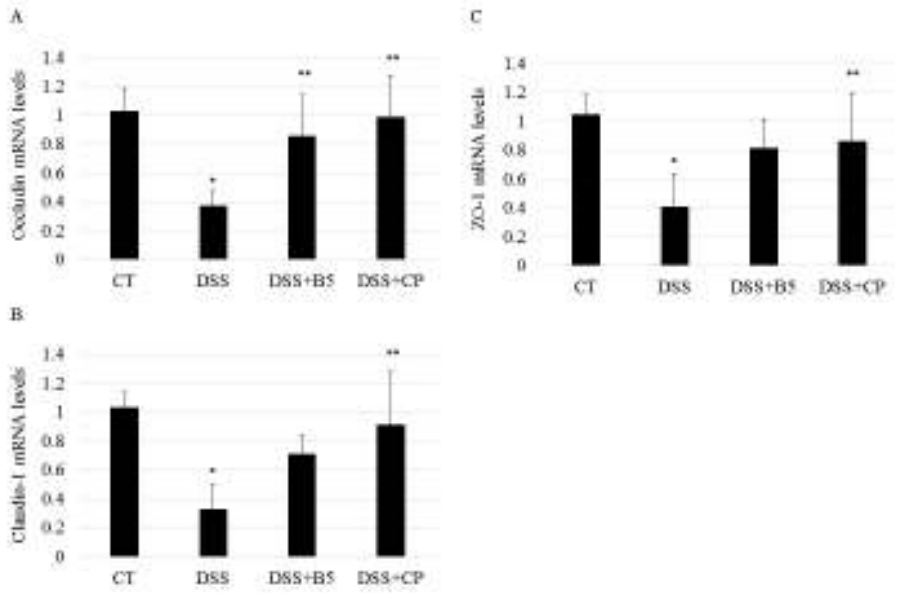

Figure 3. Effect of buteelch-5 on colonic mRNA levels of occludin, claudin-1, and ZO-1 in mice with DSS-induced colitis

Table 2. Effect of buteelch-5 on colonic mRNA levels of occludin, claudin-1 and ZO-1 in mice with DSS-induced colitis

Groups	Occludin mRNA	Claudin-1 mRNA	ZO-1 mRNA
Control (n=5)	1.05±1.02	1.03±0.1	1.04±0.13
DSS (n=5)	0.374±0.1*	0.331±0.16*	0.312±0.23*
DSS+B5 (n=5)	0.856±0.28**	0.711±0.13	0.811±0.2
DSS+CP (n=5)	0.986±0.28**	0.914±0.36**	0.864±0.33**

*p<.01vs control; **p<.05 vs DSS

not been studied so far. However, Jiumei G [15] demonstrated that buteelch-5 relieved pain and reduced vomiting in patients with reflux gastritis. Endoscopy of these patients revealed that inflammation of the gastric mucosa was significantly reduced by buteelch-5 treatment.

Some ingredients of buteelch-5 have been shown to protect against colonic injury in different colitis models. For example, T. chebula has been shown to improve colonic damage in various models of ulcerative colitis via inhibiting free radicals and MPO activity [5,6]. S. miltiorrhiza extract inhibits DSS-induced inflammatory responses and shortening of the colon in mice [8]. Moreover, salvianolic acid A, an active phenolic acid derived from *S. miltiorrhiza* has been shown to protect DSS-induced damage by increasing tight junction-related gene expression (ZO-1 and occludin) [9]. Traditionally processed calcite exhibits healing effects against acetic acid-induced gastric ulcer in rats [10]. KM1608, composed of *Aucklandia lappa DC., T. chebula* and *Zingiber officinale Roscoe* significantly improved DSS-induced colitis symptoms, such as disease activity index, colon length, and colon weight, as well as suppressed the expression of IL-6, TNF-α, and MPO in the DSS-induced colitis tissues [7].

Histology analysis revealed a successful induction of colitis in our study. DSS-treated mice displayed marked inflammatory cellular infiltration and severe epithelial injury including ulceration (Fig. 1B). These changes are consistent with the study by Chen et al [16], which showed severe epithelial injury and inflammatory cell infiltration in the mucosa and sub mucosa in a DSS-treated group. By another study, a large number of histological lesions which included loss of crypts, edema, mucosal erosions, and infiltration of inflammatory cells in the mucosa were observed in mice treated with DSS [17]. Our present study demonstrated buteelch-5 could suppress DSS-induced colitis in mice and improve acute colonic damages (Fig. 1B). Moreover, treatment with buteelch-5 significantly reduces inflammatory infiltration in colon tissue in mice administered with DSS (Fig. 1C). Consistent to our study, QHCY, a Chinese formula ameliorated the mucosal

ulceration and reduced the neutrophil cell infiltration [12]. Li et al., [18] showed that ginsenosides and berberine markedly reduced the severity of DSS-induced colitis in mice. Hans et al., [19] demonstrated that acute DSS colitis treatment (5% DSS for 7 days) with ciprofloxacin and metronidazole led to an improvement of the histological parameters (epithelial damage, $p<.05$; inflammatory infiltrate, $p<.05$) and colon length ($p<.0028$) and significantly reduced granulocyte infiltration and MPO activity in colonic biopsies. Since we established the acute DSS colitis model, we used ciprofloxacin as a comparison in our study. Similar effects to buteelch-5 were observed with ciprofloxacin.

Further, we tried to evaluate some mechanisms that may account for the therapeutic effect of buteelch-5. The intestinal epithelial barrier protects the body from pathogens and other toxic luminal substances. Tight junction proteins such as claudins, occludin, and ZOs play an important role in epithelial barrier regulation. Studies have demonstrated that occludin, claudins, ZOs and other tight junction proteins were disrupted in inflammatory bowel diseases [2,20]. In the present study, western blot analysis revealed that expression of occludin, claudin-1 and ZO-1 were significantly reduced in DSS-treated mice (Table 1, Fig.2). These results were similar to the studies by others that protein expression of ZO-1, occludin and claudin-1 in colon tissue of the DSS-induced colitis mice were significantly decreased compared to the mice in the control group [12,18]. Treatment with buteelch-5 increased the levels of these proteins in colon tissue (Table 1, Fig. 2). However, no statistical significance was observed compared to the model group. In the study by Ke et al., [12] QHCY significantly reversed the effect of DSS on the expressions of ZO-1, occludin, and claudin-1.

Further, we examined whether buteelch-5 could restore DSS-induced down-regulation of mRNA expression of these proteins. The mRNA levels of occludin, claudin-1, and ZO-1 were significantly down-regulated in DSS-treated mice in our study. In the study by Ke et al., [12] the DSS-induced colitis mice group showed a significant reduction in mRNA levels of ZO-1, occludin, and claudin-1 which was also reversed by the QHCY treatment. In our study, buteelch-5 treatment significantly increased mRNA levels of occludin and slightly increased claudin-1and ZO-1 mRNA levels compared to the DSS-treated group (Table 2, Fig. 3). The limitation of our study is the small sample size used for the analysis. Taken together these results indicate that buteelch-5 could ameliorate DSS-induced colonic injuries in mice. The protective potential of buteelch-5 against colonic damages at least partly might be due to slight restoration of tight junction proteins such as occludin, claudin-1, and ZO-1. Various herbal medicines have been shown to possess potential therapeutic effects on DSS-induced colitis in mice [21, 22]. Studies mainly focus on inflammatory cytokines and oxidative stress in order to explain possible mechanisms of action of herbal formulas [18, 23, 24]. Our study is the first to show the effect of Mongolian multicomponent medicine on epithelial damage and tight junction protein expressions in experimental colitis.

Studies have demonstrated that barrier dysfunction alone is insufficient to cause inflammatory bowel disease, it can lead to activation of immune responses that may affect disease development at later times. Pro-inflammatory cytokines such as TNF-α and IL-1β have been shown to cause barrier dysfunction due to epithelial tight junction regulation [16, 25].

Further study is needed to examine the effects of buteelch-5 on immune response in inflammatory bowel disease models.

Conflict of interests

The authors state no conflict of interest.

References

1. Gajendran M, Loganathan P, Jimenes G, Catinella AP, Ng N, Ziade N, et al. A comprehensive review and update on ulcerative colitis. Dis Mon 2019. DOI:10.1016/j.disamonth.2019.02.004.
2. 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.
3. Damomnrangbaluo SG. The guidebook of Tibetan pharmacology. New Delhi, India: Drud publication; 2003. p 224-5.
4. Yutog YG. The oral instruction tantra of the secret quintessential instructions on the eight branches of the ambrosia essence tantra. Ulaanbaatar, Mongolia: National printing; 1990. p 161-74.
5. Gautam MK, Goel S, Ghatule RR, Singh A, Nath G, Goel RK, et al. Curative effect of Terminalia chebula extract

on acetic acid-induced experimental colitis: Role of antioxidants, free radicals and acute inflammatory marker. Inflammopharmacol 2013; 21(5): 377-83.

6. Gautam MK, Ghatule RR, Singh A, Kumar M, Goel RK. Healing effect of Terminalia chebula fruit extract on trinitrobenzene sulfonic acid induced colitis in rat. Indian J Physiol Pharmacol 2013; 57: 325-36.
7. Shin M, Kim S, Lee J, Choi HS, Park J, Park JY, et al. Beneficial effect of herbal formulation KM1608 on inflammatory bowel diseases: A preliminary experimental study. Molecules 2018. DOI:10.3390/molecules23082068.
8. Wen X, Wang C, Yu C, Zhang Z, Calway T, Wang Y, et al. Salvia miltiorrhiza (Dan Shen) significantly ameliorates colon inflammation in dextran sulfate sodium induced colitis. Am J Chin Med 2013; 41: 1097-108.
9. Wang K, Yang Q, Wang B, Wan Z, Chen M, Wu L. Protective effects of salvanic acid A against dextran sodium sulfate-induced acute colitis in rats. Nutrients 2018. DOI: 10.3390/nu10060791.
10. Soyolt T. Effect of calcite processed by traditional method on gastric ulcer model [dissertation]. Ulaanbaatar, Mongolia: Health Sciences University of Mongolia; 2010.
11. Wang L, Liu W, Pei L, Ke Y, Cui J, LiS, et al. Garidisan: Improving the quality of ulcer healing in rats with ulcerative colitis. Evid Based Complement Alternat Med 2017. DOI:10.1155/2017/8721257.
12. Ke X, Liu L, Zhao P, Chen Y, Peng J, Fang W, et al. The effects of Qing Hua Chang Yin on the epithelial tight junctions of mice with inflammatory bowel disease. Int J Clin Exp Med 2019; 12: 6864-73.
13. Zou Y, Lin J, Li W, Wu Z, He Z, Huang G, et al. Huangqin-tang ameliorates dextran sodium sulphate-induced colitis by regulating epithelial cell homeostasis, inflammation and immune response. Sci Rep 2016. DOI: 10.1038/srep39299.
14. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-unduced colitis in mice. Curr Protoc Immunol 2014; 104: 1-14.
15. Jiumei G. Tibetan medicine jiebai pill (Rigel) was used to treat 56 cases of bile reflux gastritis. Chinese journal of ethnic medicine 2004. DOI:10.16017j.korki.cnl5-1175.2004.01.005.
16. Chen G, Yang Y, Hu C, Cheng X, Xu Y, Cai X, et al. Protective effects of Huangqin Decoction against ulcerative colitis and associated cancer in mice. Oncotarget 2016; 7: 61643-55.
17. Li X, Wang Q, Xu H, Tao L, Li J, Chai L, et al. Somatostatin regulates tight junction proteins expression in colitis mice. Int J Clin Pathol 2014; 7: 2153-62.
18. Li J, Zhong W, Wang W, Hu S, Yuan J, Zhang B, et al. Ginsenoside metabolite compound k promotes recovery of dextran sulfate sodium-induced colitis and inhibits inflammatory responses by suppressing NF-κB activation. Plos One 2014. DOI:10.1371/journal.pone.0087810. eCollection2014.
19. Hans W, Schölmerich J, Gross V, Falk W. The role of the resident intestinal flora in acute and chronic dextran sulfate sodium-induced colitis in mice. Eur J Gastroenterol Hepatol 2000; 12: 267-73.
20. Edelblum KL, Turner JR. The tight junction in inflammatory disease: communication break down. Curr Opin Pharmacol 2009; 9: 715-20.
21. Ke F, Yadav PK, Ju LZ. Herbal medicine in the treatment of ulcerative colitis. Saudi J Gastroenterol 2012; 18: 3-10.
22. Teschke R, Wolff A, Frenzel C, Eickhoff A, Schulze C. Herbal traditional Chinese medicine and its evidence base in gastrointestinal disorders. World J Gastroenterol 2015; 21: 4466-90.
23. Wan G, Xie M, Zhang X, Li M. Chang-wei-qing: a Chinese herbal formula ameliorates colitis-associated tomour development via inhibiting NF-κB and STAT3 signaling pathway. Pharm Biol 2019; 57: 231-7.
24. Song Y, Dunkin D, Dahan S, Iuga A, Ceballos C, Hoffstadter-Thal K, et al. Anti-inflammatory effects of the Chinese herbal formula FAHF-2 in experimental and human IBD. Inflamm Bowel Dis 2014; 20: 144-53.
25. Bhat AA, Uppada S, Achkar IW, Hashem S, Yadav AK, Shanmugakonar M, et al. Tight junction proteins and signaling pathways in cancer and inflammation: A functional cross talk. Front Physiol 2019. DOI:10.3389/fphys.2018.01942.