International Immunopharmacology 72 (2019) 454–458

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Saikosaponin A protects against dextran sulfate sodium-induced colitis in mice

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ARTICLE INFO

Keywords:
Colitis
DSS
Saikosaponin A
LXRα

ABSTRACT

Ulcerative colitis, one of the most important inflammatory bowel diseases, affects millions of people worldwide. The aim of this study was to investigate the effects of Saikosaponin A on dextran sulfate sodium (DSS)-induced colitis in mice. The mice were treated with 2.5% DSS for 5 d to induce acute colitis. Saikosaponin A was given 3 d before and during DSS treatment by intragastric administration. The results showed that Saikosaponin A significantly inhibited DSS-induced body weight loss and shortening of colon length. DSS-induced colonic histological changes and MPO activity were also prevented by treatment of Saikosaponin A. The levels of TNF-α and IL-1ß were increased by DSS and dose-dependently inhibited by Saikosaponin A. Furthermore, Saikosaponin A significantly inhibited DSS-induced NF-κB activation and up-regulated the expression of LXRα. Taken together, our results indicated that Saikosaponin A had protective effects against DSS-induced colitis. Saikosaponin A protected DSS-induced colitis through inhibiting inflammatory response.

1. Introduction

Ulcerative colitis is a lifelong disease that characterized by an exaggerated mucosal immune response in the colon [1]. It is predominantly occurred in the developed countries of the world, such as United Kingdom and North America [2]. The mechanism of ulcerative colitis is complex and studies showed that inflammatory response was involved the pathogenesis of ulcerative colitis [3]. During the development of ulcerative colitis, inflammatory cytokines such as TNF-α and IL-1ß were increased and these inflammatory mediators could induce inflammatory activation of the mucosal immune system [4]. Nowadays, the treatment of ulcerative colitis mainly relies on traditional drugs, such as corticosteroids and immunosuppressive agents [5]. However, in long-term treatment, these drugs may have side effects [6]. In recent years, natural herbal medicines have been developed as an important complementary treatment for ulcerative colitis [7].

Saikosaponin A (SKA), a triterpene saponin derived from *Radix bupleuri*, has been reported to have anti-inflammatory and anti-oxidative effects [8]. SKA has been reported to suppress LPS-induced inflammatory cytokines production mouse macrophages [9]. SKA also protected traumatic brain injury (TBI) rats after controlled cortical impact [10]. Furthermore, SKA was found to inhibit LPS-induced inflammatory response in human umbilical vein endothelial cells [11]. SKA also had protective effects against LPS-induced acute lung injury in mice [12]. In addition, SKA could protect mice against experimental sepsis [13]. However, the protective effects of SKA on DSS-induced colitis remain unclear. The present study was to investigate the protective effects and clarify the mechanism of SKA on DSS-induced colitis.

2. Materials and methods

2.1. Materials

Saikosaponin A and DMSO were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dextran sodium sulfate was purchased from MP Biomedicals (Solon, OH). TNF-α and IL-1β ELISA kits were purchased from BD (Minneapolis, MN, USA). MPO determination kit was provided by the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All the antibodies were purchased from Cell Signaling Technology Inc. (Beverly, MA).

2.2. Animals and experimental design

Male C57BL/6 mice (6–8 week old) were purchased from Medical Experimental Animal Center of Xinxiang Medical University. The mice were housed in the room with a temperature of 23∘C ± 1 ˚C and a

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https://doi.org/10.1016/j.intimp.2019.04.024
Received 7 October 2018; Received in revised form 9 April 2019; Accepted 10 April 2019

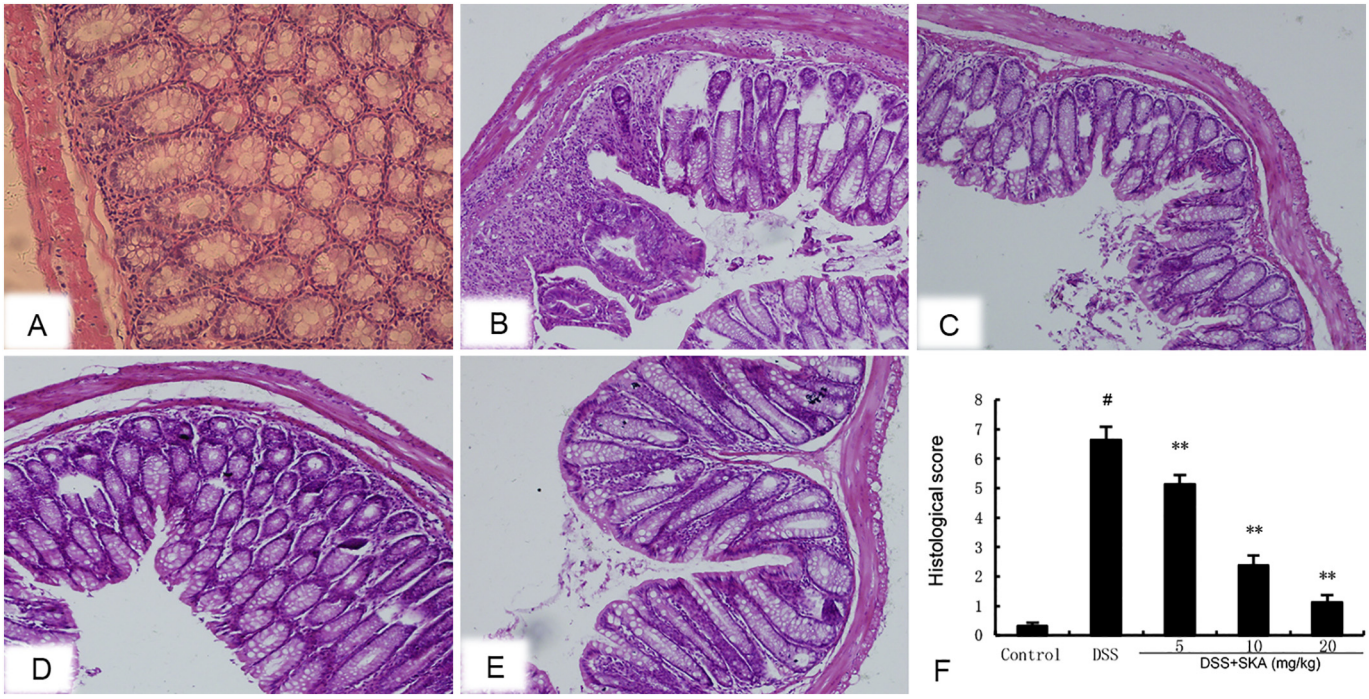

Fig. 1. Effects of SKA on histopathological changes in colon tissues in DSS-induced colitis. Representative histological changes of colon tissues obtained from mice of different groups. A: Control group, B: DSS group, C: DSS + SKA (5 mg/kg) group, D: DSS + SKA (10 mg/kg) group, E: DSS + SKA (20 mg/kg) group (hematoxylin and eosin staining, magnification 200 ×).

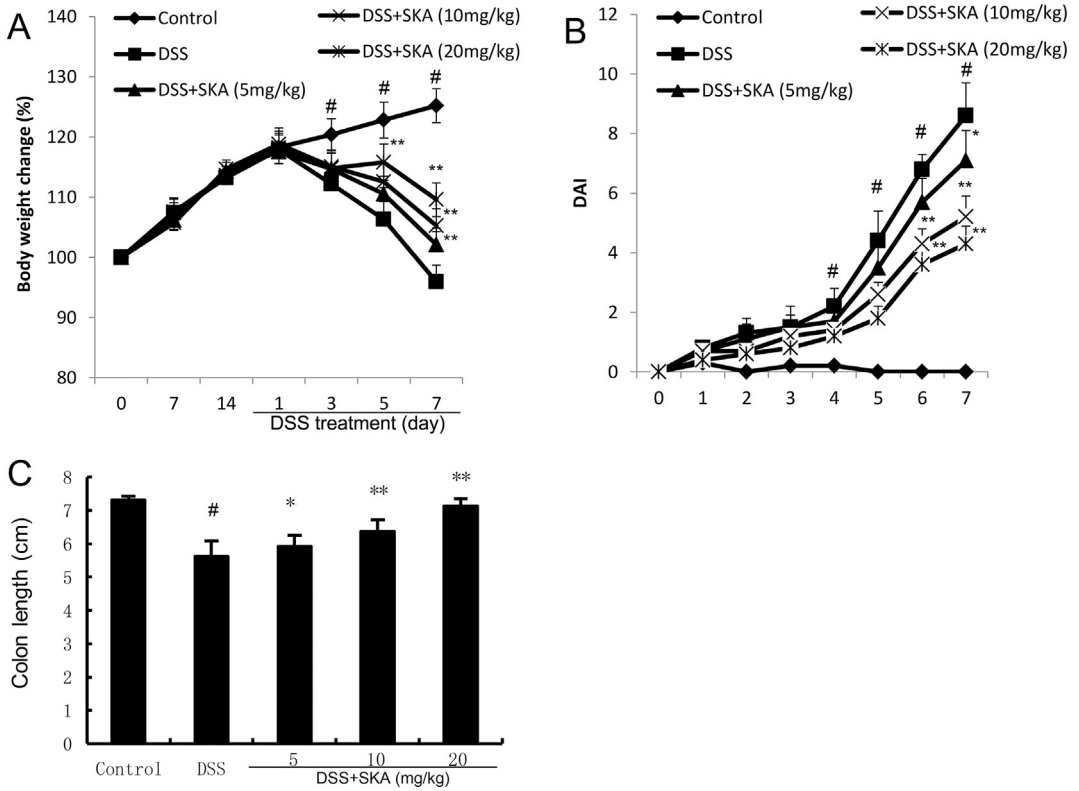

Fig. 2. Effects of SKA on DSS-induced colitis. (A) Body weight change of each group. (B) Disease activity index. (C) The lengths of colons of each group. The values presented are the mean ± SEM (n = 12 in each group) of three parallel measurements. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. DSS group.

relative humidity of 50% ± 10%. The mice were fed with food and water ad libitum.

The experimental protocol was approved by the Institutional Animal Ethics Committee of Xinxiang Medical University. All mice were randomly divided into five groups: control group, DSS group, DSS + SKA (5, 10, 20 mg/kg) groups. The mice of DSS group were administrated 2.5% (wt/vol) DSS for 7 day. The mice of DSS + SKA groups were received SKA (5, 10, 20 mg/kg) oral once a day for 14 day

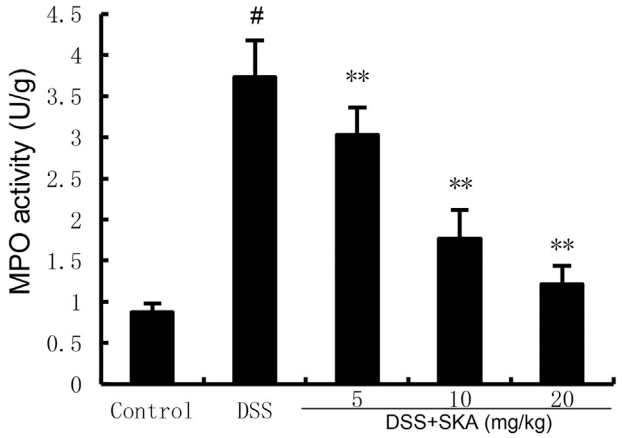

Fig. 3. Effects of SKA on DSS-induced MPO activity. 7 days after DSS treatment, the colonic tissues were collected and the MPO activity was measured. The values presented are the mean ± SEM (n = 12 in each group) of three parallel measurements. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. DSS group.

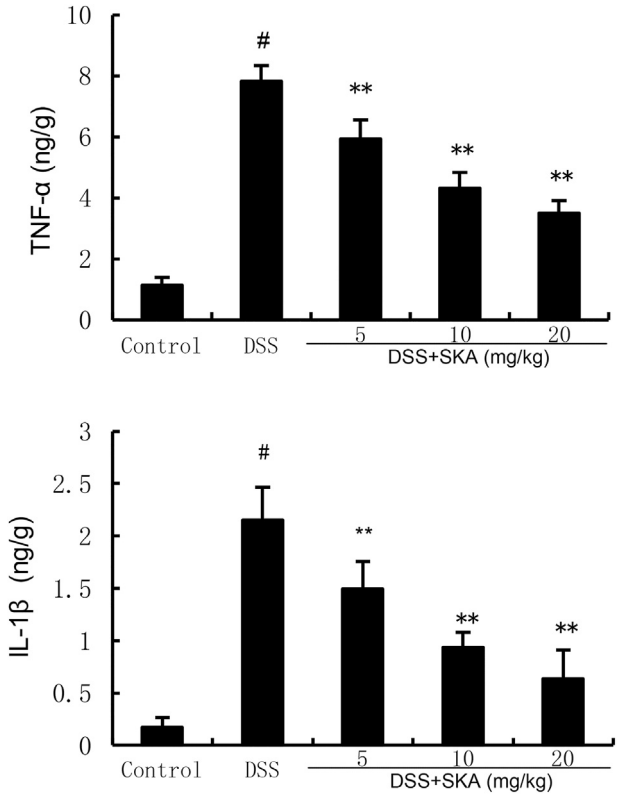

Fig. 4. Effects of SKA on DSS-induced TNF-α and IL-1β in the colonic tissues. 7 days after DSS treatment, the colonic tissues were collected and the levels of TNF-α and IL-1β in the colonic tissues were measured by ELISA. The values presented are the mean ± SEM (n = 12 in each group) of three parallel measurements. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. DSS group.

before and during DSS treatment. The body weight was recorded once a day and the clinical disease activity index (DAI) was the sum of the clinical score. On the last day, the mice were sacrificed and the length of colon was measured and the colon was collected for subsequent experiments.

2.3. Histological analysis

The colonic tissues were fixed with 10% paraformaldehyde, dehydrated, and embedded in paraffin. Then, the tissues were cut into 5 mm sections and stained with H&E staining. The sections were observed using a light microscope (Nikon Eclipse TE2000-U, NIKON, Japan) at 400 × magnification. Histological scores were assessed by using a previously described method [14].

2.4. ELISA assay

The colonic tissues were homogenized and the supernatant were collected. After the above treatment, the levels of TNF-α and IL-1ß in the supernatant were detected by ELISA according to the manufacturer provided instruction (BD, Minneapolis, MN, USA).

2.5. MPO assay

The colonic tissues were homogenized and the supernatants were collected. The MPO activity in the supernatant was detected using MPO assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol.

2.6. Western blot analysis

The proteins of colonic tissues were prepared using the T-PER Tissue Protein Extraction Reagent (Thermo, USA) and quantified by the BCA methods. The proteins were separated by 12% SDS-PAGE and transferred to PVDF membranes for 2 h at 100 V. After blocking with 5% nonfat dried milk, the membranes were probed with primary antibodies and secondary antibodies. Proteins were visualized by chemiluminescence with an ECL kit (Millipore Corp., Billerica, MA).

2.7. Statistical analysis

All results are represented as mean ± SEM. Differences between groups were analyzed by using one-way ANOVA coupled with a post hoc test. A statistically significant difference was defined as $p < 0.05$.

3. Results

3.1. Saikosaponin A protects DSS-induced colitis in mice

In this study, the protective effects of Saikosaponin A on colitis were detected by measuring colonic histopathological changes, body weight loss, disease activity index (DAI), and colonic length shortening. As shown in Fig. 1, massive epithelial destruction, severe crypt destruction, and inflammatory cell infiltration were observed in the colonic tissues of DSS treated group. Treatment of Saikosaponin A dose-dependently attenuated these histopathological changes induced by DSS.

As shown in Fig. 2, compared with the control group, the levels of body weight and colonic length were found significantly decreased by DSS treatment. Conversely, the levels of body weight and colonic length of the Saikosaponin A treatment groups were markedly decreased when compared with DSS group. Furthermore, the level of DAI of DSS-treated mice increased significantly when compared with the control mice. However, Saikosaponin A dose-dependently inhibited the levels of DAI.

3.2. Saikosaponin A attenuates DSS-induced MPO activity in colonic tissues

MPO activity was measured in this study to investigate the effects of Saikosaponin A on neutrophil infiltration. As shown in Fig. 3, compared with the control group, the level of MPO activity in colonic tissues were found significantly increased by DSS treatment. Conversely, the level of MPO activity in colonic tissues of the Saikosaponin A treatment groups were markedly decreased when compared with DSS group.

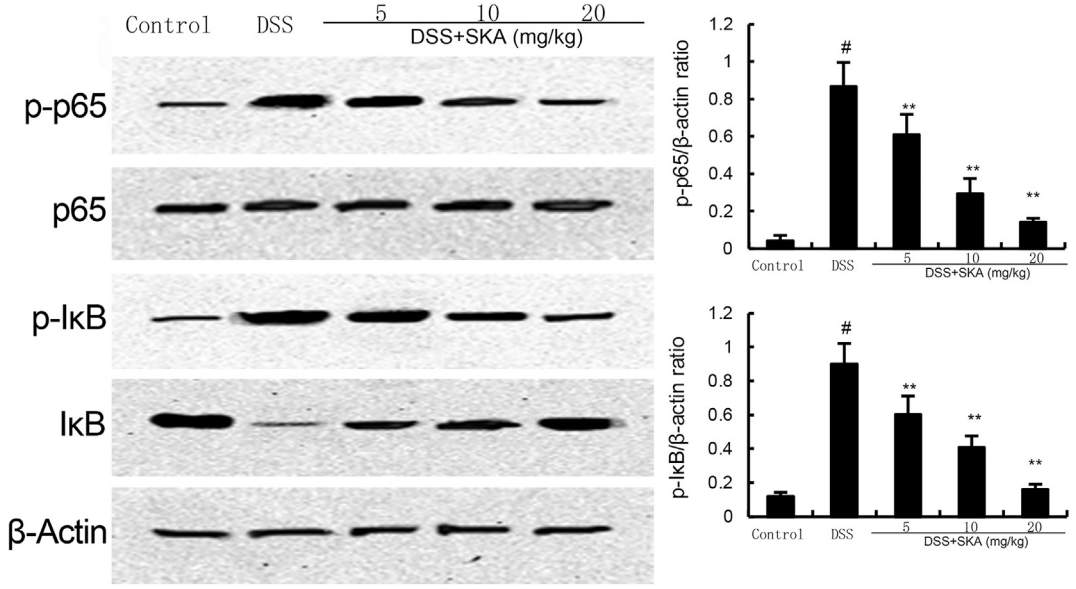

Fig. 5. Effects of SKA on DSS-induced NF-κB activation in the colonic tissues. 7 days after DSS treatment, the colonic tissues were collected and the NF-κB activation in the colonic tissues was measured by western blot analysis. The values presented are the mean ± SEM (n = 12 in each group) of three parallel measurements. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. DSS group.

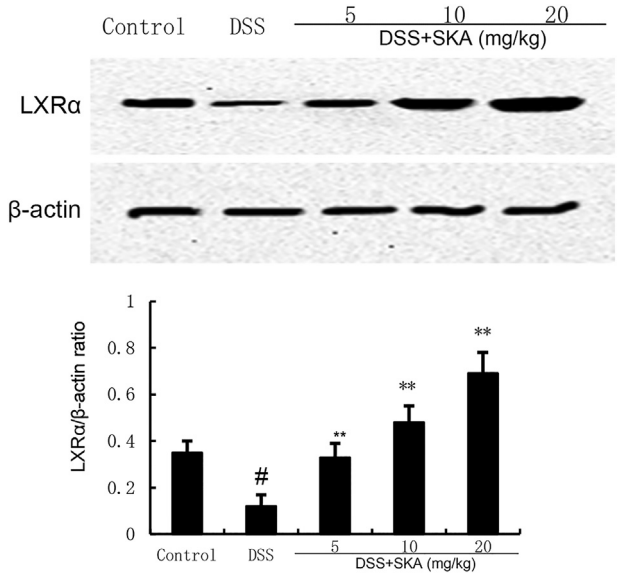

Fig. 6. Effects of SKA on LXRα expression in the colonic tissues. 7 days after DSS treatment, the colonic tissues were collected and the expression of LXRα in the colonic tissues was measured by western blot analysis. The values presented are the mean ± SEM (n = 12 in each group) of three parallel measurements. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. DSS group.

3.3. Saikosaponin A attenuates DSS-induced TNF-α and IL-1β production in colonic tissues

In order to investigate the anti-inflammatory activities of Saikosaponin A, the levels of TNF-α and IL-1β in colonic tissues were measured in this study. As shown in Fig. 4, compared with the control group, the levels of TNF-α and IL-1β in colonic tissues were found significantly increased by DSS treatment. Conversely, the levels of TNF-α and IL-1β in colonic tissues of the Saikosaponin A treatment groups were markedly decreased when compared with DSS group.

3.4. Effects of Saikosaponin A on DSS-induced NF-κB activation

The effects of Saikosaponin A on DSS-induced NF-κB activation were detected in this study. As shown in Fig. 5, compared with the control group, the levels of phosphorylated NF-κB and IκBα in colonic tissues were found significantly increased by DSS treatment. Conversely, the levels of phosphorylated NF-κB and IκBα in colonic tissues of the Saikosaponin A treatment groups were markedly decreased when compared with DSS group. These results suggested that Saikosaponin A could inhibit DSS-induced NF-κB activation.

3.5. Effects of Saikosaponin A on LXRα expression in colonic tissues

To investigate the anti-inflammatory mechanism of Saikosaponin A, the effects of Saikosaponin A on LXRα expression were detected in this study. As shown in Fig. 6, compared with the control group, the expression of LXRα in colonic tissues were found significantly increased by DSS treatment. Conversely, the expression of LXRα in colonic tissues of the Saikosaponin A treatment groups were markedly decreased when compared with DSS group.

4. Discussion

Herbal products have been widely used in the treatment of many diseases, such as colitis [15]. Saikosaponin A (SKA), a triterpene saponin derived from *Radix bupleuri*, has been known to have anti-inflammatory activity. In the present study, Saikosaponin A significantly attenuated DSS-induced colitis in mice. Saikosaponin A may be used as an agent for the treatment of colitis.

DSS-induced colitis is characterized by the accumulation of neutrophils in colonic tissues [16]. The activation of neutrophils could release the inflammatory cytokines, such as TNF-α and IL-1β. Overactivation of neutrophils could lead to the injury of colonic tissues [17,18]. Furthermore, the production of inflammatory cytokines have been known to be involved in the pathogenesis of colitis [19]. In the present study, our results showed that the accumulation of neutrophils in colonic tissues were inhibited by the treatment of Saikosaponin A, as confirmed by the decreased MPO activity in colonic tissues. Previous studies showed that inhibition of inflammatory mediators could attenuate DSS-induced colitis [20]. In this study, our results showed that

Saikosaponin A protected against DSS-induced colitis through inhibiting inflammatory cytokines TNF-α and IL-1β production. These results indicated that Saikosaponin A protected against DSS-induced colitis via suppressing inflammatory response.

Previous studies showed that activation of NF-κB signaling pathway promoted the injury of colonic tissues. NF-κB activation was involved in the development of colitis [21]. Given the fact that NF-κB could regulate the expression of inflammatory cytokines [22], we investigated the effects of Saikosaponin A on DSS-induced NF-κB activation to clarify the mechanism of Saikosaponin A. Our results showed that Saikosaponin A significantly suppressed DSS-induced NF-κB activation. This was consistent with previous published articles, which showed Saikosaponin A could inhibit NF-κB activation. LXRα, the members of the nuclear hormone receptor superfamily, have the ability to regulate the metabolic conversion of cholesterol to bile acids [23]. Also, LXRα has been involved in the regulation of inflammatory response [24]. A previous study demonstrated activation of LXR could protect mice against DSS- and TNBS-induced colitis [25]. Furthermore, activation of LXRα could attenuate the activation of NF-κB [26]. And a large body of natural herbal compounds exhibited anti-inflammatory effects through activating LXRα [27]. Therefore, to clarify the mechanism of Saikosaponin A, the expression of LXRα were measured. Our results showed that Saikosaponin A could increase the expression of LXRα.

In conclusion, our research discovered that Saikosaponin A protected DSS-induced colitis in mice. Saikosaponin A inhibited DSS-induced colitis through suppressing inflammatory response via activation of LXRα. Saikosaponin A has the potential to be used as a agent for the treatment of intestinal inflammatory diseases.

Conflict of interest

All authors declare that they have no conflict of interest.

References

[1] M. Bosani, S. Ardizzone, G.B. Porro, Biologic targeting in the treatment of inflammatory bowel diseases, Biologics: targets & therapy 3 (2009) 77–97.

[2] F. Farrokhyar, E.T. Swarbrick, E.J. Irvine, A critical review of epidemiological studies in inflammatory bowel disease, Scand. J. Gastroenterol. 36 (2001) 2–15.

[3] R.B. Sartor, Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis, Nature clinical practice Gastroenterology & hepatology 3 (2006) 390–407.

[4] K. Mitsuyama, A. Suzuki, N. Tomiyasu, O. Tsuruta, S. Kitazaki, T. Takeda, et al., Pro-inflammatory signaling by Jun-N-terminal kinase in inflammatory bowel disease, Int. J. Mol. Med. 17 (2006) 449–455.

[5] S.J. Bickston, Infliximab for ulcerative colitis induction of remission and maintenance therapy, Gastroenterology & hepatology 3 (2007) 55–56.

[6] A. Kornbluth, D.B. Sachar, Practice parameters Committee of the American College of G. ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee, Am. J. Gastroenterol. 105 (2010) 501–523 (quiz 24).

[7] S.J. Hur, S.H. Kang, H.S. Jung, S.C. Kim, H.S. Jeon, I.H. Kim, et al., Review of natural products actions on cytokines in inflammatory bowel disease, Nutr. Res. 32 (2012) 801–816.

[8] Z. Wei, J. Wang, M. Shi, W. Liu, Z. Yang, Y. Fu, Saikosaponin A inhibits LPS-induced inflammatory response by inducing liver × receptor alpha activation in primary mouse macrophages, Oncotarget 7 (2016) 48995–49007.

[9] J. Zhu, C. Luo, P. Wang, Q. He, J. Zhou, H. Peng, Saikosaponin A mediates the inflammatory response by inhibiting the MAPK and NF-kappaB pathways in LPS-stimulated RAW 264.7 cells, Experimental and therapeutic medicine 5 (2013) 1345–1350.

[10] X. Mao, G. Miao, X. Tao, S. Hao, H. Zhang, H. Li, et al., Saikosaponin A protects TBI rats after controlled cortical impact and the underlying mechanism, Am. J. Transl. Res. 8 (2016) 133–141.

[11] Y. Fu, X. Hu, Y. Cao, Z. Zhang, N. Zhang, Saikosaponin A inhibits lipopolysaccharide-oxidative stress and inflammation in human umbilical vein endothelial cells via preventing TLR4 translocation into lipid rafts, Free Radic. Biol. Med. 89 (2015) 777–785.

[12] H.W. Wang, M. Liu, T.D. Zhong, X.M. Fang, Saikosaponin-D attenuates ventilator-induced lung injury in rats, Int. J. Clin. Exp. Med. 8 (2015) 15137–15145.

[13] H. Zhao, S. Li, H. Zhang, G. Wang, G. Xu, H. Zhang, Saikosaponin A protects against experimental sepsis via inhibition of NOD2-mediated NF-kappaB activation, Experimental and therapeutic medicine 10 (2015) 823–827.

[14] L.A. Dieleman, M.J. Palmen, H. Akol, E. Bloemena, A.S. Peña, S.G. Meuwissen, E.P. Van Rees, Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines, Clin. Exp. Immunol. 114 (3) (1998) 385–391.

[15] A. Triantafyllidi, T. Xanthos, A. Papalois, J.K. Triantafillidis, Herbal and plant therapy in patients with inflammatory bowel disease, Ann. Gastroenterol. 28 (2015) 210–220.

[16] C.L. Zindl, J.F. Lai, Y.K. Lee, C.L. Maynard, S.N. Harbour, W. Ouyang, et al., IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 12768–12773.

[17] M.B. Grisham, L.A. Hernandez, D.N. Granger, Xanthine oxidase and neutrophil infiltration in intestinal ischemia, Am. J. Phys. 251 (1986) G567–G574.

[18] M.B. Grisham, D.N. Granger, Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites, Dig. Dis. Sci. 33 (1988) 6S–15S.

[19] R.J. Xavier, D.K. Podolsky, Unravelling the pathogenesis of inflammatory bowel disease, Nature 448 (2007) 427–434.

[20] H.S. Kwon, S.M. Oh, J.K. Kim, Glabridin, a functional compound of liquorice, attenuates colonic inflammation in mice with dextran sulphate sodium-induced colitis, Clin. Exp. Immunol. 151 (2008) 165–173.

[21] I. Atreya, R. Atreya, M.F. Neurath, NF-kappaB in inflammatory bowel disease, J. Intern. Med. 263 (2008) 591–596.

[22] M.F. Neurath, I. Fuss, G. Schurmann, S. Pettersson, K. Arnold, H. Muller-Lobeck, et al., Cytokine gene transcription by NF-kappa B family members in patients with inflammatory bowel disease, Ann. N. Y. Acad. Sci. 859 (1998) 149–159.

[23] D.J. Peet, S.D. Turley, W. Ma, B.A. Janowski, J.M. Lobaccaro, R.E. Hammer, et al., Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha, Cell 93 (1998) 693–704.

[24] S.B. Joseph, A. Castrillo, B.A. Laffitte, D.J. Mangelsdorf, P. Tontonoz, Reciprocal regulation of inflammation and lipid metabolism by liver × receptors, Nat. Med. 9 (2003) 213–219.

[25] T. Jakobsson, L.L. Vedin, T. Hassan, N. Venteclef, D. Greco, M. D'Amato, E. Treuter, J.Å. Gustafsson, K.R. Steffensen, The oxysterol receptor LXRβ protects against DSS- and TNBS-induced colitis in mice, Mucosal Immunol. 7 (6) (2014) 1416–1428.

[26] O. Cheng, R.P. Ostrowski, W. Liu, J.H. Zhang, Activation of liver × receptor reduces global ischemic brain injury by reduction of nuclear factor-kappaB, Neuroscience 166 (2010) 1101–1109.

[27] Y. Fu, E. Zhou, Z. Wei, W. Wang, T. Wang, Z. Yang, N. Zhang, Cyanidin-3-O-β-glucoside ameliorates lipopolysaccharide-induced acute lung injury by reducing TLR4 recruitment into lipid rafts, Biochem. Pharmacol. 90 (2) (2014) 126–134.