# Ginsenoside Rg1 Attenuates Dextran Sodium Sulfate-Induced Ulcerative Colitis in Mice

**Yuan CHEN[1,2], Qian ZHANG[3], Lifeng SUN[1,2], Haiyan LIU[1,2], Jizhen FENG[4], Jiamei LI[5], Zhiyi WANG[6]**

[1]Department of Pediatrics, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, P.R. China, [2]Shandong Provincial Hospital, Shandong Provincial Clinical Reasearch Center for Children's Health and Disease office, Jinan, Shandong, China, [3]Department of Endocrinology, Jinan Shizhong Distract People's Hospital, Jinan, Shandong, China, [4]Department of Radiology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China, [5]Department of Pathology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China, [6]Department of Hepatobiliary Surgery , Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China

Received July 2, 2023
Accepted August 30, 2023

**Summary**

Ulceration colitis (UC) is a chronic and recurrent inflammatory disorder in the gastro-intestinal tract. The purpose of our study is to explore the potential mechanisms of ginsenoside Rg1 (GS Rg1) on dextran sulfate sodium (DSS)-induced colitis in mice and lipopolysaccharide (LPS)-induced RAW 264.7 cells. Acute colitis was induced in male C57BL/6 mice. In vitro model of LPS-induced RAW 264.7 cells to simulate enteritis model. The disease activity index (DAI), colon length, body weight and histopathological analysis were performed in vivo. Pro-inflammatory cytokines and markers for oxidative and anti-oxidative stress, MPO level were measured in vivo and in vitro. Nuclear erythroid 2-related factor 2 (Nrf2) and NF-κB p65 protein levels were analyzed using western blotting. Our results indicated that the UC models were established successfully by drinking DSS water. GS Rg1 significantly attenuated UC-related symptoms, including preventing weight loss, decreasing DAI scores, and increasing colon length. GS Rg1 ameliorated the DSS-induced oxidative stress. IL-1β, IL-6, and TNF-α levels were significantly increased in serum and cell supernatant effectively, while treatment with the GS Rg1 significantly reduced these factors. GS Rg1 reduced MPO content in the colon. GS Rg1 treatment increased SOD and decreased MDA levels in the serum, colon, and cell supernatant. GS Rg1 restored the Nrf-2/HO-1/NF-κB pathway in RAW 264.7 cells and UC mice, and these changes were blocked by Nrf-2 siRNA. Overall, GS Rg1 ameliorated inflammation and oxidative stress in colitis via Nrf-2/HO-1/NF-κB pathway. Thus, GS Rg1 could serve as a potential therapeutic agent for the treatment of UC.

**Key words**

Ulcerative colitis • Ginsenoside Rg1 • Inflammation • Oxidative stress

**Corresponding authors**

Zhiyi Wang, Department of Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, no 324 Jingwu Road, Jinan 250021, P.R. China. E-mail: zhiyiw888@126.com; Jiamei Li, Department of Pathology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250021, Shandong, P.R. China. E-mail: Jiameili2012@126.com

## Introduction

Ulcerative colitis (UC) is a chronic nonspecific inflammatory disease. Primary symptoms of UC include hemorrhagic diarrhea, weight loss, and abdominal cramps [1-3] with lesions restricted to the colon and rectum. What is more, several studies illustrated that UC was an independent risk factor of colon-rectal cancer [4-6]. The incidence and prevalence of UC have recently steadily increased all over the world [7]. Previous studies reveal that UC pathogenesis is focused on cytokines and inflammatory mediators responsible for the immune response and inflammatory processes in UC [8,9]. The etiology and pathogenesis of UC are still unclear to date.

Studies have shown the role of oxidative stress in UC development. Nuclear factor E2 related factor 2 (Nrf-2) plays a crucial role in oxidative stress processes. External injury factors can activate Nrf-2 and transfer it to stone, thus initiating transcription of various antioxidant enzymes to enhance the antioxidant capacity of local tissues, and help reduce the extent of oxidative stress response induced by external injury factors [10]. To clarify the role of NRF-2 mediated oxidative stress in the occurrence and changes of UC.

Ginsenoside Rg1 is a natural stem extract and a major active ingredient in ginseng [11,12]. It also possesses several pharmacological activities, including anti-inflammation, anti-apoptosis, [13] anti-oxidative, and neuroprotective effects [14]. GS Rg1 at 200 mg/kg can suppress the release of IL-1β and TNF-α via NLRP12 upregulation in mice with colitis [15].

In this study, we investigated the effect of ginsenoside Rg1 treatment on the development of colitis, with the classic IBD drug 5-aminosalicylic acid (5-ASA) as a positive control drug and reference substance to evaluate the therapeutic effect of GS Rg1. Our results indicated that ginsenoside Rg1 significantly decreased inflammatory responses by regulating Nrf-2/HO-1/NF-κB pathway during DSS- induced mice colitis.

## Materials and methods

*Animals*

Male C57BL/6 mice (8~10 weeks, 20~23g, n=40) were purchased from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd. All mice were fed with water and food ad libitum and were housed in an animal room at standard conditions (21 °C~24 °C; humidity, 45 %~60 %; 12~12 h light/dark cycle). After acclimation for 7 days, the mice were randomly divided into four equal groups: NC group (normal group, n=10), DSS group (DSS-induced colitis without treatment, n=10), DSS+Rg1 (DSS-induced colitis+ treatment with GS Rg1, n=10), DSS+5-ASA (DSS-induced colitis+treatment with 5-aminosalicycle acid, n=10) groups. All procedures for this experiment were approved by the Animal Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University.

*Colitis model*

According to the previously established classic mouse UC model [11,12]. All groups of mice were allowed free access to standard chow and sterilized water in the first three days. Then, a) NC group: drinking sterile water on the 4th day for 7 days; b) DSS group: drinking water was changed to sterile water with 3 % DSS (w/v, dissolved in sterilized water) on the 4th day for 7 days; c) Rg1 group: drinking water was changed to sterile water with 3 % DSS on the 4th day for 7 days, in addition, the mice were administered GS Rg1 daily for 10 days in the dosage of 200mg/kg b.w; [13,14], d) 5-ASA group: drinking water was changed to sterile water with 3 % DSS on the 4th day for 7 days, in addition, the mice were administered 5-ASA daily for 10 days in the dosage of 300mg/kg b.w [15]. Mice were sacrificed under sodium pentobarbital anesthesia on day 11 for subsequent analysis.

After the blood was collected through the abdominal aorta, the anus was taken to the colon of the ileocecal section. The gross morphological changes were observed under the anatomical microscope.

*Disease activity index (DAI) evaluation*

Throughout the experiment, weight loss, stool condition as well as rectal bleeding of the mice were observed and recorded daily. The DAI score was determined as an average of the scores for the parameters mentioned (DAI = (Weight loss + stool condition + gross bleeding)/3 based on previously reported method.

*Cell culture*

RAW 264.7 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), and cultured in DMEM (ThermoFisher Scientific, Waltham, MA, USA) medium containing 10 % fetal bovine serum (ThermoFisher Scientific), 100U/ml penicillin (ThermoFisher Scientific) and 100μg/ml streptomycin (ThermoFisher Scientific). These cells were cultured at 37 ℃, 5 % $CO_2$ in a humidified atmosphere.

*MTT assessment*

Cell viability was determined by MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay method. RAW 264.7 cells ($5x10^3$ cells/well) were plated in 96-well plates. After 24h, these cells were treated with LPS and GS Rg1 at different concentrations. After a 24h incubation period, 10μl of MTT solution (5mg/ml) was added to each well and the plate was incubated for 4 h at 37 ℃. Thereafter, the media in the wells were aspirated and a volume of 100μl of dimethyl sulfoxide (DMSO) was added to solubilize the formazan salt formed. The OD value was measured at a wavelength of 450 mm using a microplate reader (TECAN, Vienna, Austria), indirectly

reflecting the number of viable cells.

*Nrf2 silencing by siRNA*

RAW 264.7 cells were cultured in 6-well plates and allowed to grow to 70 % confluence. The LipofectamineTM 2000 (Invitrogen Ltd., Carlsbad, CA) transfection reagent was used for transient transfection following the manufacturer's instructions. Specific siRNA for Nrf2 isoforms, Forward primer 5'-GGGUAAGUCGAGAAGUG UUTT-3' and 5'-AACACUUCUCGACUUACCCTT-3' and scrambled siRNA control were designed by Gene Pharma Co. (Shanghai, China). The reaction mixture containing 5μl siRNA, 5μl LipofectamineTM 2000, and 95μl serum-free culture medium (Opti-MEM, Invitrogen) was mixed at room temperature, and incubated for 20 min. Later, 800μl of Opti-MEM medium was added drop-wise to each culture well containing the RAW 264.7 cells, and the reaction mixture added. After transfection for 6 h, the cell culture medium was replaced, and cells incubated for another 24h before exposure to lipopolysaccharide (LPS).

*Evaluation of myeloperoxidase (MPO) activity in the colon and ROS detection*

MPO activity was determined following an established protocol (7). For ROS detection, RAW 264.7 cells or and Nrf2 siRNA treated RAW 264.7 cells were pre-treated with GS Rg1 for 24 h, the cells were followed by washing with serum-free medium, and incubated with (2,7-Dichlorodihydrofluorescein diacetate) DCFH-DA (10 μM) for 20 min at 37 ℃. The fluorescence intensity was measured at an excitation wavelength of 488 nm and an emission wavelength of 525 nm.

*Measurement of pro-inflammatory cytokines、oxidative and anti-oxidative stress levels in the serum, colon, and cell supernatant*

The SOD、MDA、IL-1β, IL-6, and TNF-α content in serum, colon, and cell supernatant were detected following the kit instructions (BD Bioscience, San Diego, CA, USA).

*Western blotting analysis*

Colon tissue and cells were homogenized and lysed using cold RIPA lysis buffer (KeyGEN BioTECH, Nanjing, China), for 15 min on the ice. Then these lysates were centrifuged at 12,000 g for 10 min at 4 °C, and then supernatants were transferred to distilled tube. Protein samples (40 μg) were separated by 10 % SDS-polyacrylamide gel electrophoresis, and transferred onto a PVDF membrane (Pierce Biotechnology, Rockford, IL, USA). The membrane was blocked with 5 % non-fat milk for 1 h at room temperature. Then, the membrane was incubated overnight at 4 °C with primary antibodies (dilution, 1:1000) against. Next day, the membrane was rinsed 3 times with TBST buffer and incubated for 1 h with horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse secondary antibody (dilution, 1:8000) at room temperature, and rewashed with TBST buffer. The bolt bands were reacted with an enhanced chemiluminescence solution (Bio-Rad, CA, USA) and detected using western imaging system (Tanon 5200, Beijing, China). The intensity of the protein bands was quantified using Image Lab software (Bio-Rad, CA, USA).

*Statistical analysis*

Statistical analysis was analyzed by using Graph Pad Prism software 7.0 and expressed as the mean ± S.D (standard deviation). Statistical significance was identified by using one-way analysis of variance (ANOVA) and Bonferroni's post-hoc analysis. A $P<0.05$ was statistically significant.

## Results

*GS Rg1 protects cells against LPS-induced cytotoxicity*

The effect of GS Rg1 on RAW 264.7 cell viability was assessed through a MTT assay. When the different concentrations (1~160 μM) of GS Rg1 was used to simulate the RAW 264.7 cells, we found that GS Rg1 has no effect on RAW 264.7 cell viability (Fig. 1A), while incubation with LPS (0~320 μM) remarkably damaged the cell viability of RAW 264.7 cells (Fig. 1B). Then, we assessed the effect of GS Rg1 on LPS induced cellular injury in RAW 264.7 cells. As expected, GS Rg1 pretreatment restored the cell viability considerably (Fig. 1C).

*GS Rg1 decreased the ROS production in LPS-induced RAW 264.7 cells*

Our result demonstrated that the ROS levels was significantly increased in LPS-induced RAW 264.7 cells, however, GS Rg1 treatment reduced the ROS levels. Moreover, Nrf-2 siRNA transfection eliminated the effect of GS Rg1 on ROS levels in LPS-induced RAW 264.7 cells (Fig. 2).

*Ginsenoside Rg1 alleviates the colitis symptoms in DSS-*

*induced colitis mice*

As shown in Figure 3B, the experimental schedule was used to establish the DSS-induced colitis in mice. Previous study has reported that the characteristics of DSS-induced colitis are body weight loss, diarrhea, and severe bloody stools. Therefore, in our study, mice in the DSS group showed significant body weight loss compared with the normal group (Fig. 3C). However, GS Rg1 (200mg/kg) attenuated body weight loss during the progression of colitis in mice (Fig. 3C). In the model mice, the DAI score was significantly increased by the presence of DSS, but was dramatically decreased by GS Rg1 (Fig. 3D). Besides, we also observed that the colonic shortening induced by DSS was restored by GS Rg1 (Fig. 3E, 3F). The spleen is the most important lymphoid organ and is enlarged in response to infection or inflammation in the body. Therefore, we investigated the spleen index (spleen weight/body weight (g)) after treatment with GS Rg1. As expected, the increase in spleen index induced by DSS was restored by GS Rg1 (Fig. 3G).

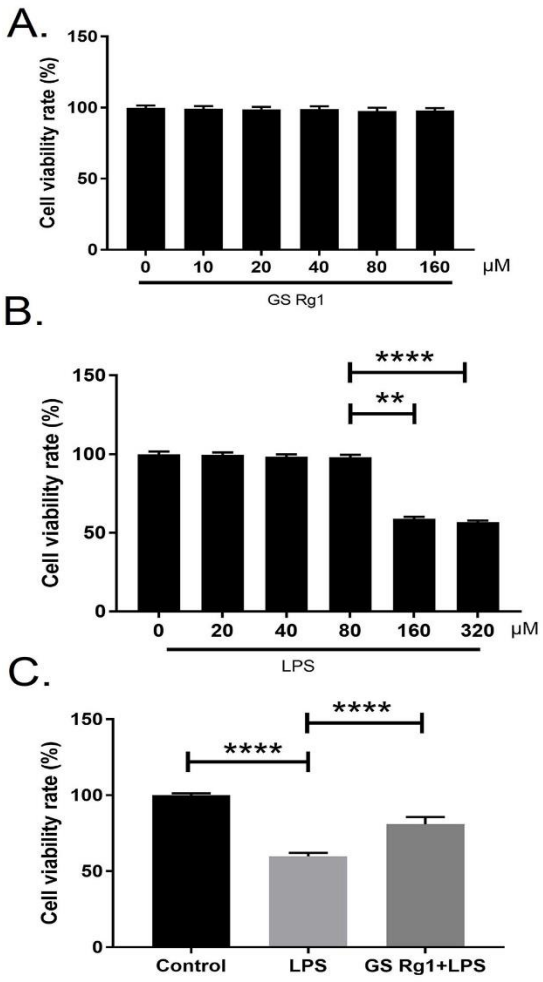


**Fig. 1.** GS Rg1 exhibited preventive effects on LPS-induced cytotoxicity. (**A**) RAW 264.7 cells were treated with the different concentrations of GS Rg1 for 24 h. (**B**) RAW 264.7 cells were treated with the different concentrations of LPS for 24 h. **p<0.01; ****p<0.0001. (**C**) RAW 264.7 cells were co-treated with the GS Rg1 and LPS for 24 h. ****p<0.0001. GS Rg1: Ginsenoside Rg1; LPS: lipopolysaccharide. One-way ANOVA followed by Bonferroni's *post-hoc* test.

*GS Rg1 decreased the histopathological change and MPO in colon*

Consistent with previous reports, inflammatory cell infiltration into the distal colon mucosa, crypt loss, and epithelial cell destruction in colon tissue of mice in the DSS group was significantly increased compared to the control group mice. However, GS Rg1 treatment considerably restored the colon tissue (Fig. 4A). GS Rg1 treatment had a stronger effect than the 5-ASA (Fig. 4A). Moreover, GS Rg1 also decreased the DSS mediated induction of MPO activation (Fig. 4B).

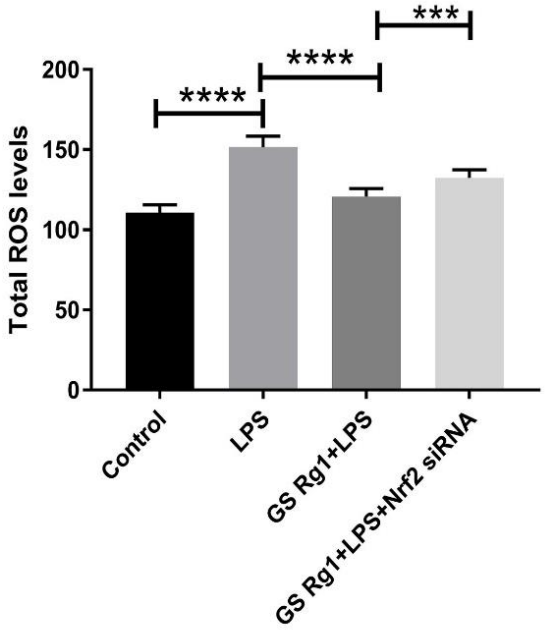


**Fig. 2.** GS Rg1 decreased the ROS production in LPS-induced RAW 264.7 cells. The vaules were presented as the mean ± SD. ***p<0.001; ****p<0.0001. One-way ANOVA followed by Bonferroni's *post-hoc* test. GS Rg1: Ginsenoside Rg1; ROS: reactive oxygen species; LPS: lipopolysaccharide.

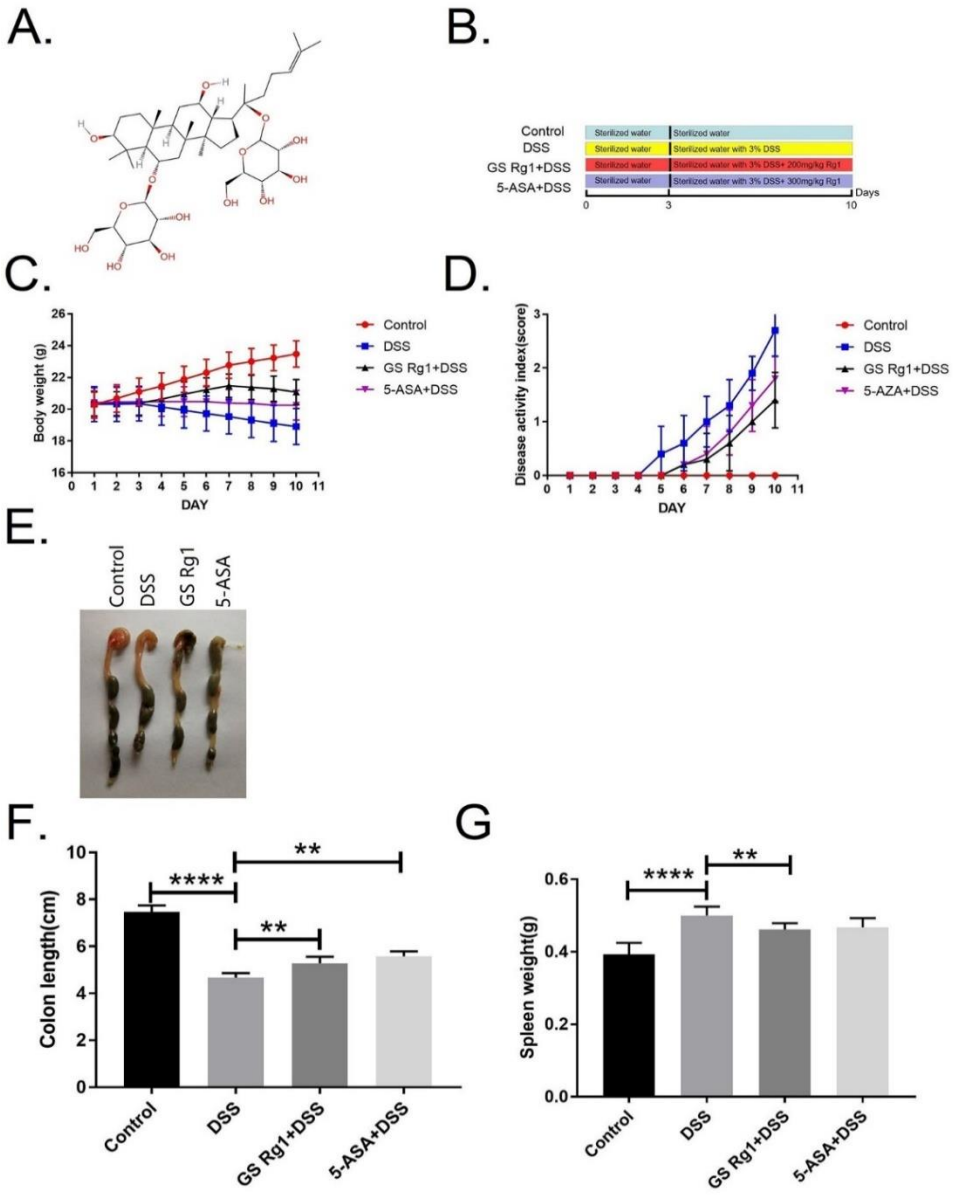


**Fig. 3.** GS Rg1 alleviates colitis symptoms in DSS-induced colitis mice. (**A**) The chemical structures of GS Rg1. (**B**) Experiment schedule for the model of DSS-induced colitis in mice. (**C**) Body weight change. (**D**) DAI score. (**E, F**) Photographs of the colon and colon length. (**G**) The spleen index. All values are presented as the mean ± SD. The data were analyzed by One-way ANOVA followed by Bonferroni's *post-hoc* test. GS Rg1, ginsenosides Rg1; DSS, dextran sulphate sodium. ****p<0.0001.

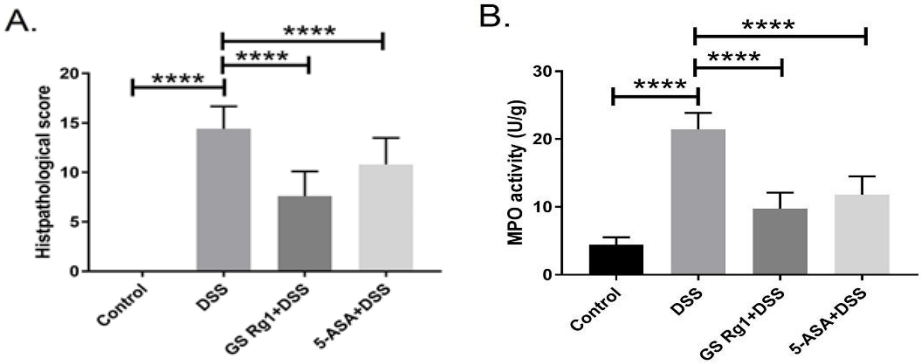


**Fig. 4**. GS Rg1 decreased the histopathological change and MPO in colon. (**A**) The histopathological score. (**B**) The effect of GS Rg1 on MPO in colon. The values are presented as the mean ± SD. ****P<0.0001. One-way ANOVA followed by Bonferroni's *post-hoc* test. GS Rg1, ginsenosides Rg1; MPO, myeloperoxidase; DSS, dextran sulphate sodium.

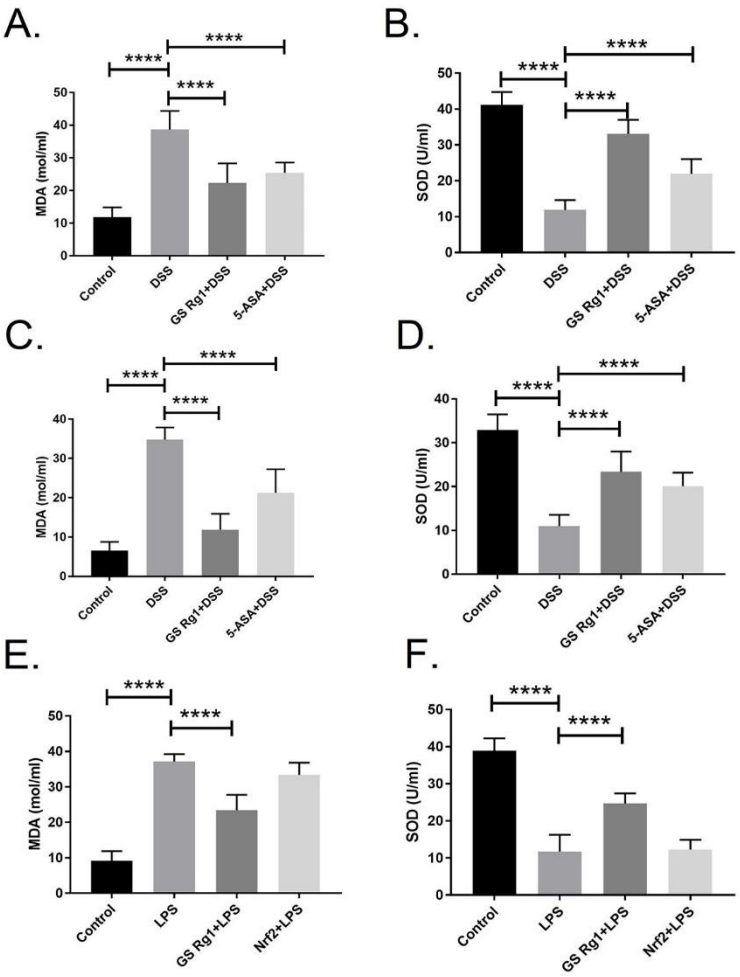


**Fig. 5.** GS Rg1 decreases the MDA content and increases SOD activity in serum, colon and the cell supernatants. The expression of the MDA content (**A**) and SOD activity (**B**) in serum sections were detected. The expression of the MDA content (**C**) and SOD activity (**D**) in colon section were measured. The expression of the MDA content (**E**) and SOD activity (**F**) in RAW 264.7 cells was detected. The values are presented as the mean ± SD. ****P<0.0001. One-way ANOVA followed by Bonferroni's *post-hoc* test. GS Rg1, ginsenoside Rg1; DSS, dextran sulphate sodium; LPS, lipopolysaccharid; MDA, malon-dialdehyde; SOD, superoxide dismutase.

*GS Rg1 reduces MDA content and enhances SOD activity levels in serum, colon and the cell supernatants*

Oxidative stress in the intestinal tissues leads to the production of increased oxidative stress in the blood. Hence, it is critical to monitor intestinal oxidative stress products in the serum. As shown in Fig. 5, increase MDA content and decrease SOD activity levels in the serum (Fig. 5A, 5B) and colon (Fig. 5C, 5D) of DDS-induced mice compared to control mice, and GS Rg1 treatment reversed the effects on MDA and SOD. Moreover, LPS treatment increased MDA (Fig. 5E) and decreased SOD (Fig. 5F) levels in RAW 264.7 cells, and as expected, pretreatment with GS Rg1 (640 μM) increased SOD and decreased the MDA level, and Nrf-2 siRNA treatment of cells blocked this effect.

*GS Rg1 reduces the inflammatory cytokines levels in serum, colon and the cell supernatants*

We assessed the levels of pro-inflammatory cytokines in the serum, colon and the cell supernatants. We found that IL-1β (Fig. 6A, 6D), IL-6 (Fig. 6B, 6E) and TNF-α (Fig. 6C, 6F) levels were significantly increased in serum (Fig. 6A, 6B, 6C) and colon (Fig. 6D, 6E, 6F) of DSS-induced mice, and GS Rg1 treatment decreased IL-1β, IL-6 and TNF-α levels in serum and colon significantly. Besides, LPS simulation increased IL-1β (Fig. 6G), IL-6 (Fig. 6H), and TNF-α (Fig. 6I) levels in RAW 264.7 cells. GS Rg1 (640 μM) treatment decreased the levels of IL-1β, IL-6, and TNF-α, and Nrf-2 siRNA reversed this effect.

*GS Rg1 regulates the Nrf-2/HO-1/NF-κB pathway in DSS-induced mice and LPS-induced RAW 264.7 cells*

As shown in Fig. 7A, we found that decreased levels of Nrf-2 (Fig. 7B; $p<0.001$, $p<0.0001$) and HO-1 (Fig. 7C; $p<0.001$, $p<0.0001$) and increased p-NF-κBp65 (Fig. 7D; $p<0.05$, $p<0.001$) in the colon of DSS-induced mice. Administration of GS Rg1 increased the levels of Nrf-2 and HO-1 and decreased p-NF-κBp65 level significantly (Fig. 7B, C, D). Besides, the Nrf-2 (Fig. 7E, F) and HO-1 (Fig. 7E, G) levels decreased while p-NF-κBp65 level increased in LPS-induced RAW 264.7 cells, and GS Rg1 (640 μM) increased Nrf-2 and HO-1 levels and decreased the levels of p-NF-κBp65 significantly.

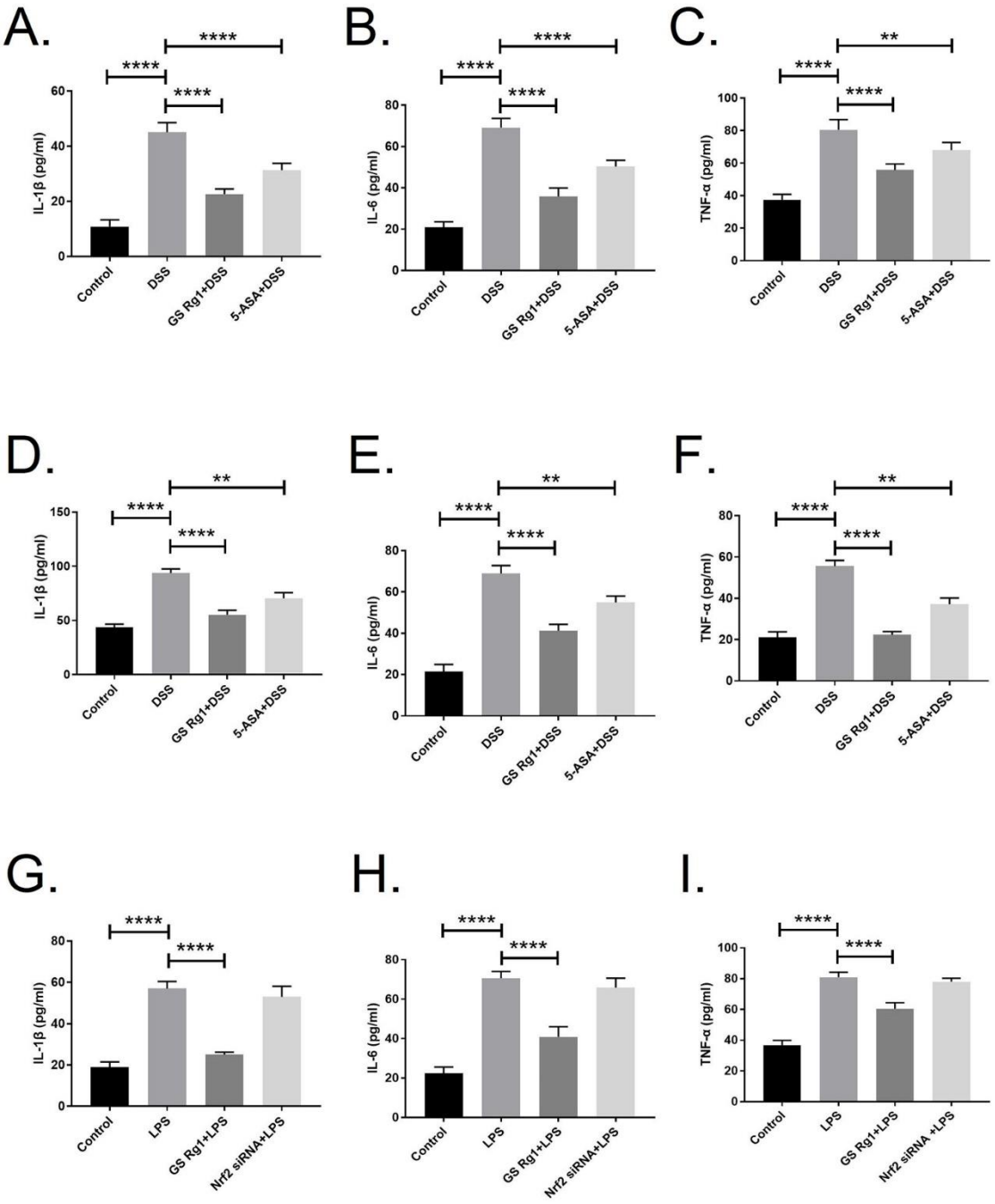


**Fig. 6.** GS Rg1 reduces the inflammatory cytokines levels in serum, colon and the cell supernatants. The expression of IL-1β (**A**), IL-6 (**B**), and TNF-α (**C**) in colon sections were detected by ELISA. The expression of IL-1β (D), IL-6 (**E**), and TNF-α (**F**) in serum were measured by ELISA. The expression of IL-1β (**G**), IL-6 (**H**), and TNF-α (**I**) in RAW 264.7 cells. The values are presented as the mean ± SD. **P<0.01; ****P<0.0001. One-way ANOVA followed by Bonferroni's *post-hoc* test. GS Rg1, ginsenoside Rg1; DSS, dextran sulphate sodium; LPS, lipopolysaccharide.

## Discussion

Ginsenoside Rg1 (GS Rg1) is mainly extracted and purified from the root of ginseng [16]. As the main constituents of Panax ginseng, ginsenoside Rg1 is well-known for its anti-inflammatory effect. It has been shown to significantly decrease the inflammatory cytokines IL-6 and TNF-α release in LPS-stimulated RAW264.7 cells. Some researchers had shown that GS Rg1 could effectively treat patient and animal colitis [15,17]. In the present study, after mice with UC administered GS Rg1 for 7 consecutive days, colon weight loss, colon shortening, increased colon weight, inflammatory cell infiltration, and ulcer formation were effectively reversed. Ginsenoside Rg1 could also inhibit the expression of iNOS (inducible nitric oxide synthase), TNF-α in mice

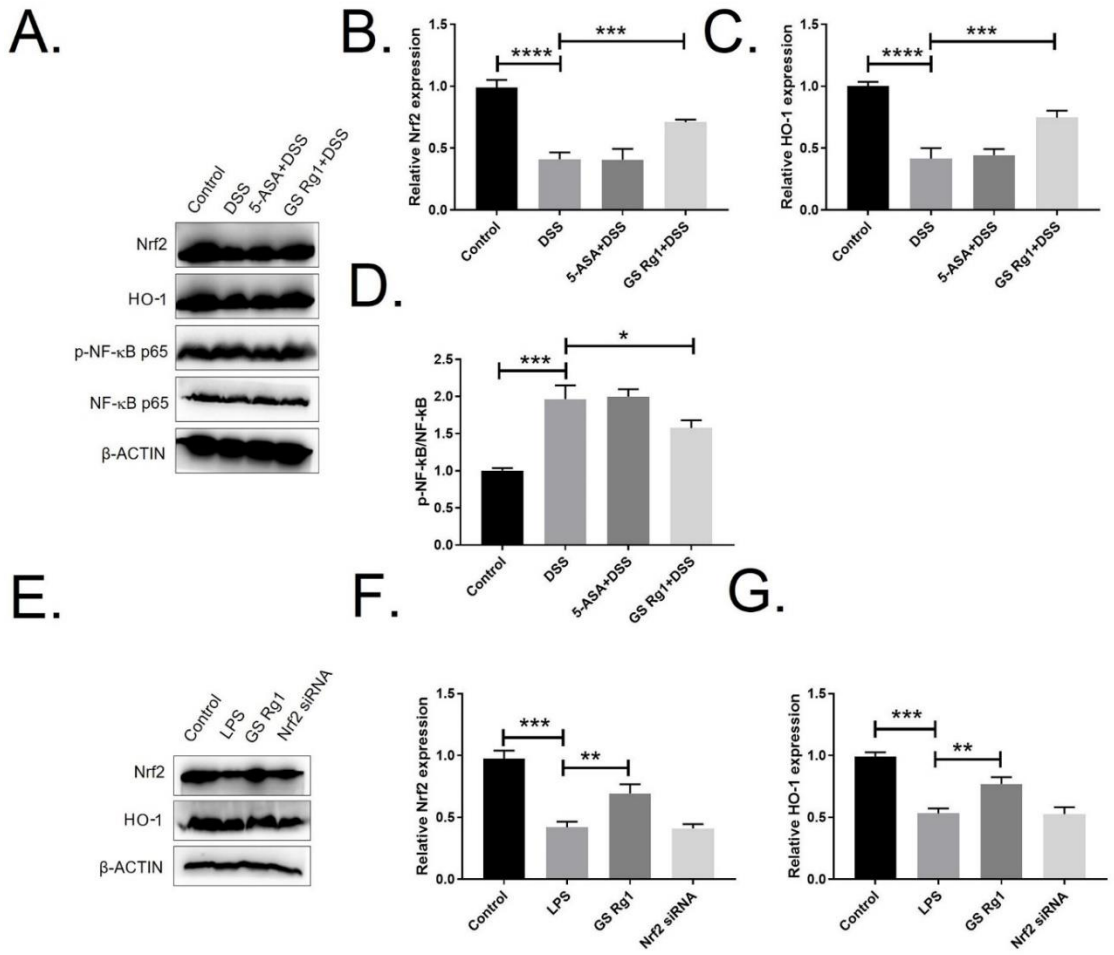


**Fig. 7.** The effect of GS Rg1 on Nrf-2/HO-1/NF-κB pathway in DSS-induced mice and LPS-induced RAW 264.7 cells. (**A, B, C, D**) The effect of GS Rg1 on Nrf-2/HO-1/NF-κB pathway in DSS-induced mice. (**E, F, G**) The effect of GS Rg1 on Nrf-2/HO-1/NF-κB pathway in LPS-induced RAW 264.7 cells. The values are presented as the mean ± SD. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. One-way ANOVA followed by Bonferroni's *post-hoc* test. GS Rg1, Ginsenosides Rg1; Nrf-2, nuclear factor E-2 related factor; HO-1, heme oxygenase-1; NF-κB, nuclear factor kappa-B; DSS, dextran sulphate sodium.

hippocampus injected by LPS by disrupting NF-κB and MAPK pathways [18]. Ginsenoside Rg1 was showed to have a potent protective role against LPS-induced sepsis [19]. As to the role of Rg1 on colitis, a recent study demonstrated that ginsenoside Rg1 and its metabolites protected mice from TNBS- induced colitis. In addition, ginsenosides significantly inhibited NF-κB activation through a pregnane X receptor (PXR) [20]. In the present study, we further investigated the molecular mechanisms lying behind the anti-inflammatory effect of GS Rg1 during colitis, GS Rg1 reduced IL-6 and TNF-α elevations. This suggests that GS Rg1 is effective for ameliorating DSS-induced colitis in mice.

Nrf2 is an oxidative stress receptor and an important transcription factor in maintaining cellular redox balance [21]. Therefore, it also plays an important role in the process of oxidative stress in cells. At the same time, HO-1 serves as a target gene for the Nrf2/HO-1 signaling pathway, in which Nrf2 can induce an upregulation of HO-1 expression, thereby inhibiting the production and release of inflammatory mediators, and thereby playing an anti-inflammatory, antioxidant, and anti-apoptotic role [22,23]. Previous literature has reported that Nrf2/HO-1 signaling pathways are involved in the pathogenesis of many diseases. For example, upregulation of Nrf2/HO-1 signaling pathway proteins can alleviate ischemia-reperfusion injury and the production of inflammatory mediators [24,25]. In our study, in a DSS induced mouse model, it was found that the expression of Nrf2/HO-1 pathway protein was significantly reduced, while it was upregulated in the Nrf2-siRNA group. Therefore, we infer that Nrf2/HO-1 signaling pathway is involved in the pathogenesis of UC.

NF-κB as a regulatory factor that specifically binds to the enhancer sequence of the B-lymphocyte immunoglobulin K light chain gene, NF-κB has strong

transcriptional regulatory activity and is involved in the development of many inflammatory diseases [26]. NF-κB also plays an important role in regulating inflammatory cells, apoptosis, and immune regulatory gene expression [27-29]. Later research found that any κB Genes at the B site can pass NF-κB regulates transcription, including its association with immune, inflammatory, and transcriptional regulation. Activation of NF-κB under non physiological conditions, NF-κB can undergo nuclear translocation, leading to the secretion of a large number of inflammatory cytokines. In addition, NF-κB can also be further activated by these cytokines, which can exacerbate the intestinal immune response and inflammatory damage in UC patients. NF-κB activation and expression of B may be one of the key steps in the development of UC. NF-κB P65 is NF-κB One of the five members of the B family and an important proinflammatory factor in UC. In our study, we found that NF-κB pathway was activated in the DSS induced mouse group, while it was downregulated in the Nrf-2 siRNA and GS Rg1 groups. Therefore, we infer that NF-κB signal pathway is also involved in the development of UC.

In conclusion, our findings demonstrated that GS Rg1 protects mice from DSS-induced colitis through activation of the Nrf2/HO-1 signaling pathway for enhancing the antioxidant capacity and inhibiting the pro-inflammatory mediators in the colon. In addition, our research provides evidence that Nrf-2 siRNA targeting regulates the NF-κB pathway and alleviates DSS-induced colitis in mice and LPS-induced RAW 264.7 cells. However, our research is preliminary, and further studies are warranted.

## Conflict of Interest

There is no conflict of interest.

## Acknowledgements

Funding by Natural Science Foundation of Shandong Province (ZR2022QH076); Natural Science Foundation of Shandong Province (ZR2021MH381).

## References

1. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet 2007; 369: 1641-1657. https://doi.org/10.1016/S0140-6736(07)60751-X
2. Salaritabar A, Darvishi B, Hadjiakhoondi F, et al. Therapeutic potential of flavonoids in inflammatory bowel disease: a comprehensive review. World Journal of Gastroenterology 2017; 23: 5097-5114. https://doi.org/10.3748/wjg.v23.i28.5097
3. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet 2017; 389: 1756-1770. https://doi.org/10.1016/S0140-6736(16)32126-2
4. Rubin DT, Parekh MJ. Colorectal cancer in inflammatory bowel disease: molecular and clinical considerations. Current Treatment Options in Gastroenterology. 2006; 9: 211-220. https://doi.org/10.1007/s11938-006-0040-5
5. McKenna NP, Bews KA, Behm KT, Mathis KL, Lightner AL, Habermann EB. Do patients with Inflammatory bowel disease have a higher postoperative risk of venous thromboembolism or do they undergo more high-risk operations. Annals of Surgery 2020; 271: 325-331. https://doi.org/10.1097/SLA.0000000000003017
6. Gu J, Remzi FH, Shen B, Vogel JD, Kiran RP. Operative strategy modifies risk of pouch-related outcomes in patients with ulcerative colitis on preoperative anti-tumor necrosis factor-α therapy. Diseases of the Colon & Rectum 2013; 56: 1243-1252. https://doi.org/10.1097/DCR.0b013e3182a0e702
7. Ng SC, Shi HY, Hamidi N et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. The Lancet 2017; 390: 2769-2778. https://doi.org/10.1016/S0140-6736(17)32448-0
8. Tatiya-Aphiradee N, Chatuphonprasert W, Jarukamjorn K. Immune response and inflammatory pathway of ulcerative colitis. J Basic Clin Physiol Pharmacol 2018; 30: 1-10. https://doi.org/10.1515/jbcpp-2018-0036
9. Torres M, Rios A. Current view of the immunopathogenesis in inflammatory bowel disease and its implications for therapy. World Journal of Gastroenterology: WJG. 2008; 14: 1972. https://doi.org/10.3748/wjg.14.1972
10. Yuan Z, Yang L, Zhang X, et al. Huang-Lian-Jie-Du Decoction ameliorates acute ulcerative colitis in mice via regulating NF-kB and Nrf2 signaling pathways and enhancing intestinal barrier function. Front Pharmacol 2019;10:1354. https://doi.org/10.3389/fphar.2019.01354

11. Zhang Q, Zhao YH. Therapeutic angiogenesis after ischemic stroke: Chinese medicines, bone marrow stromal cells (BMSCs) and their combinational treatment. Am J Chin Med. 2014; 42:61-77. https://doi.org/10.1142/S0192415X14500049
12. Chu SF, Zhang Z, Zhou X, He WB, Chen C, Luo P, Liu DD, Ai QD, Gong HF, Wang ZZ, et al. Ginsenoside Rg1 protects against ischemic/reperfusion-induced neuronal injury through miR-144/Nrf2/ARE pathway. Acta Pharmacol Sin. 2019; 40:13-25. https://doi.org/10.1038/s41401-018-0154-z
13. He M, Halima M, Xie Y, Schaaf MJM, Meijer AH, Wang M. Ginsenoside Rg1 acts as a selective glucocorticoid receptor agonist with anti-inflammatory action without affecting tissue regeneration in Zebrafish Larvae. Cells 2020;9,1107. https://doi.org/10.3390/cells9051107
14. Gao QG, Zhou LP, Lee VH, Chan HY, Man CW, Wong MS. Ginsenoside Rg1 activates ligand-independent estrogenic effects via rapid estrogen receptor signaling pathway. J. Ginseng Res. 2019; 43:527-538. https://doi.org/10.1016/j.jgr.2018.03.004
15. Zhu G, Wang H, Wang T, Shi F. Ginsenoside Rg1 attenuates the inflammatory response in DSS-induced mice colitis. Int. Immunopharmacol. 2017; 50: 1-5. https://doi.org/10.1016/j.intimp.2017.06.002
16. Zhang Y, Ding S, Chen Y, Sun Z, Zhang J, Han Y, et al. Ginsenoside Rg1 alleviates lipopolysaccharide-induced neuronal damage by inhibiting NLRP1 inflammasomes in HT22 cells. Exp. Ther. Med. 2021;22:782.
17. Lee SY, Jeong JJ, Eun SH, Kim DH. Anti-inflammatory effects of ginsenoside Rg1 and its metabolites ginsenoside Rh1 and 20(S)-protopanaxatriol in mice with TNBS-induced colitis. Eur. J. Pharmacol. 2015; 762: 333-343. https://doi.org/10.1016/j.ejphar.2015.06.011
18. Lee DC, Lau AS. Effects of Panax ginseng on tumor necrosis factor-α-mediated inflammation: a mini-review. Molecules 2011; 16:2802-2816. https://doi.org/10.3390/molecules16042802
19. Su F, Xue Y, Wang Y, Zhang L, Chen W, Hu S. Protective effect of ginsenosides Rg1 and Re on lipopolysaccharide-induced sepsis by competitive binding to toll-like receptor 4, Antimicrob. Agents Chemother. 2015; 59: 5654-5663. https://doi.org/10.1128/AAC.01381-15
20. Zhang J, Cao L, Wang H, Cheng X, Wang L, Zhu, T. Yan T, Xie Y, Wu Y, Zhao M. Ginsenosides regulate PXR/NF-κB signaling and attenuate dextran sulfate sodium-induced colitis. Drug Metab. Dispos. 2015; 43: 1181-1189. https://doi.org/10.1124/dmd.115.063800
21. Bellezza I, Giambanco I, Minelli A, et al. Nrf2-Keap1 signaling in oxidative and reductive stress. Biochim Biophys Acta Mol Cell Res 2018;1865:721-733. https://doi.org/10.1016/j.bbamcr.2018.02.010
22. Zeng T, Zhang CL, Song FY, et al. The activation of HO-1/Nrf-2 contributes to the protective effects of diallyl disulfide (DADS) against ethanol-induced oxidative stress. Biochim Biophys Acta 2013;1830:4848-4859. https://doi.org/10.1016/j.bbagen.2013.06.028
23. Park EJ, Kim YM, Park SW, et al. Induction of HO-1 through p38 MAPK/Nrf2 signaling pathway by ethanol extract of Inula helenium L. reduces inflammation in LPS-activated RAW 264.7 cells and CLP-induced septic mice. Food Chem Toxicol 2013;55:386-395. https://doi.org/10.1016/j.fct.2012.12.027
24. Liu C, Zhu C, Wang G, et al. Higenamine regulates Nrf2-HO-1-Hmgb1 axis and attenuates intestinal ischemia-reperfusion injury in mice. Inflamm Res 2015; 64: 395-403. https://doi.org/10.1007/s00011-015-0817-x
25. Wang J, Hu X, Xie J, et al. Beta-1-adrenergic receptors mediate Nrf2-HO-1- HMGB1 axis regulation to attenuate hypoxia/reoxygenation-induced cardiomyocytes injury in vitro. Cell Physiol Biochem 2015;35:767-777. https://doi.org/10.1159/000369736
26. Sun SC. The non-canonical NF-κB pathway in immunity and inflammation. Nat Rev Immunol 2017;17:545-558. https://doi.org/10.1038/nri.2017.52
27. Mitchell JP, Carmody RJ. NF-κB and the Transcriptional Control of Inflammation. Int Rev Cell Mol Biol 2018;335:41-84. https://doi.org/10.1016/bs.ircmb.2017.07.007
28. Timucin AC, Basaga H. Pro-apoptotic effects of lipid oxidation products: HNE at the crossroads of NF-κB pathway and anti-apoptotic Bcl-2. Free Radic Biol Med 2017;111:209-18. https://doi.org/10.1016/j.freeradbiomed.2016.11.010
29. Hayden MS, Ghosh S. NF-κB in immunobiology. Cell Res 2011;21:223-44. https://doi.org/10.1038/cr.2011.13