Ginsenoside Rg1 attenuates dextran sodium sulfate-induced ulcerative colitis in mice

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ABSTRACT

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

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 G-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 Lipofectamine™ 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 Lipofectamine™ 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 488nm and an emission wavelength of 525nm.

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 (Figure 1A), while incubation with LPS (0~320 μM) remarkably damaged the cell viability of RAW 264.7 cells (Figure 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 (Figure 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 (Figure 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. We known 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 (Figure 3C). However, GS Rg1 (200mg/kg) attenuated body weight loss during the progression of colitis in mice (Figure 3C). In the model mice, the DAI score was significantly increased by the presence of DSS, but was dramatically decreased by GS Rg1 (Figure 3D). Besides, we also observed that the colonic shortening induced by DSS was restored by GS Rg1 (Figure 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 (Figure 3G).

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 (Figure 4A). GS Rg1 treatment had a stronger effect than the 5-ASA (Figure 4A). Moreover, GS Rg1 also decreased the DSS mediated induction of MPO activation (Figure 4B).

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 Figure 5, increase MDA content and decrease SOD activity levels in the serum (Figure 5A, 5B) and colon (Figure 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 (Figure 5E) and decreased SOD (Figure 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 (Figure 6A, 6D), IL-6 (Figure 6B, 6E) and TNF-α (Figure 6C, 6F) levels were significantly increased in serum (Figure 6A, 6B, 6C) and colon (Figure 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 (Figure 6G), IL-6 (Figure 6H), and TNF-α (Figure 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 Figure 7A, We found that decreased levels of Nrf-2 (Figure 7B; $p<0.001$, $p<0.0001$) and HO-1 (Figure 7C; $p<0.001$, $p<0.0001$) and increased p-NF-κBp65 (Figure 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 (Figure B, C, D). Besides, the Nrf-2 (Figure 7E, F) and HO-1 (Figure 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.

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 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.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Legend

Figure 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.

Figure 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.

Figure 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.

Figure 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.

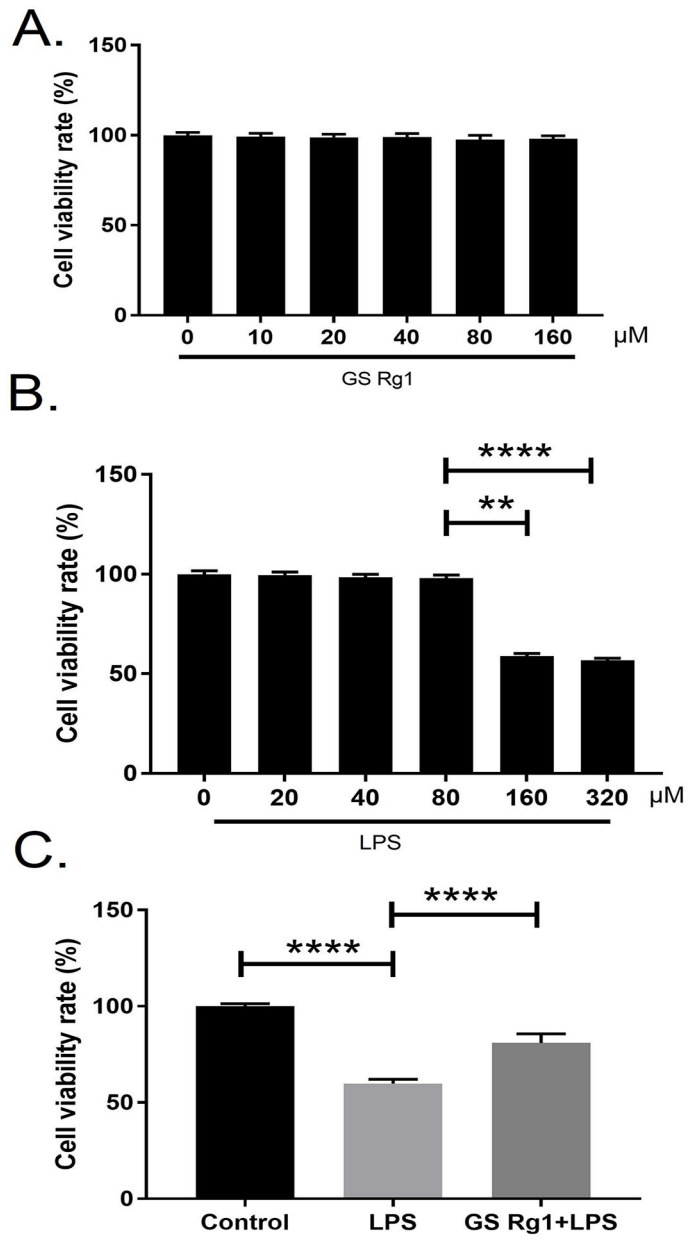
Figure 5. GS Rg1 decreases the MDA content and increases SOD activity in serum, colon and the cell supernatants. The expression of the MDA content (A) and SOD

activity (B) in colon sections were detected. The expression of the MDA content (C) and SOD activity (D) in serum 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, malondialdehyde; SOD, superoxide dismutase.

Figure 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.

Figure 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.

Fig. 1



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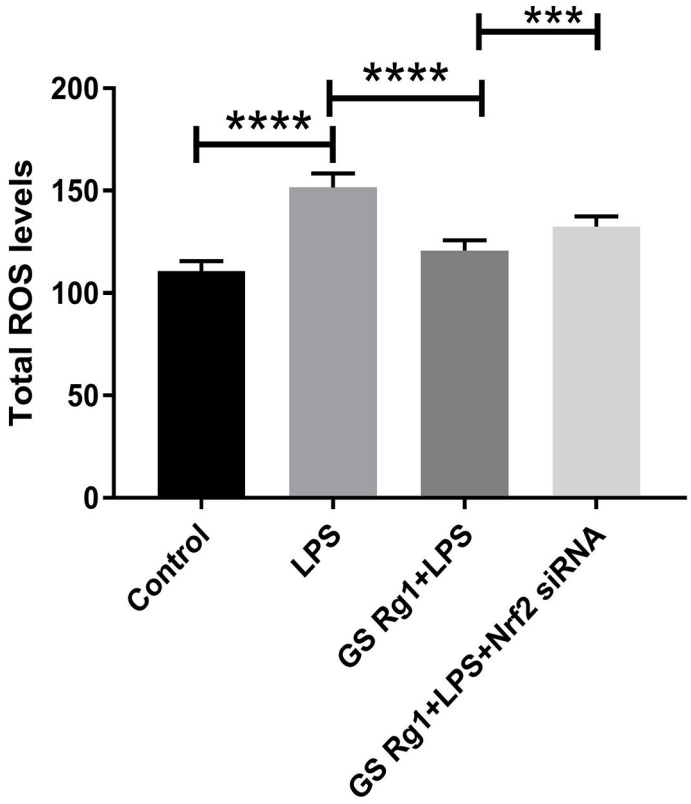
466 Fig. 2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200
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Total ROS levels
Control
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GS Rg1+LPS
GS Rg1+LPS+Nrf2 siRNA

Fig. 3

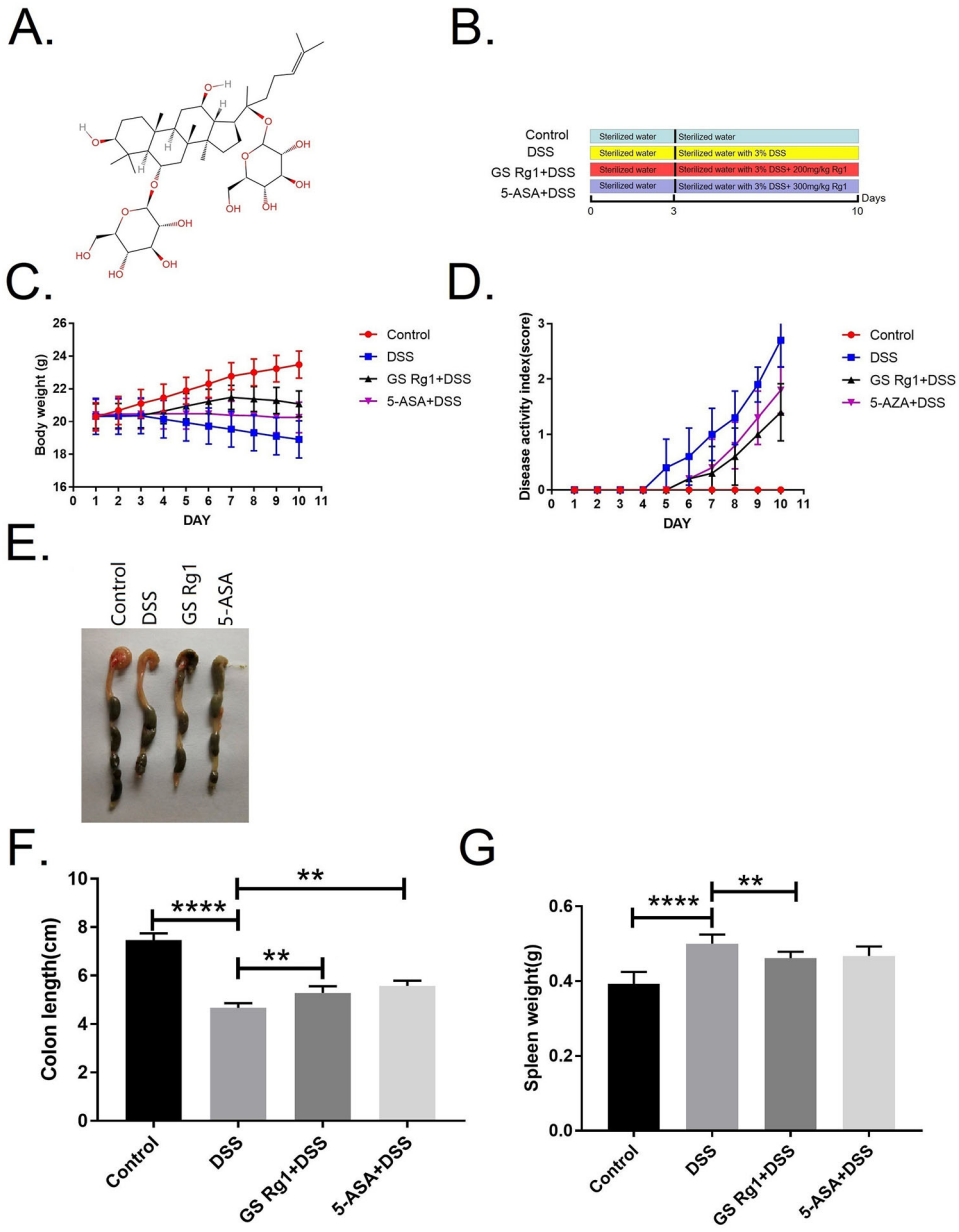
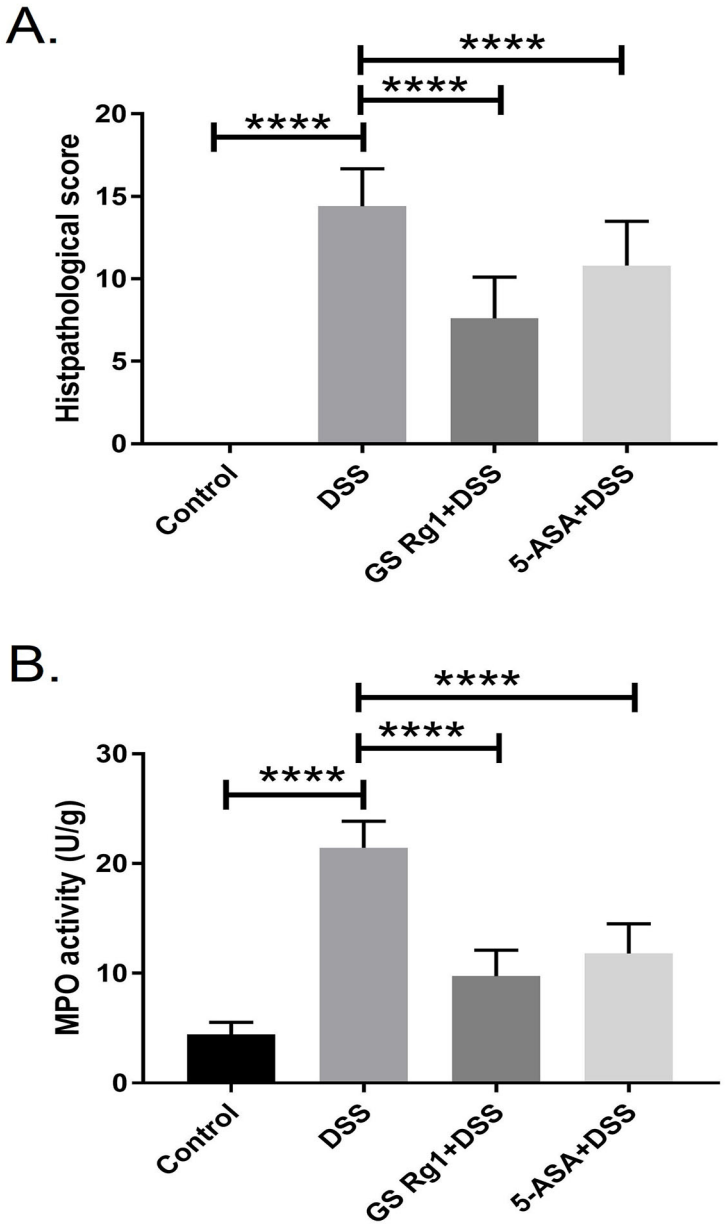

A.
B.
Control
DSS
GS Rg1+DSS
5-ASA+DSS
Sterilized water
Sterilized water with 3% DSS
Sterilized water with 3% DSS+ 200mg/kg Rg1
Sterilized water with 3% DSS+ 300mg/kg Rg1
Days
C.
Body weight (g)
DAY
D.
Disease activity index(score)
5-AZA+DSS
E.
GS Rg1
5-ASA
F.
Colon length(cm)
G
Spleen weight(g)

Fig. 4



492
493
494
495
496
497
498
499

500 Fig. 5

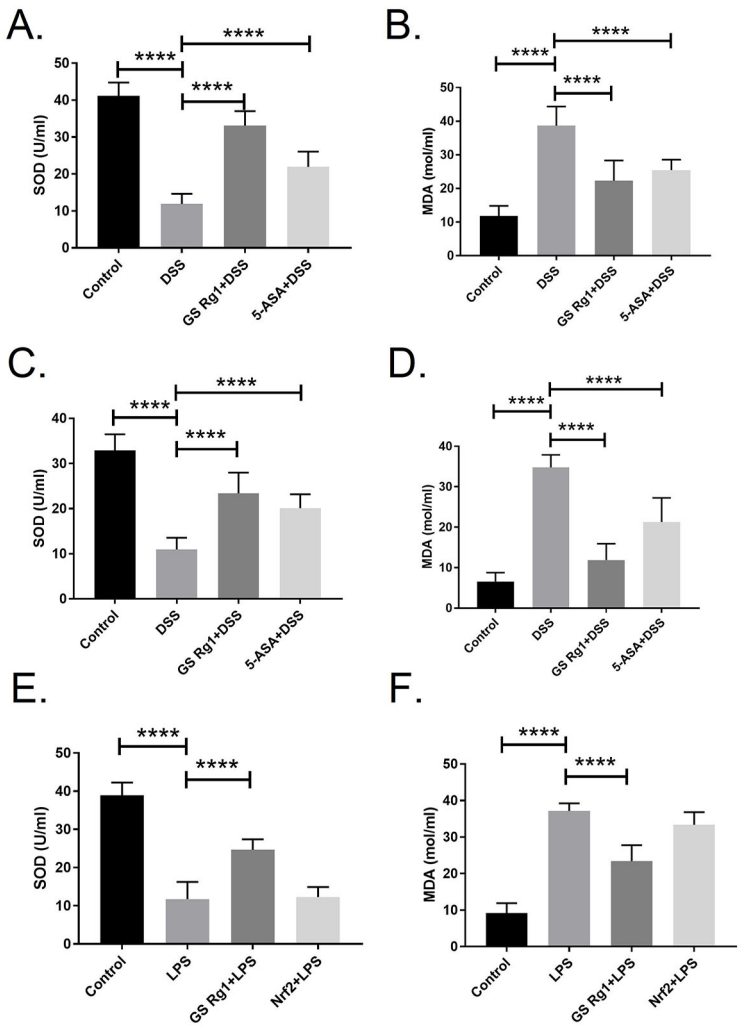

A.
B.
C.
D.
E.
F.
SOD (U/ml)
MDA (mol/ml)
****
Control
DSS
GS Rg1+DSS
5-ASA+DSS
LPS
GS Rg1+LPS
Nrf2+LPS

Fig. 6

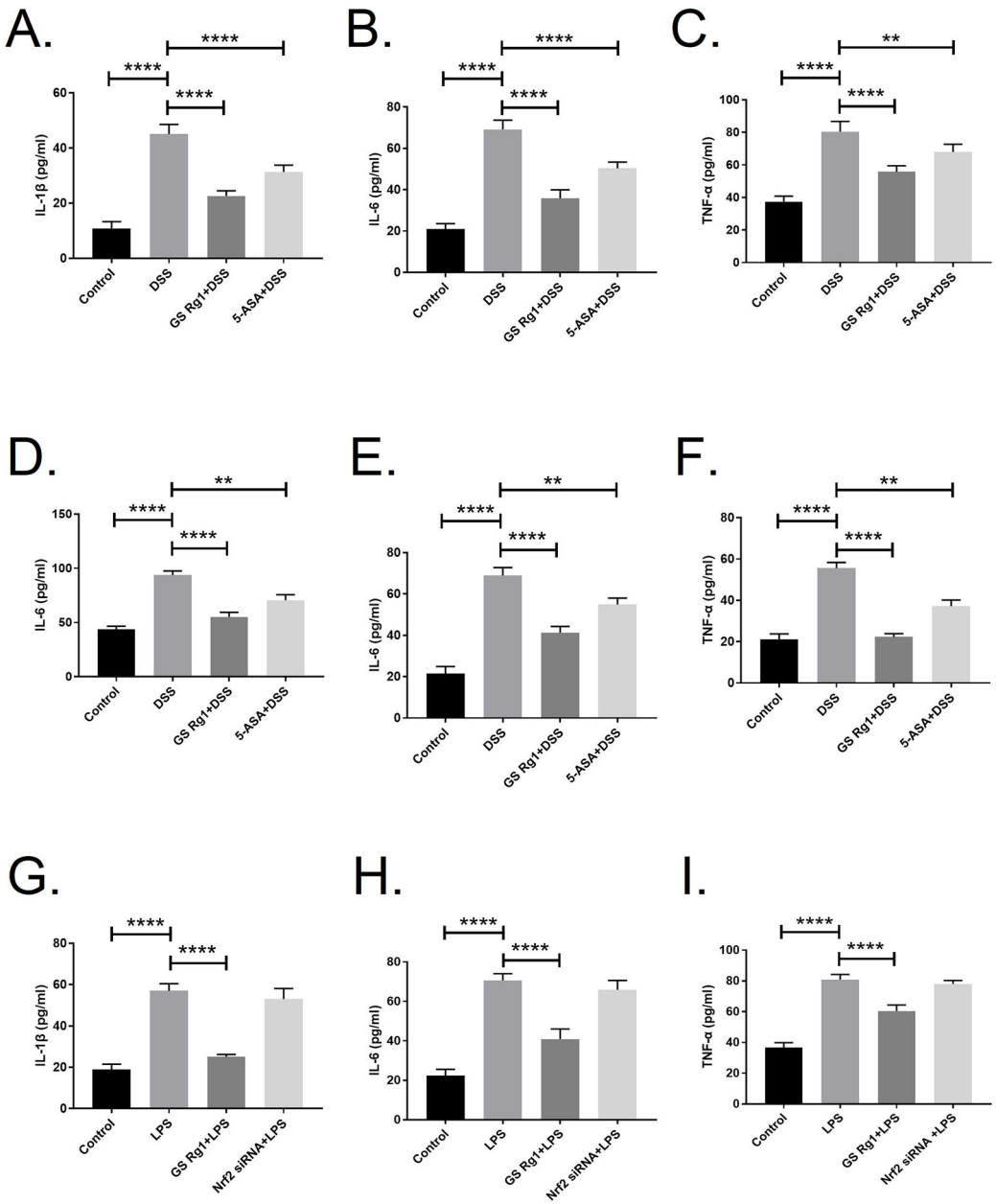
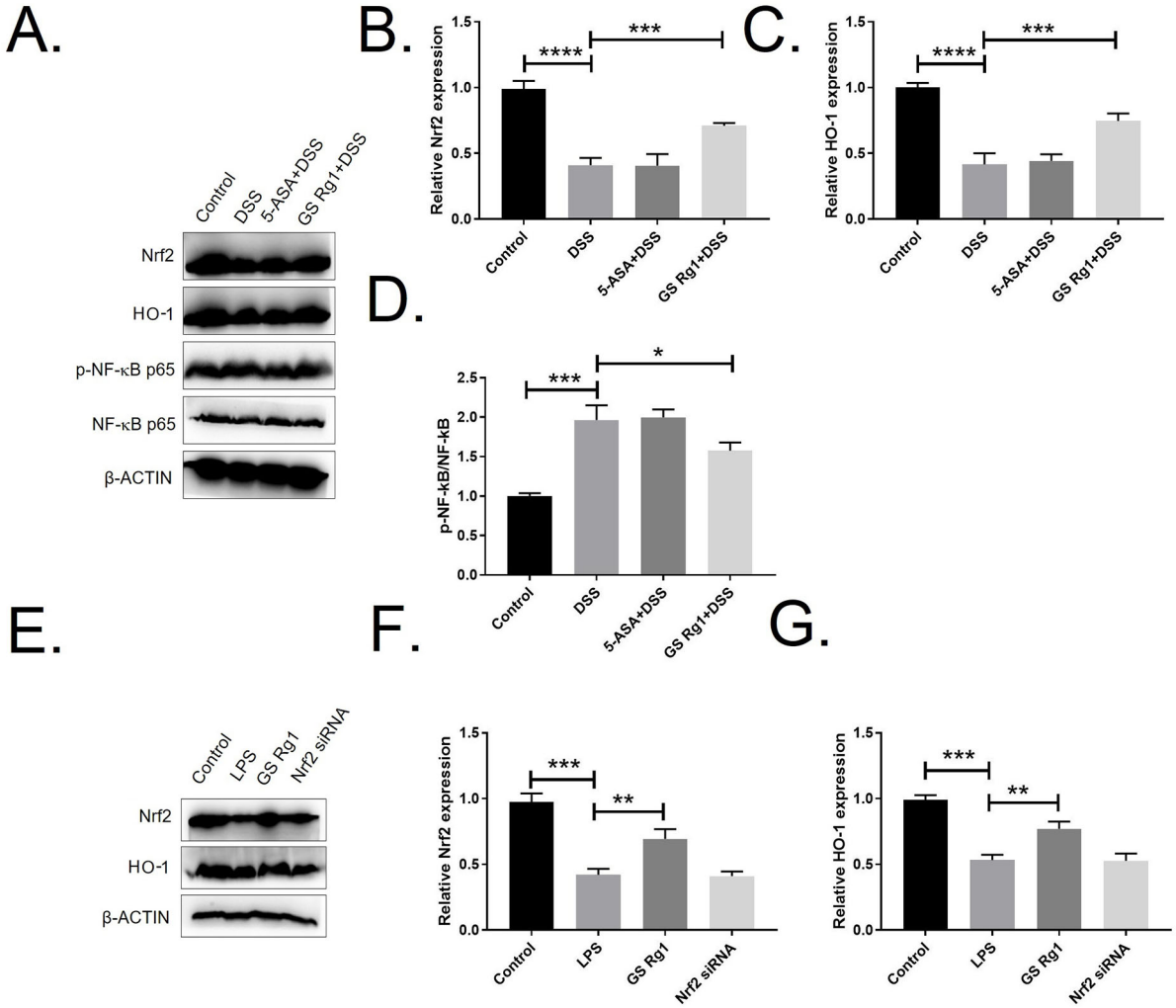

A.
B.
C.
D.
E.
F.
G.
H.
I.
IL-1β (pg/ml)
IL-6 (pg/ml)
TNF-α (pg/ml)
Control
DSS
GS Rg1+DSS
5-ASA+DSS
LPS
GS Rg1+LPS
Nrf2 siRNA+LPS
****
**

Fig. 7


A.
Control
DSS
5-ASA+DSS
GS Rg1+DSS
Nrf2
HO-1
p-NF-κB p65
NF-κB p65
β-ACTIN
B.
Relative Nrf2 expression
****
***
C.
Relative HO-1 expression
D.
p-NF-kB/NF-kB
***
*
E.
Control
LPS
GS Rg1
Nrf2 siRNA
F.
G.
**

523

524