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Effect of dehydroepiandrosterone on the immune response and gut microbiota in dextran sulfate sodium-induced colitis mice

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ABSTRACT

Dehydroepiandrosterone (DHEA) possess anti-inflammatory, anti-oxidant and immune-regulating function in animals and humans, but there is not enough information about the mechanisms underlying its beneficial effects. The present study investigated the effect and mechanism of DHEA in dextran sulfate sodium (DSS)-induced colitis mice. The findings showed that DHEA relieved the decreasing of body weight, the increasing of disease activity index, the enhancing of spleen weight, the shortening of colon length and the rising of myeloperoxidase activity; meanwhile, histopathological analysis showed that DHEA maintained a relatively intact structure of colon in DSS-induced colitis mice. DHEA decreased the malondialdehyde content, superoxide dismutase activity and inducible nitric oxide synthase protein level; meanwhile, DHEA also inhibited the secretion of tumor necrosis factor-α, interleukin-1β and interleukin-6 in DSS-induced colitis mice. Importantly, our results showed that DHEA blocked the activation of nuclear factor-kappa B (NF-κB) and p38 mitogen-activated protein kinase (MAPK) pathways; and it inhibited the Nod-like receptor protein 3 inflammasome activation in DSS-induced colitis mice. Furthermore, DHEA markedly promoted the intestinal barrier function by up-regulation zonula occludens-1 expression level. The 16S rDNA gene sequencing demonstrated that DHEA decreased the *Pseudomonas* abundance in DSS-induced colitis mice. In conclusion, our data demonstrated that DHEA reduces oxidative damage through regulating antioxidant enzyme activity; inhibits pro-inflammatory cytokines production by blocking the activation of p38 MAPK and NF-κB signal pathway; protects colon barrier integrity *via* increasing tight junction protein expression and modulating gut microbiota taxa; all that finally alleviates DSS-induced experimental colitis in mice.

1. Introduction

Ulcerative colitis (UC) is a main form of the inflammatory bowel diseases (IBD) and characterizes by body weight loss, abdominal pain, diarrhea and rectal bleeding (Conrad et al., 2014). UC mainly occurs in the colonic mucosa and lesions mostly develop from the distal end of colon, and it causes millions of patients in the worldwide (Cosnes et al., 2011). It was reported that the pathogenesis of UC is associated with several signal pathways, such as p38 MAPK and NF-κB signal pathways (Hwang et al., 2018). In addition, the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammatory plays critical role in diverse inflammatory diseases (Shen et al., 2018; Wree et al., 2018), and the NLRP3 expression requires NF-kB phosphorylation and its nuclear translocation (Xue et al., 2019). Meanwhile, researcher certified that NLRP3 can regulate the intestinal homeostasis (Cassel et al., 2009), and the inhibitors of NLRP3 may be used as the potential therapeutic drug for IBD treatment (Perera et al., 2017). In addition, the disturbance of intestinal microbiota and epithelial barrier damage were along with UC (Rubio et al., 2018). It well known that a good tight junction (TJ) of intestines is essential to maintain intestinal function and prevents the pathogenic microorganism's invasion, thereby exerting a barrier function and safeguarding the body health. Although many studies reported that environmental factors (Ananthakrishnan et al., 2018), intestinal microbial disorders (Suskind et al., 2018), intestinal mucosal damage and mucosal immune disorders (Gonçalves et al., 2018) are closely related with the onset of IBD, while the underlying precise physiological mechanism is still unclear.

Dehydroepiandrosterone (DHEA), a multifunction small molecule active substance, is produced in the cholesterol metabolism pathway in humans; and which is considered to be a relatively safe supplemental

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nutrient (Krzysztof et al., 2014). One of characteristics about DHEA is its age-dependent secretion pattern; and this reduction has been shown to be related to the physical health of human (Goldman and Glei, 2007). Importantly, previous study showed that decreasing of serums DHEA level is related to many inflammatory diseases such as IBD (Hildreth et al., 2013); and it affect inflammatory mediators secretion and then regulate immune function (Malutan et al., 2014). Although evidence showed that DHEA acts an immunomodulatory effect in mice (Alves et al., 2016) and humans (Andus et al., 2015), the mechanisms underlying these effects remain unclear. Thus, this study aimed to investigate the effect of DHEA on immune function and gut microbiota in DSS-induced mice; and the results will clarify the detailed mechanisms of DHEA as an immunomodulatory agent to control inflammatory bowel diseases in animals and humans.

2. Materials and methods

2.1. Reagents

Dextran sulfate sodium (DSS, 36–50 kDa) was obtained from Meilun Biotech Co., Ltd. (Dalian, China). DHEA was obtained from Sigma (St Louis, MO, USA). 5-amino salicylic acid (5-ASA, a positive drug for UC therapy) was obtained from Yuanye Biotech Co., Ltd. (Shanghai, China). TRIZOL reagent kit, reverse transcriptase kit and SYBR Green Master Mix were obtainedfrom Vazyme Biotech Co., Ltd. (Nanjing, China). The MPO, MDA and SOD detection kits were obtained from Jiancheng Bioengineering Institute (Nanjing, China). The cytokine enzyme-linked immunosorbent assay (ELISA) kits were obtained from Hengyuan Biotechnology Co., Ltd. (Shanghai, China). The Rabbit anti-p65, IkB-α, p38, phospho(p)-p65, p-IkB-α, p-p38 and iNOS were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). The Rabbit anti-GAPDH, β-Tublin and HRP goat anti-rabbit IgG were obtained from Bioword Technology Co., Ltd. (Nanjing, China).

2.2. Ethics statements

The animal experiment was conducted in a strict accordance with protocols approved by Animal Welfare & Ethical Committee of Nanjing Agricultural University (Permit Number: PZ2018012).

2.3. Induced and evaluated of colitis

C57BL/6 mice (male, 6–8 weeks old, weighing 21–23 g) were randomly divided into 7 groups, with 10 mice per group: normal control, DSS treatment, 10 mg/kg DHEA + DSS treatment, 50 mg/kg DHEA + DSS treatment, 100 mg/kg DHEA + DSS treatment, 100 mg/kg 5-ASA + DSS treatment and 50 mg/kg DHEA treatment group. The mice in containing DHEA or 5-ASA treatment group were administrated with respective different dose of DHEA or 100 mg/kg 5-ASA that suspended in 0.5 % carboxymethylcellulose (CMC) body weight *via* gavage once per day for 10 days, and other mice received with equal vehicle. After DHEA or 5-ASA treatment for 3 days, 2.5 % (w/v) DSS were added into drinking water in all containing DSS treatment groups, and other without DSS treatment group received normal drinking water for the subsequently 7 days. During experiment, the body weight at 0, 3, 4, 5, 6, 7, 8, 9 and 10 days was recorded; and the disease activity index (DAI) was counted according to a previously described scoring system (Philip et al., 2009). Mice were sacrificed by cervical dislocation under anesthesia at the end of experiment point; the blood was centrifuged to obtain the serum, the serum and spleen tissues were stored at -80 ℃ for further analysis. The colon length were measured, and the specimens of colon were fixed with 10 % formalin for haematoxylin and eosin (H&E), and then the histological scoring was analyzed in accordance with the previously method (Dieleman et al., 2010).

2.4. Measurement of myeloperoxidase (MPO) activity

The colon tissues were homogenized on ice with PBS (1:9, w/v), and the supernatants were obtained for further analysis. The MPO activity was detected using a commercial kit in accordance with the instruction.

2.5. Determination of antioxidant parameters

The MDA content and SOD activity in serum or homogenate samples of colon were measured using MDA and SOD detection kits following the manufacturer's instructions.

2.6. Measurement of cytokine level

The content of three pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) in serum or homogenate samples of colon tissue were detected using ELISA kits in accordance with the instruction.

2.7. Lymphocyte proliferation assay

At the end of experiment, the proliferation activity of peripheral blood lymphocytes (PBLs) and spleen lymphocytes (SLs) were determined by CCK-8 method. Briefly, the peripheral blood (0.5 mL) and spleens were collected, the peripheral blood and spleen lymphocytes were separated using lymphocyte separation kits (Hao Yang Biotechnology Co. LTD, Tianjin, China) following the manufacture's instruction. A total of 100 μL lymphocyte suspension (5×10^6 cells/mL) was added to RPMI-1640 medium (containing 10 % fetal calf serum, 2 mM glutamine, 50 U/mL of penicillin and 50 g/mL of streptomycin) and incubated in 96-well plates at 37 ˚C. T-lymphocyte mitogen Con-A (Sigma, St Louis, MO, USA) was added with a final concentration of 5 μg/mL for 48 h, then 10 μL of CCK-8 was added into each well and incubated for another 1 h, and the optical density at 450 nm was measured. The results were presented as a stimulation index (SI) which calculated as: $SI = (OD_{450\ \text{stimulated well}} - OD_{450\ \text{blank well}}) / (OD_{450\ \text{unstimulated well}} - OD_{450\ \text{blank well}})$.

2.8. Real-time quantitative RT-PCR

Total RNA was extracted from the colon and spleen tissues using the TRIZOL reagent kit according to the manufacturer's instructions. Then, 2 μg RNA were reverse transcribed into cDNA using the Superscript II kit according to the manufacturer's recommendation. The cDNA samples were added with 10 μL of SYBR Green PCR Master Mix and 10 pmol each of primers for inducible nitric oxide synthase (*iNOS*), cysteinyl aspartate-specific proteases 1 (*caspase1*), cyclooxygenase-2 (*COX-2*), Nod-like receptor protein 3 (*NLRP3*), interleukin-1β (*IL-1β*), interleukin-6 (*IL-6*), zonula occludens-1 (*ZO-1*) and *β-actin* (internal control) (Table 1). All samples were analyzed by using the ABI 7500 Real-time Detection System (Applied Biosystems, USA) and programmed to conduct one cycle (95 ˚C for 3 min) and 40 cycles (95 ˚C for 10 s, 60 ˚C for 30 s, and 72 ˚C for 20 s). The $2^{-\Delta\Delta CT}$ method was used to calculated the relative gene expression levels. The primers were synthesized by GenScript Biotechnology Co., Ltd (Nanjing, China).

2.9. Western blotting

The colon tissues were homogenized, and total protein was collected with the RIPA lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) and the protein concentration was determined using BCA protein assay kit according to the protocol. Extracted protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12 % gel and transferred into PVDF membranes. The membranes were blocked with buffer containing 5 % non-fat milk for 2 h, then washed the membranes three times with PBS (pH7.4) and incubated overnight at 4℃ with rabbit polyclonal antibodies against p-

Table 1
Sequences of target genes and β-actin primers.

Target gene	GenBank accession number	Primers sequence (5′-3′)
iNOS	NM_010927.4	F: CCTTGTTCAGCTACGCCTTC R: CTGAGGGCTCTGTTGAGGTC
COX-2	L25925.1	F: GCTTCGGGAGCACAACAGAG R: CAGCGGATGCCAGTGATAGAG
NLRP3	NM_145827.4	F: TATCCTCAGTGGGCAAGGTGTT R: AAGGTATGGGCAAAGGTCAAAA
Caspase1	NM_021283.2	F: CTGTGCCCATCTATGAAGGCT R: ATTTCTCTCTCGGCTGTGGTG
IL-1β	NM_008361.4	F: ACCTGTGTCTTTCCCGTGG R: TCATCTCGGAGCCTGTAGTG
IL-6	NM_031168.2	F: TGGAGTCACAGAAGGAGTGGCTAAG R: AGATAGTTGCCCTCCTCG
ZO-1	NM_016674.4	F: GTTCCGGGGAAGTTACGTGC R: AAGTGGGACAAAAGTCCGGG
β-actin	NM_007393.5	F: CTACCGTCGTGACTTCGC R: GGGTGACATCTCCCTGTT

IkB-α (1:1000 dilution), IkB-α (1:1000), p-p65 (1:1000), p65 (1:1000), p-p38 MAPK (1:1000), p38 MAPK (1:1000) and iNOS (1:1000). After washing five times with TBST, the membranes were incubated with HRP goat anti-rabbit IgG (1:10,000) for another 2 h at the room temperature. Protein bands were visualized using the clarity ECL chemiluminescent substrate (Tanon Technology Co., Ltd., Shanghai, China), and the ImageJ software was used to quantify the protein abundance. The GAPDH (1:10,000) or β-Tublin (1:10,000) was used as control, and the results were quantified by calculating the band intensity of target protein relate to control protein.

2.10. Immunofluorescence

Immunofluorescence was performed on the paraffin-embedded colon tissues. The sections of colon tissues were deparaffinized, rehydrated and washed with PBST (PBS with 0.1 % Tween-20). After that, the sections were treated with 3 % hydrogen peroxide, blocked with 10 % goat serum and incubated with rabbit polyclonal antibody against ZO-1 in PBST containing 1 % BSA for 1 h at 37°C. After that, the sections were washed and incubated with species-specific fluorescently labeled secondary antibodies under dark condition for 1 h. The slides were stained with DAPI and analyzed with a confocal laser microscope.

2.11. Gut microbiota DNA isolation and 16S rDNA gene sequence analysis

Colonic contents in each group (normal control, DSS treatment, 50 mg/kg DHEA + DSS treatment) were collected and kept at -80 ℃. The fecal genomic DNA was extracted with the QIAamp DNA Stool Mini Kit (Qiagen, Inc., Shanghai, China). The purity and concentration of the DNA samples were evaluated using NanoDrop2000 (Thermo Scientific, Waltham, Mass). The PCR amplification of 16S rDNA for V3-V4 domain of intestinal microflora rDNA, library construction, sequencing on the Ion S5 (Life Technologies, Carlsbad, USA) and bioinformatics analysis were performed by Novel Bio Co., Ltd (Shanghai, China).

2.12. Data analysis and statistics

Data were showed as mean values ± standard error of mean (SEM). Significant differences among different group were analyzed by the student *t*-test and one-way analysis of variance (ANOVA) ($P < 0.05$ represented statistically significant).

3. Results

3.1. Apparent effect of DHEA on DSS-induced colitis in C57BL/6 mice

DSS alone treatment had caused an obviously body weight losing compared with the normal control ($P < 0.05$); however, different doses of DHEA or 5-ASA treatment attenuated the body weight losing induced by DSS in mice ($P < 0.05$) (Fig. 1A). The DAI, which indicated as the severity of weight loss, blood in stool and stool consistency, was obviously reduced in different doses of DHEA or 5-ASA treatment than that in DSS alone treatment ($P < 0.05$) (Fig. 1B). Meanwhile, DSS treatment significantly caused the increasing of spleen weight and shortening of colon length ($P < 0.05$), and these changes were reversed in DSS-induced colitis mice treated with different doses of DHEA or 5-ASA (Fig. 1C–E). Histopathological analysis showed that the epithelial erosion, goblet cell loss, crypt abscesses and immune cell infiltration were observed in DSS alone treatment group; but different dose DHEA or 5-ASA treatment maintained a relatively intact structure in colon when compared to the DSS alone treatment group (Fig. 1F and G). Compared with the normal control, DSS treatment obviously increased the MPO activity in mice ($P < 0.01$); while different doses of DHEA or 5-ASA treatment significantly decreased the MPO activity when compared to DSS treatment mice ($P < 0.01$) (Fig. 1H).

3.2. Effect of DHEA on the oxidative stress

The iNOS protein expression level was significantly enhanced in mice after DSS challenged when compared to the normal mice ($P < 0.05$); different doses of DHEA or 5-ASA treatment significantly decreased the iNOS protein expression level in DSS-induced colitis mice when compared to DSS alone treatment group ($P < 0.01$) (Fig. 2A). Compared with control group, a significant increasing of SOD activity and MDA content in serum and colon tissues was observed in mice treated with DSS alone ($P < 0.01$) (Fig. 2B–E). 50 mg/kg DHEA or 5-ASA treatment significantly decreased serum MDA content ($P < 0.05$); and different doses of DHEA or 5-ASA treatment significantly reduced MDA content in colon tissues in DSS-induced colitis mice ($P < 0.05$) (Fig. 2B and C). Similar, different doses of DHEA treatment obviously decreased the SOD activity in serum and colon tissues when compared to DSS alone treatment group ($P < 0.01$), except that serum SOD activity in 10 mg/kg DHEA treatment group ($P > 0.05$) (Fig. 2D and E).

3.3. Effect of DHEA on the inflammation related cytokines secretion

In colon tissues, the inflammation related cytokines (TNF-α, IL-1β and IL-6) level was obviously enhanced in mice after DSS challenged when compared to normal mice ($P < 0.01$); while DHEA treatment obviously decreased these inflammatory cytokine levels when compared to DSS alone treatment group ($P < 0.05$), except that IL-6 level in 100 mg/kg DHEA treatment ($P > 0.05$) (Fig. 3A–C). In serum, no statistically significant difference was observed on TNF-α level, while IL-1β and IL-6 level were dramatically enhanced in DSS-induced mice when compared to normal mice ($P < 0.01$); similar, DHEA (10 and 50 mg/kg) or 5-ASA treatment obviously decreased the IL-1β and IL-6 levels when compared to DSS alone treatment group ($P < 0.05$) (Fig. 3D–F).

3.4. Effect of DHEA on the immune function

Compared with the normal mice, the *iNOS*, *COX-2*, *IL-6*, *caspase1*, *NLRP3* and *IL-1β* mRNA levels were obviously up-regulated in mice treated with DSS alone ($P < 0.05$) (Fig. 4A–F). Compared with DSS alone treatment, different doses of DHEA or 5-ASA treatment significantly decreased the *iNOS*, *COX-2*, *IL-6*, *caspase1* and *NLRP3* mRNA levels in DSS-induced colitis mice ($P < 0.01$); similar, the *IL-1β* mRNA

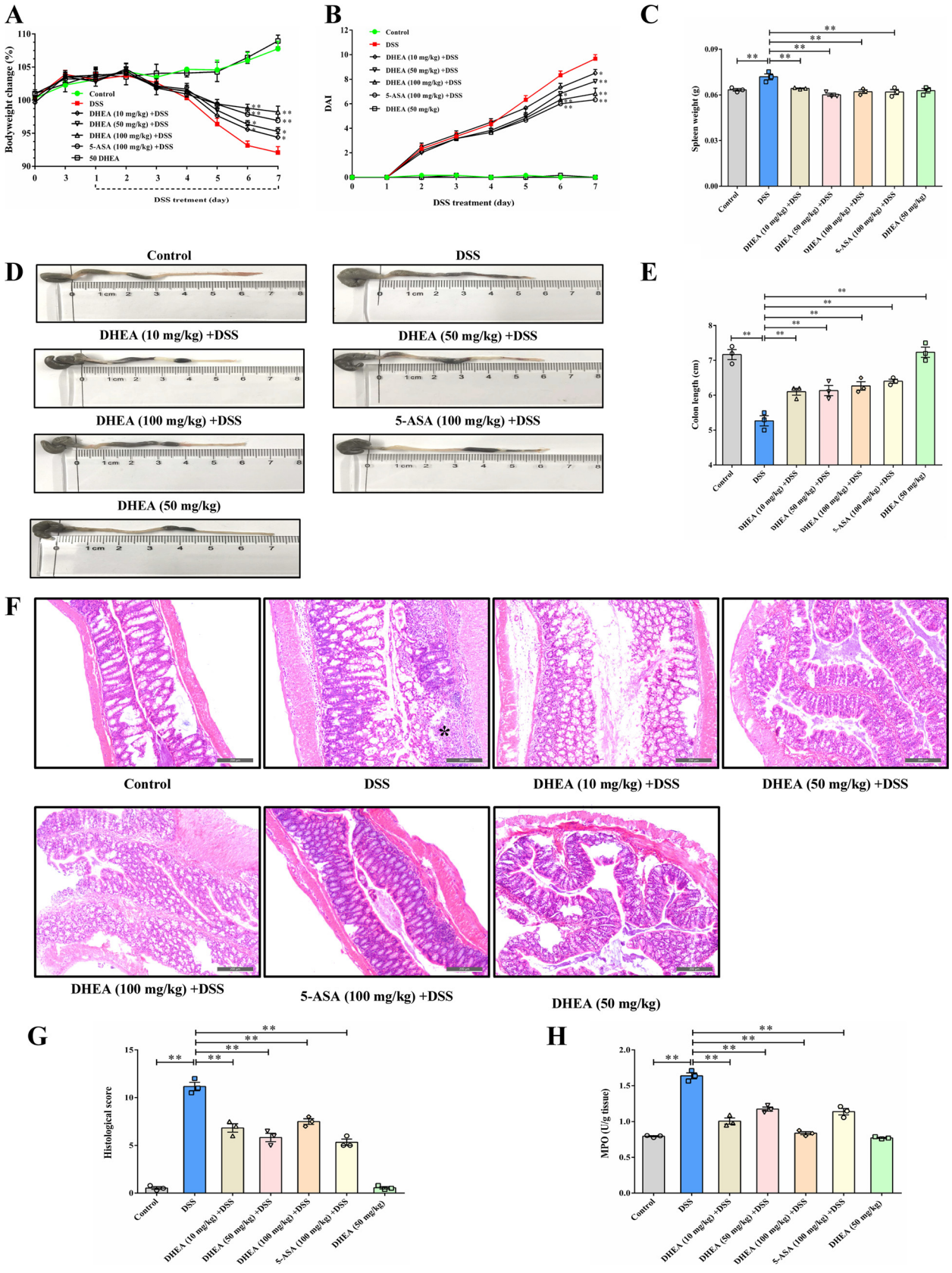

Fig. 1. DHEA attenuated DSS-induced colitis in C57BL/6 mice.
A: Body weight loss; B: Disease activity index (DAI); C: Spleen weight; D-E: Representative images and the data of colon length; F: Histopathological examination of colon (H & E staining ×100; * loss of epithelium surface and cryptal glands); G: Histopathological scores; H: Myeloperoxidase (MPO) activity. Values presented as means ± SEM. * $P < 0.05$, ** $P < 0.01$, compared with the respectively control.

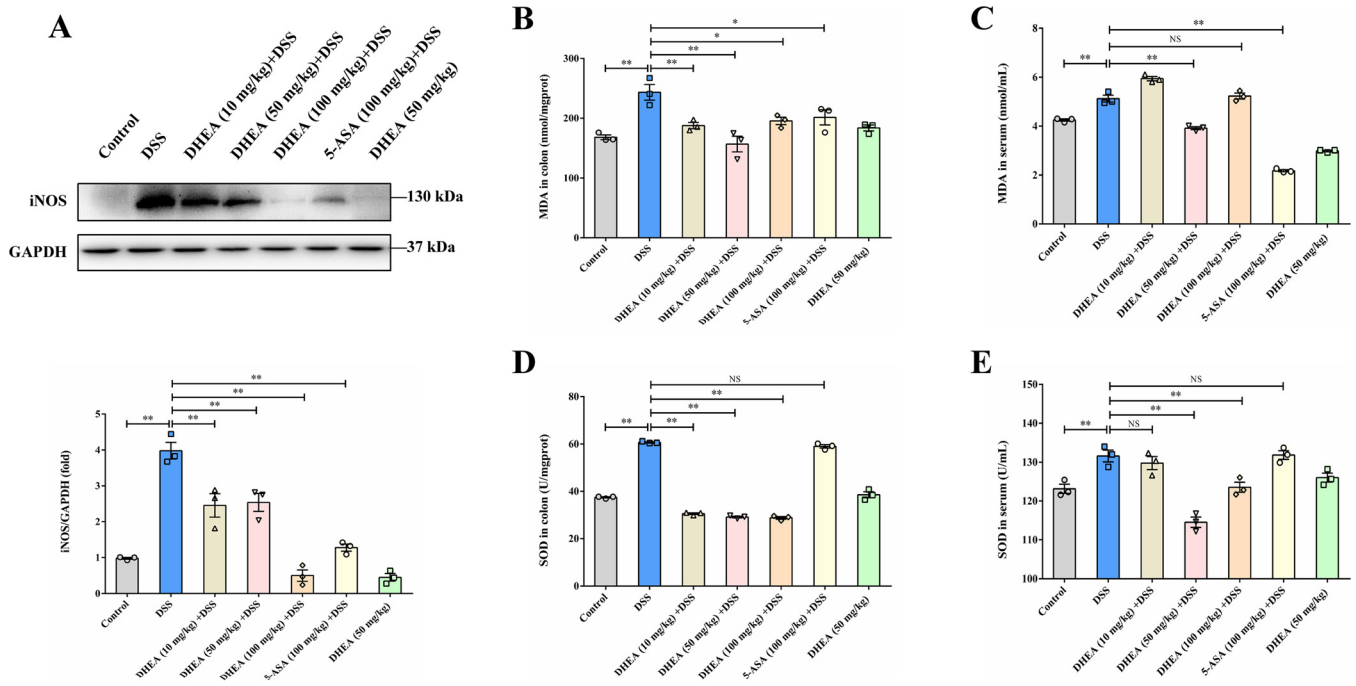

Fig. 2. DHEA ameliorated the oxidative stress index in DSS-induced colitis mice.
A: Inducible nitric oxide synthase (iNOS) protein level; B: Malondialdehyde (MDA) content in colon; C: Serum MDA content; D: Superoxide dismutase (SOD) activity in colon; E: Serum SOD activity. Values presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the respectively control. NS: no significance between the indicated groups.

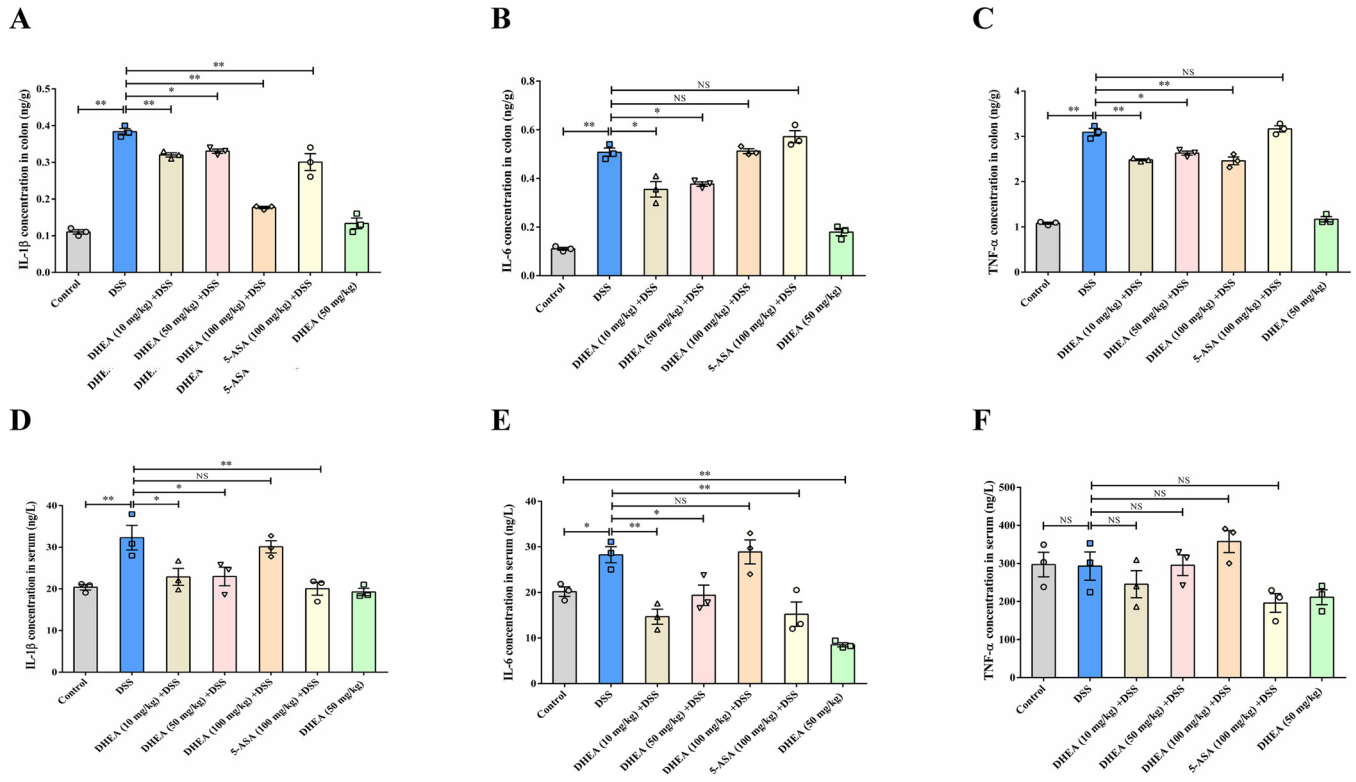

Fig. 3. DHEA decreased the inflammation related cytokines production in DSS-induced colitis mice.
A-C: The interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) content in colon tissues, respectively; D-F: The content of IL-1β, IL-6 and TNF-α in serum. Values presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the respectively control. NS: no significance between the indicated groups.

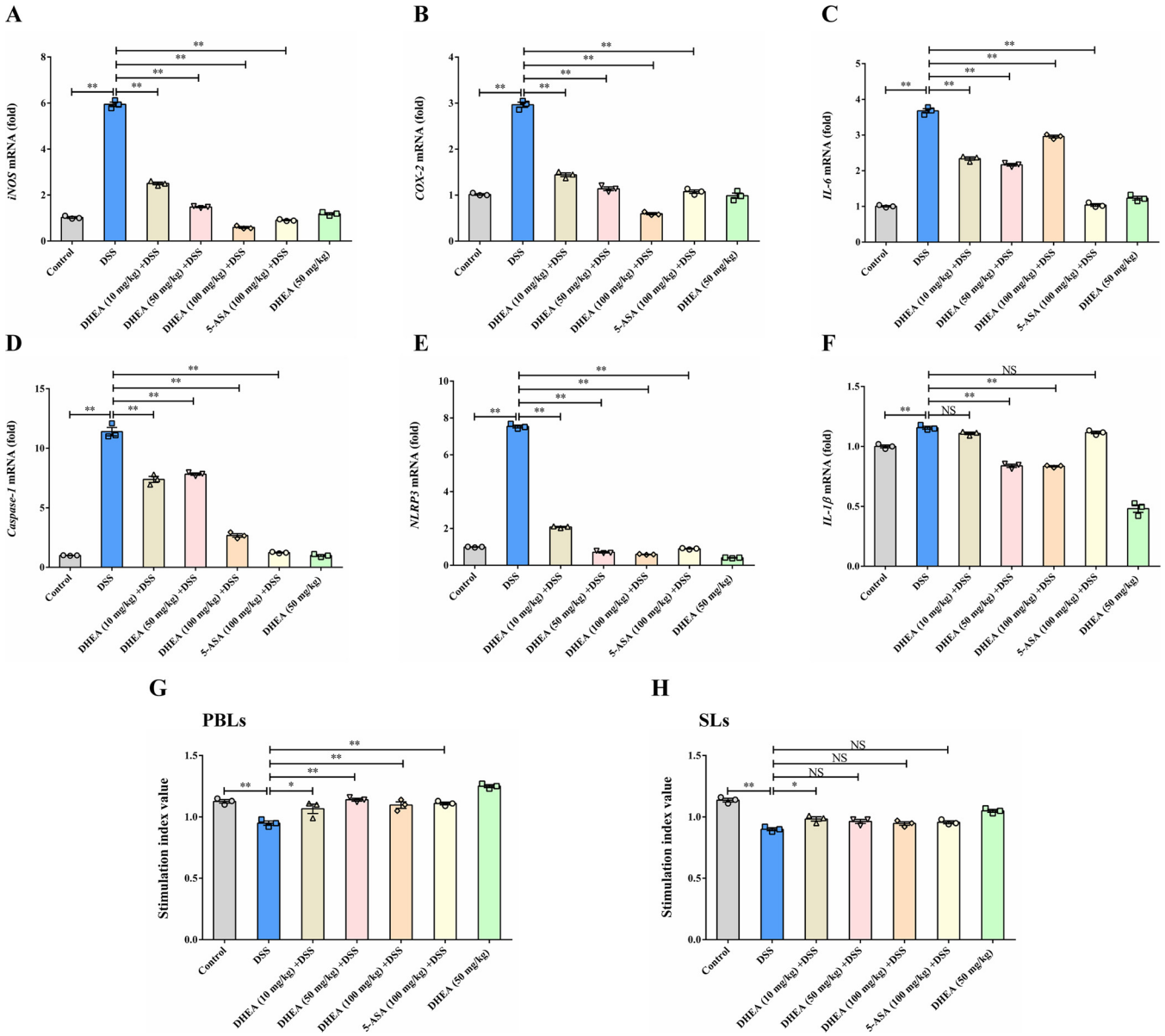

Fig. 4. DHEA improved the immune function in DSS-induced colitis mice.
A-F: The mRNA level of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), cysteinyl aspartate-specific proteases 1 (caspase1), Nod-like receptor protein 3 (NLRP3) and interleukin-1β (IL-1β) in spleen; G: Proliferation activity of peripheral blood lymphocytes (PBLs); H: Proliferation activity of spleen lymphocytes (SLs). Values presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the respectively control. NS: no significance between the indicated groups.

level was obviously decreased in 50 and 100 mg/kg DHEA treatment when compared to DSS alone treatment mice ($P < 0.05$) (Fig. 4A–F).

Compared with the normal mice, the average stimulation indexes (SI) of PBLs and SLs were obviously decreased in mice after DSS challenged ($P < 0.01$) (Fig. 4G and H). Compared with DSS alone treatment group, different doses of DHEA or 5-ASA treatment significantly increased the average SI of PBLs in DSS-induced mice ($P < 0.05$) (Fig. 4G); while only 10 mg/kg DHEA treatment group enhanced the average SI of SLs in DSS-induced mice ($P < 0.05$) (Fig. 4H). These results indicated that DHEA increases the proliferative activities of peripheral blood lymphocytes in DSS-induced colitis mice.

3.5. Effect of DHEA on the p38 MAPK and NF-κB pathways

The phosphorylation protein level of NF-κB p65, IκB and MAPK p38 in DSS alone treatment mice were obviously increased than that of normal mice ($P < 0.01$) (Fig. 5). DHEA (50 and 100 mg/kg) or 5-ASA treatment obviously decreased the p65 phosphorylation protein level ($P < 0.01$) (Fig. 5A and B); meanwhile, DHEA (10 and 50 mg/kg) treatment significantly decreased the IκB phosphorylation protein level in DSS-induced colitis mice ($P < 0.01$) (Fig. 5A and C). In addition, different doses of DHEA or 5-ASA treatment significantly reduced the MAPK p38 phosphorylation protein level in DSS-induced colitis mice when compared to DSS alone treatment group ($P < 0.01$) (Fig. 5D and E).

3.6. Effect of DHEA on the epithelial tight junction architecture

Compared with the normal mice, the mRNA level and protein abundance of ZO-1 were obviously decreased in mice treated with DSS alone ($P < 0.01$) (Fig. 6A and B). 10 and 50 mg/kg DHEA or 5-ASA treatment significantly increased the ZO-1 mRNA level and protein

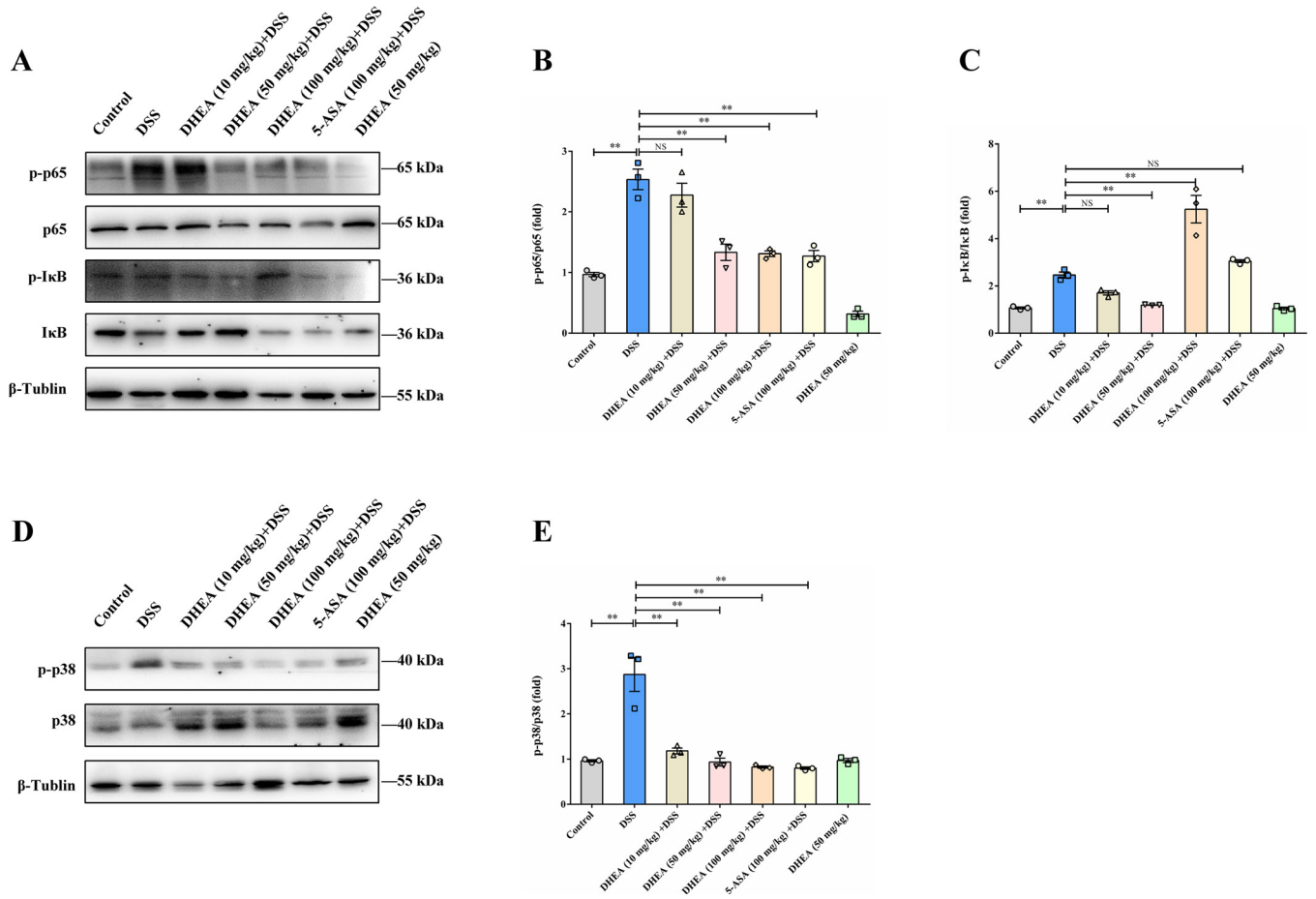

Fig. 5. DHEA blocked the activation of NF-κB and p38 MAPK pathways in DSS-induced colitis mice.
A: Immunoblot of NF-κB p65 and IκB protein level in colon tissue; B: p-p65 protein expression level; C: p- IκB protein level; D: Immunoblot of MAPK p38 protein level in colon tissue; E: p-38 MAPK protein level. Values presented as means ± SEM. $^{**}P < 0.01$, compared with the respectively control. NS: no significance between the indicated groups.

abundance in DSS-induced colitis mice when compared to DSS alone treatment group ($P < 0.05$) (Fig. 6A and B). The IF analysis further demonstrated that the ZO-1 protein was localized at the surface of colonic epithelium in different groups; and DHEA treatment dramatically prevented the substantial loss of ZO-1 staining intensity when compared to DSS alone treatment group (Fig. 6C).

3.7. Effect of DHEA on the gut microbiota

The Venn diagram showed that 310 OTUs overlapped among three groups, and the distinctive OTU numbers in control, DSS and DHEA + DSS treatment group was 1873, 740 and 1670, respectively (Fig. 7A). The index of Shannon and Simpson which represented α-diversity for three treatment groups were shown in Fig. 7B and C, the results showed that DSS significantly reduced the diversity than that of normal mice ($P > 0.05$), while DHEA could alleviate this phenomenon in DSS-induced colitis mice. The principal component analysis (PCA) which represented β-diversity for three groups were shown in Fig. 7D, and the results showed that the gut microbiota were obviously changed in mice treated with DSS. The phylum level of bacterial composition (Fig. 7E) showed that DSS treatment up-regulated the relative abundance of *Firmicutes*, but it had no statistical significance ($P > 0.05$). Meanwhile, DSS alone treatment significantly increased the relative abundance of *Actinobacteria* and reduced the relative abundance of *Bacteroidetes* when compared to the normal mice ($P < 0.01$) (Fig. 7F). To clarify the detailed impacts of DHEA on gut microbiota in DSS-induced colitis, we investigated 120 significant difference species using LEfSe analysis. The results of hierarchically clustered heat map analysis showed that DSS obviously enhanced the abundance of genus *Pseudomonas* when compared to the normal mice; while DHEA decreased the genus level in DSS-induced mice when compared to DSS alone treatment group (Fig. 8). Besides the taxonomic composition analysis, the differences of functional profiles among three groups (Control *vs* DSS, Control *vs* DHEA + DSS) were predicted by the PICRUSt (Fig. 9). The DSS alone treatment mainly caused the changes in some metabolism pathways, such as inorganic transport and metabolism, amino acid transport and metabolism and secondary metabolites biosynthesis (Fig. 9A); however, the DHEA treatment can not reverse these changes induced by DSS in mice (Fig. 9B).

4. Discussion

Ulcerative colitis (UC), a chronic and relapsing inflammatory disease of gastrointestinal tract with a high prevalence especially in developed countries (Rand et al., 2010). Currently, patients with UC are mainly treated with immunosuppressive or anti-inflammatory drugs (Katsanos et al., 2018), while most of these agents are inadequate to prevention the UC. Therefore, it is important to develop the new therapy (*e.g.* probiotics or other immunomodulatory agents) for inflammatory bowel diseases (IBD) treatment (Toumi et al., 2013; Toumi et al., 2014). As an intermediate in cholesterol metabolism, the age-related decline of DHEA level had attracted public health-related researchers interest due to its beneficial effects on oxidative stress (Chandra et al., 2019), anti-inflammatory (Alicja, 2010) and anti-

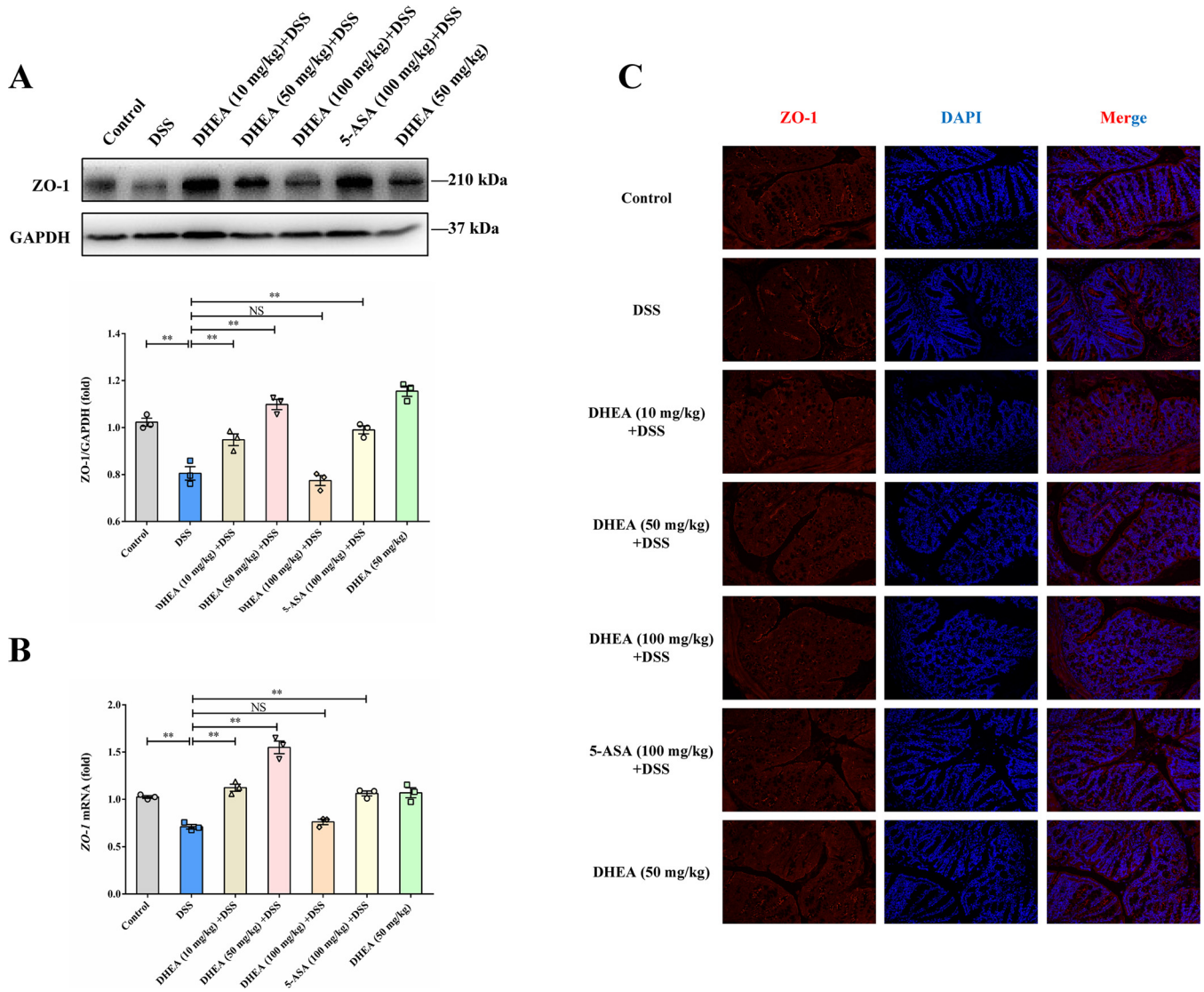

Fig. 6. DHEA enhanced the ZO-1 expression level in colon of DSS-induced colitis mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
A: Zonula occludens-1 (ZO-1) protein level; B: *ZO-1* mRNA expression level; C: Sections of colon tissue were immunostained with anti-ZO-1 (red) and DAPI (blue). Values presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the respectively control. NS: no significance between the indicated groups.).

microbial properties (Zhao et al., 2019). Although previous study had investigated the protective effects of DHEA on patients with ulcerative colitis (Andus et al., 2015), but the potential mechanisms of DHEA as an immunomodulatory agent to alleviate ulcerative colitis is still unclear.

Body weight loss and DAI are mainly used to evaluate the severity in IBD (Mendall et al., 2018; Zhu et al., 2018). In our study, DSS alone treatment significantly decreased the body weight gain, increased the DAI and enhanced the spleen weight; all which indicated that DSS treatment can trigger experimental colitis in mice. However, DHEA treatment effectively blocked the losing of body weight, increasing of DAI and enhancing of spleen weight, which demonstrated that DHEA attenuate the inflammation response in DSS-induced murine experimental colitis. In addition, we found that the colonic shortening, a directly index of colitis, was obviously relieved in DSS-induced mice treated with DHEA. Consist with this results, DHEA maintained a relatively intact structure in colon of DSS-induced mice. Moreover, DHEA significantly decreased the MPO activity in DSS-induced colitis mice. MPO is usually implicated as an inflammatory biomarker in IBD (Hansberry et al., 2017). Thus, these results indicated that DHEA posse a protection effects in mice challenged with DSS.

The decline of immune response or imbalance between free radical production and antioxidant defense can induce and exacerbate the UC (Khairy et al., 2018; Serra et al., 2018). Previous study reported that free radical content in gut is closely related with DAI, and which implied that oxidative stress plays a significance role in inflammation response (Alzoghaibi, 2013). In addition, the depletion of antioxidant defense has been postulated to be a major reason for oxidative stress (Kaya et al., 2018). Our study clearly showed the protective effect of DHEA on oxidative stress since it significant averted the elevation of malondialdehyde (MDA) in DSS-induced mice. MDA content not only reflect the free radical level and status of lipid peroxidation in tissue, but also indirectly reflect the degree of cellular damage (Tripathi et al., 2018). Our data emphasized that the antioxidant effect of DHEA mainly achieved through regulating the SOD activity in DSS-induced mice. This result is consist with Pélissier et al. (2006) reported that DHEA against oxidative stress in the DSS-induced colitis rats. Many studies illustrated that DHEA has an anti-oxidant effect (Ding et al., 2017). Our previous study also indicated that DHEA enhance the activity of total antioxidant capacity (T-AOC) and SOD in the LPS-stimulated mice (Cao et al., 2019). Therefore, all these findings demonstrated that DHEA reduce the

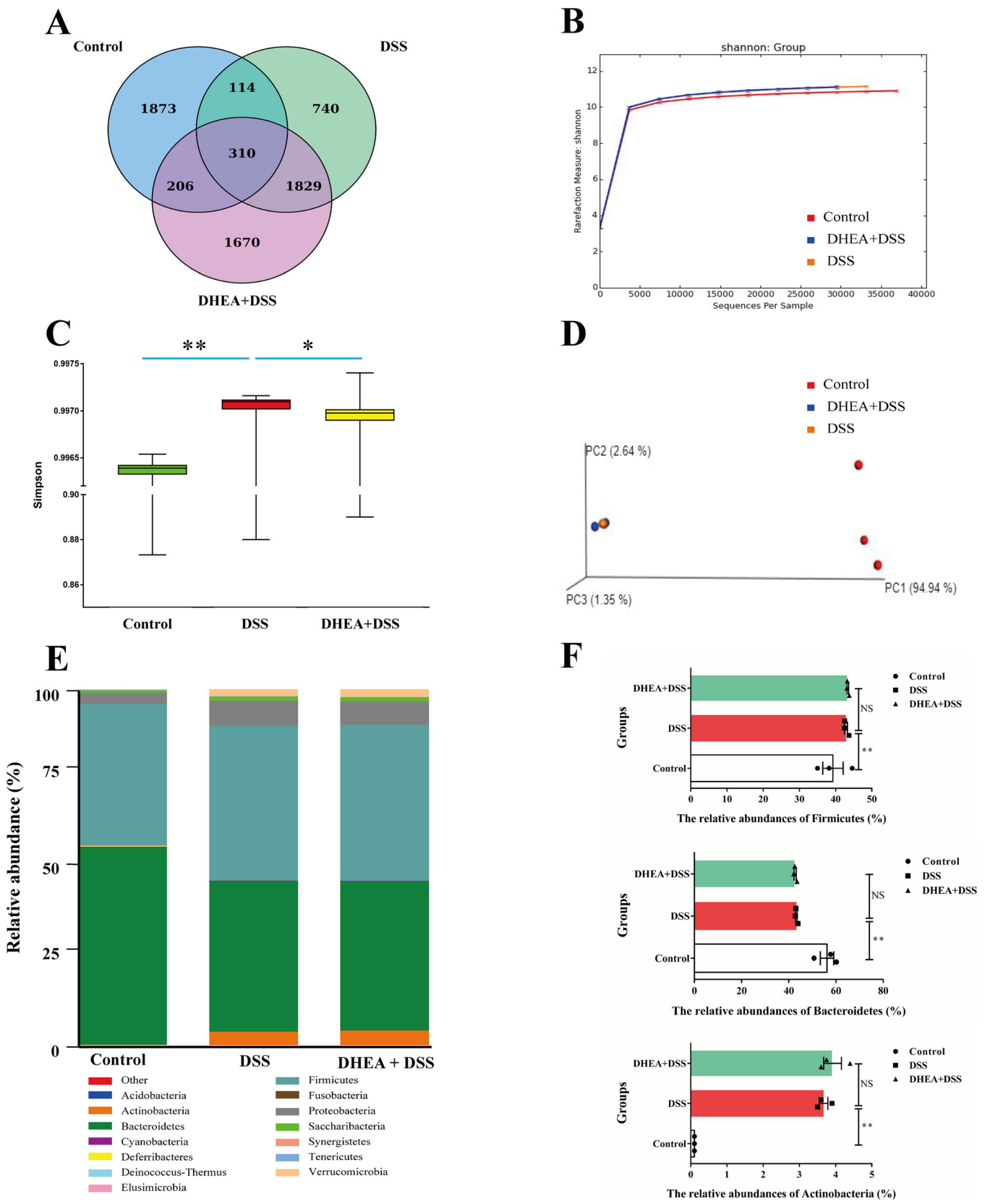

Fig. 7. DHEA modulated the gut microbiota diversity in DSS-induced colitis mice.
A: Species richness accessed by observed OTUs; B: Comparison of alpha diversity accessed by Shannon indexes; C: Comparison of alpha diversity accessed by Simpson indexes; D: Comparison of beta diversity accessed by weighted UniFrac principal coordinates analysis (PCA); E: Microbial composition (phylum level); F: Relative abundances of *Firmicutes*, *Actinobacteria* and *Bacteroidetes*. Values presented as means ± SEM. $^{**}P < 0.01$, compared with the respectively control. NS: no significance between the indicated groups.

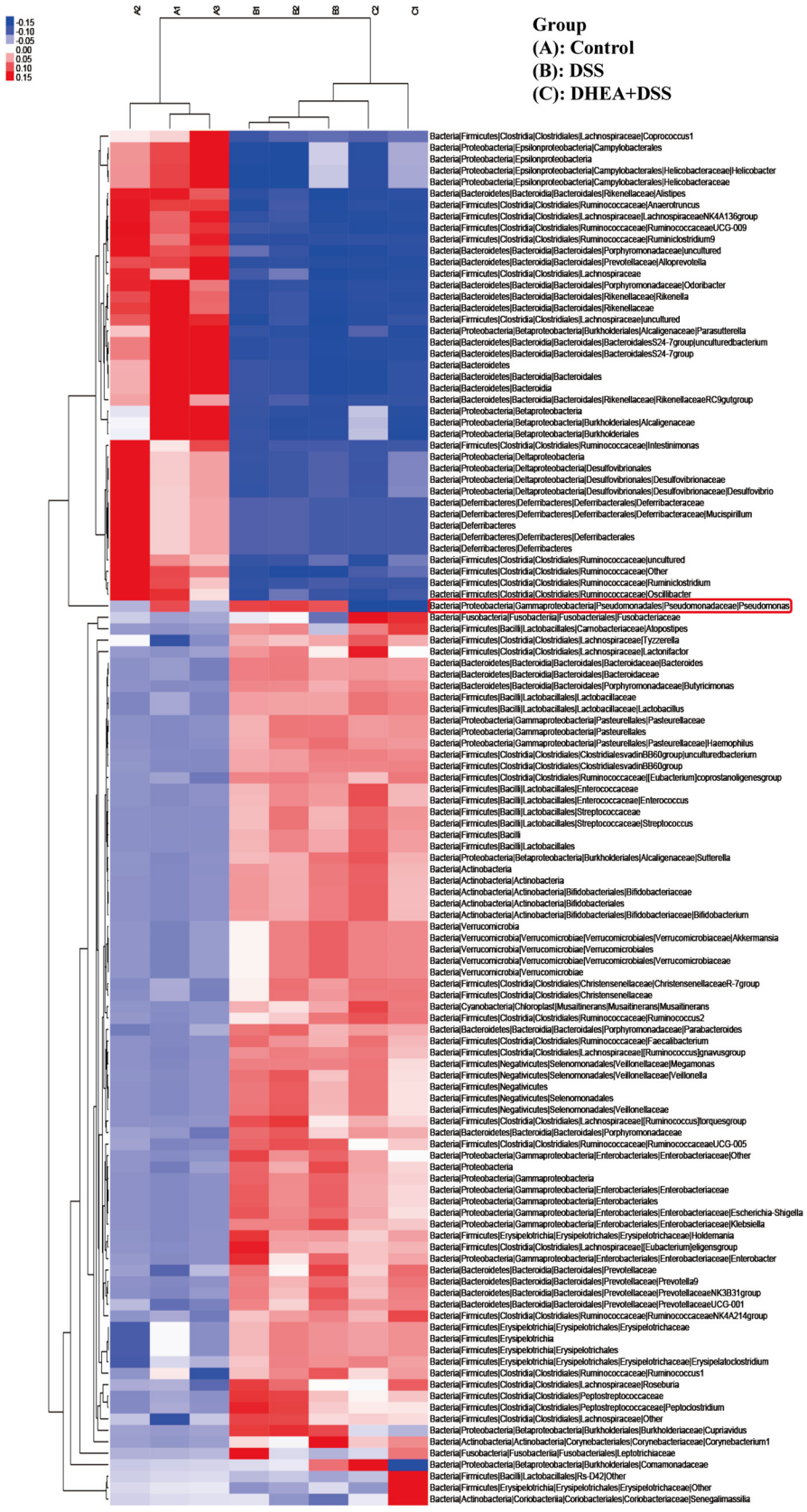

Fig. 8. DHEA altered the composition of gut microbiota in DSS-induced colitis mice.

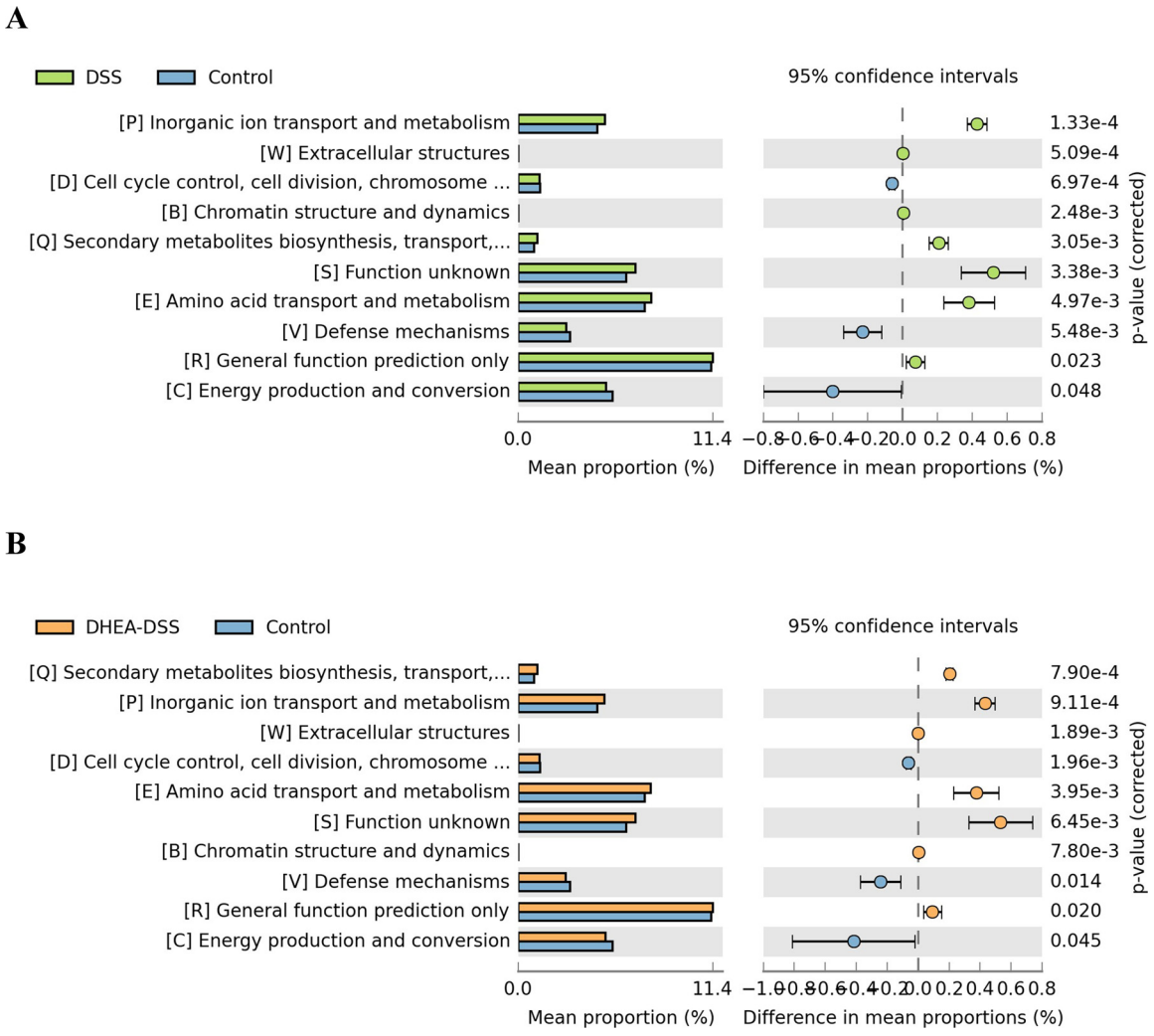

Fig. 9. The functions of gut microbial community predicted by PICRUSt.

oxidative stress and which finally attenuate inflammation response in DSS-induced colitis mice.

It is well known that the iNOS produces a large amounts of nitric oxide (NO) in response to pro-inflammatory cytokines (*e.g.* TNF-α, IL-1β and IL-6) and contributes to inflammatory disease (Soufli et al., 2016). Our results showed that DHEA dramatically decreased the iNOS expression level and the inflammation related cytokines secretion in DSS-induced colitis mice. In patients with IBD, the TNF-α, IL-1β and IL-6 levels were selected as the important markers for the intestinal mucosal immune response (Hwang et al., 2018). It reported that DHEA affect immunity function through regulating the inflammation related cytokines secretion (Malutan et al., 2014). These results demonstrated that DHEA attenuate inflammation response through suppression pro-inflammatory cytokines production in DSS-induced colitis mice. T cells are positively correlated with the body's cellular immune function (Lewis et al., 2019), and its proliferation can reflect the basic status of cell-mediated immune response (Soufli et al., 2016). The effects of DHEA on T-lymphocytes functions are controversial in previous studies. Some research suggested that DHEA can activate the proliferation of T-lymphocytes (Cao et al., 2019), whereas other investigator showed that DHEA inhibit proliferation of T-lymphocytes (Niki, 2010). In order to evaluate the effect of DHEA on cellular immune response, the proliferation activity of peripheral blood lymphocytes (PBLs) and spleen lymphocytes (SLs) were analyzed in DSS-induced colitis mice. We found that DHEA markedly enhanced the proliferative activities of peripheral blood lymphocytes, which indicated that DHEA enhanced the T lymphocyte function in DSS-induced colitis mice. Besides, it reported that DHEA can elicit an immune-protective effect regarding Th1 and Th2 cell responses (Pratschke et al., 2014). Taken the above results, we conjectured that DHEA improve the immune function *via* decreasing pro-inflammatory cytokines production and enhancing the T lymphocyte function in DSS-induced colitis mice.

NF-κB plays a critical role in inflammatory bowel diseases (Lee et al., 2018), and it can be activate by several cellular kinases, such as p38 MAPK (Chen et al., 2018); once activated, NF-κB can induce the production of pro-inflammatory cytokines (Yuan et al., 2018). Therefore, we conjectured that the inhibitory effects on the cytokines production by DHEA might be related with the p38 MAPK and NF-κB activation in DSS-induced colitis mice. As expected, DHEA blocked the activation of p38 MAPK and NF-κB pathways induced by DSS treatment in mice. Importantly, our results showed that DHEA significantly decreased the NLRP3 expression level in DSS-induced colitis mice. It reported that the NLRP3 is involved in activation of caspase 1 (Marchetti, 2019); and the NLRP3 can bind to apoptosis-associated speck-like protein adaptor and then recruit the pro-caspase1 (referred to as “inflammasome”), which lead the maturation and secretion of IL-1β (Xue et al., 2019). In addition, the NLRP3 expression requires the phosphorylation of NF-kB and its nuclear translocation (Xue et al., 2019). Consist with the changes of NLRP3 expression level, we also found that DHEA obviously decreased the *caspase1* and *IL-1β* genes expression level in DSS-induced colitis mice. From the above results, our data indicated that DHEA reduce pro-inflammatory cytokines production by

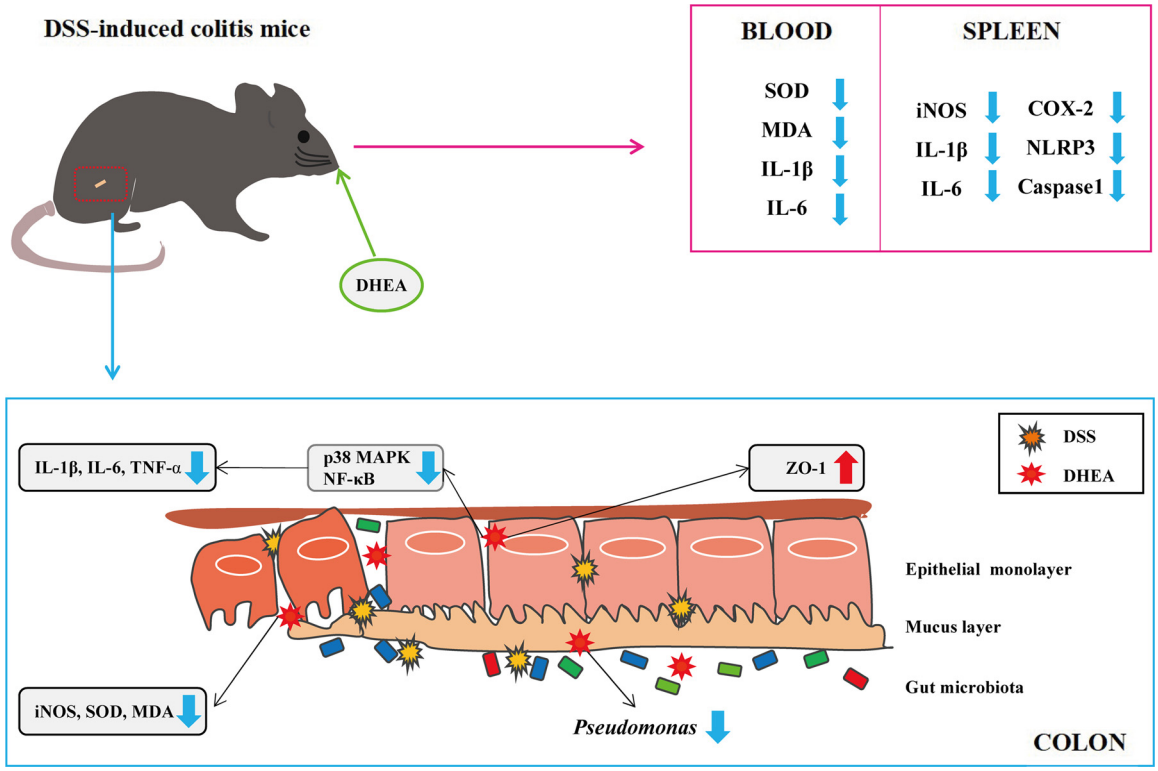

Fig. 10. Schematic diagram of possible mechanism about DHEA attenuates the DSS-induced colitis outcomes.
DHEA reduces the oxidative damage by regulating antioxidant enzyme activity; maintains colon barrier integrity by increasing the ZO-1 expression level and modulating gut microbiota taxa; regulates the immune function *via* decreasing pro-inflammatory cytokines secretion and enhancing the T lymphocyte proliferative; all that finally led the attenuation of inflammation response in DSS-induced colitis mice.

inhibiting the activation of p38 MAPK and NF-κB pathway in DSS-induced colitis mice.

Intestinal barrier and gut microbiota are two important aspects in maintaining the intestinal health (Ducray et al., 2019; Ma et al., 2019). Previous study reported that the abnormalities of tight junction protein function (*e.g.* ZO-1, Claudins or Occludin) is a major causes for the alteration of intestinal barrier in IBD (Camilleri et al., 2012; Ma et al., 2019). Morita et al. (2015) reported that the abnormalities of ZO-1 expression level can cause the alteration of intestinal permeability in IBD patients. In this study, DHEA significantly prevented the decreasing of ZO-1 expression levels caused by DSS in mice. Besides, the gut microbiota also acts a critical role in maintaining the intestinal permeability; and many studies reported that the disorder of intestinal microbiota always connected with the pathogenesis of IBD (Chiodini et al., 2018; Gilis et al., 2018). Our results found that DSS alone treatment significantly up-regulated the abundance of *Actinobacteria*, while down-regulated the abundance of *Bacteroidetes* in mice. This disorder of microbiota community composition is consist with other study reported (Rodríguez-Nogales et al., 2018). Several researches showed that *Pseudomonas* act a trigger effect in IBD (Josef et al., 2008; Wei et al., 2002). Interestingly, we found that DHEA treatment decreased the abundance of genus *Pseudomonas*, which indicated that DHEA may partly modulate the gut microbiota taxa in DSS-induced colitis mice. Therefore, we speculated that DHEA protects the barrier integrity through up-regulating the ZO-1 expression levels and regulating the gut microbiota taxa, which finally reduce the severity of colitis induced by DSS in mice.

In summary, our data demonstrated that DHEA reduces the oxidative damage by regulating antioxidant enzyme activity; maintains colon barrier integrity by increasing the ZO-1 expression level and modulating gut microbiota taxa; improves the immune function *via* decreasing pro-inflammatory cytokines secretion and enhancing the T lymphocyte proliferative; all that finally led the attenuation of inflammation response in DSS-induced colitis mice. Importantly, the inhibitory effect of DHEA's on pro-inflammatory cytokines production is achieved by blocking the activation of p38 MAPK and NF-κB pathway in DSS-induced colitis mice (Fig. 10). These information not only increase our understanding the mechanisms of DHEA's protective and preventive effects on experiment colitis mice, but also highlight the potential application of DHEA as a supplementation in therapy of IBD.

CRediT authorship contribution statement

Ji Cao: Writing - original draft, Formal analysis, Investigation, Methodology. **Huihui Zhang:** Resources, Supervision, Validation. **Zhongmiao Yang:** Investigation, Formal analysis. **Jinlong Zhao:** Investigation. **Haitian Ma:** Conceptualization, Funding acquisition, Project administration, Writing - review & editing.

Declaration of Competing Interest

All authors declare that they have no competing of interests.

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