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Dehydroepiandrosterone alleviates *E. Coli* O157:H7-induced inflammation by preventing the activation of p38 MAPK and NF-κB pathways in mice peritoneal macrophages

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ABSTRACT

As an important metabolite in cholesterol metabolism, dehydroepiandrosterone (DHEA) can modulate the immune function in animals and humans, but the underlying mechanism is still unclear. The present study investigated the effect and mechanism of DHEA's anti-inflammatory action in primary mice peritoneal macrophages infected with *E. coli* O157:H7. The finding showed that DHEA improved the phagocytic ability in *E. coli* O157:H7-infected macrophages. DHEA inhibited the cytokines (including tumor necrosis factor-α, interleukin-1β and interleukin-6) secretion in *E. coli* O157:H7-infected macrophages. The inducible nitric oxide synthase and cyclooxygenase-2 protein level were significantly decreased in *E. coli* O157:H7-infected macrophages treated with DHEA. In addition, DHEA markedly decreased the phospho (p)-p38 MAPK protein level in *E. coli* O157:H7-infected macrophages. Furthermore, DHEA prevented the nuclear translocation of NF-κB by decreasing of p-IκB-α protein level in *E. coli* O157:H7-infected macrophage; and these effects of DHEA were heightened when the cells were pre-treated with p38 MAPK inhibitor SB203580. Our data indicated that DHEA alleviates the pro-inflammatory mediator production in *E. coli* O157:H7-infected mice peritoneal macrophages; and this beneficial action associated with it prevents the activation of p38 MAPK and NF-κB signaling pathway.

1. Introduction

Dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are the most abundant circulating steroid hormones in humans. An aging-related marked decline in circulating DHEA levels is associated with autoimmune diseases, aberrations in lipid metabolism and oxidative stress-related disease (Prall and Muehlenbein, 2018). As a precursor of endogenous steroid hormones, it reported that DHEA can affects the immune function and elicits immune-regulatory effects (Hazeldine et al., 2010). Many studies had certified that DHEA exerts an influence on the immune system which results in an increasing of host resistance against the infections of bacteria (Gentilini et al., 2015), viruses (Yang et al., 2016) or parasites (Brazao et al., 2010). It well known that cytokines plays a crucial role in the progression of inflammatory diseases, such as enteritis, mastitis and neuroinflammation (Alves et al., 2016; Park et al., 2016). The imbalance between pro-inflammatory and anti-inflammatory cytokines impedes the resolution of inflammation and leads to disease perpetuation and tissue destruction (Neurath, 2014). Previous studies reported that DHEA can regulate the cytokines secretion by altering Th1/Th2 cytokine balance (Brazão et al., 2010; Namazi, 2009). Although many researchers conjectured that the anti-bacterial or anti-inflammatory effects of DHEA attributes to its ability to reduce pro-inflammatory cytokines production (Alexaki et al., 2017; Powell and Sonnenfeld, 2006), but the exact mechanisms is not fully elucidated.

Macrophages widely distribute in the body and provide an immediate defense against pathogens prior to leukocyte migration; they also can produce the inflammatory mediators, such as nitric oxide (NO), prostaglandin E2 (PGE2), tumor necrosis factor (TNF)-α and interleukin (IL) (Shin et al., 2010). Inflammatory response is characterized by the abundant inflammatory mediators or cytokines production; and the pro-inflammatory mediators are considered as important targets for the development of anti-inflammatory agents (Kiemer et al., 2003). As mentioned above, substantial researches showed that DHEA regulate the cytokines secretion, while few data are available about the effect of DHEA on the function of peritoneal macrophages.

The production of inflammation mediator is controlled by the nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs)

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at both transcriptional and post-transcriptional levels (Bak et al., 2012). It reported that NF-κB is a pivotal transcription factor in regulating various factors expression which involved in immune and acute phase inflammatory responses (Ruland, 2011). The activation of NF-κB signaling pathway is linked to the activation of MAPKs, which subsequently promote the production of pro-inflammatory mediators and cytokines (Arthur and Ley, 2013; Wu et al., 2016). Although previous studies showed that DHEA regulated the immune function (Prall and Muehlenbein, 2018) and which may be partly through inhibiting NF-κB activation (Altman et al., 2008; Hazeldine et al., 2010). Nevertheless, current researches mostly focus on the action of DHEA in lymphocytes function; how DHEA elicits its anti-inflammatory effect in peritoneal macrophages and it exerts these actions whether associated with the activation of MAPK and NF-κB signaling pathway have been elusive to define.

Based on the findings of the literature so far, the present study aimed to investigate the effect of DHEA on phagocytic ability and inflammatory mediator production in *E. coli* O157:H7-infected primary mice peritoneal macrophages, and further evaluated if this action of DHEA is related to its modulation on p38 MAPK and NF-κB signaling pathways. We believe that these findings will reveal the detailed molecular mechanism of DHEA's anti-inflammatory effect; and reinforce its potential as an immunomodulatory agent to prevent bacterial infection and relieve inflammatory responses in animals and humans.

2. Materials and methods

2.1. Reagents

DHEA and dimethyl sulfoxide (DMSO) were purchased from Sigma (St Louis, MO, USA). Fetal bovine serum and RPMI-1640 medium were obtained from Hyclone (Logan, UT, USA). The detection kit of alkaline phosphatase (AKP), N-acetyl-β-D-glucosamidase (NAG) and lactate dehydrogenase (LDH) was purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The cytokines enzyme-linked immunosorbent assay (ELISA) kits were purchased from Biogen Biotechnology Co., Ltd. (Nanjing, China). The rabbit anti-iNOS, COX-2, p38 MAPK, p65 NF-κB, IkB-α, PCNA, β-actin and goat anti-rabbit IgG horseradish peroxidase conjugate were purchased from Proteintech Group. (Rosemont, IL, USA). The rabbit anti-p-p38 MAPK, p-IkB-α were purchased from Affinity Biosciences (Cincinnati, OH, USA). The protein assay kits were obtained from the Beyotime Biotechnology Institute (Shanghai, China). The cell counting Kit-8 (CCK-8) and SB203580 were purchased from ApexBio Technology (Houston, USA).

2.2. Isolation and culture of mice peritoneal macrophages

Male ICR mice (4–6 weeks old) were obtained from the Comparative Medicine Centre of Yangzhou University (License number: SCXK (Su) 2017-0007). Mice were caged individually with a 12-h light-and-dark cycle at a room temperature, and were allowed *ad libitum* access to normal diet and water. Animal experiment was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the national laboratory animal welfare ethics, and protocols concerning animals was approved by the Ethical Committee of the Faculty of Nanjing Agricultural University. Mice were administered with 5 mL ice-cold PBS by intraperitoneal and massaged for 1 min, then the peritoneal fluid were collected using a syringe with a needle inserted into the inguinal region. The peritoneal suspensions was centrifuged at 1500 × *g* for 10 min and re-suspended in RPMI-1640 medium. The peritoneal macrophages were cultured with a density of 1×10^6 cells/mL at 37 °C in a 5% CO_2 atmosphere.

2.3. Peritoneal macrophages viability assay

The eff ;ect of DHEA on cells viability were evaluated using a CCK-8 colorimetric assay. Briefly, cells were suspended in 96-well plates at 1×10^6 cells/well and treated with 0, 0.1, 1, 10, 50 or 100 μmol/L DHEA for 24 h. Then, cells were treated with CCK-8 solution for 4 h, and the absorbance at 450 nm was measured using a model microplate reader (Bio-Rad, California, USA).

2.4. Measurement of peritoneal macrophages damage caused by E. Coli O157:H7 infection

The *E. coli* O157:H7 strain was a generous gift from Key Laboratory of Animal Bacteriology (Nanjing, China). The macrophages were treated with 0, 0.1, 1 or 10 μmol/L DHEA for 24 h, and then cells were infected with *E. coli* O157:H7 in mid-exponential phase at a multiplicity of infection (MOI) of 10 for 1 h, 2 h, 3 h or 4 h. After treatment, the cells supernatant were collected; and the activity of **N**-acetyl-β-D-glucosidase (NAG), alkaline phosphatase (AKP) and lactate dehydrogenase (LDH) were measured according to the manufacturer's protocol.

2.5. Peritoneal macrophages phagocytosis assay

Macrophages were treated with 0, 0.1, 1, or 10 μmol/L DHEA for 24 h, and then cells were infected with *E. coli* O157:H7 in mid-exponential phase at a multiplicity of infection (MOI) of 10 for 3 h. After that, the suspension was removed and cells were washed six times with RPMI 1640, and then cultured with RPMI 1640 containing 100 μg/mL gentamicin for 90 min. Cells were washed three times with RPMI 1640 and lysed by 1 mL 0.5% Triton X-100. Then, suspensions were serially diluted and plated on LB plate incubation at 37 °C for 24 h in order to quantify viable intracellular bacteria.

2.6. Measurement cytokine level

Macrophages were incubated with 0, 0.1, 1 or 10 μmol/L DHEA for 24 h. Then, cells were infected with *E. coli* O157:H7 in mid-exponential phase at a multiplicity of infection (MOI) of 10 for 3 h. After treatment, the supernatant were harvested and the TNF-α, IL-1β and IL-6 level were assessed using ELISA kits according to the manufacturer's instructions.

2.7. Western blot

One population of macrophages were treated with 0, 0.1, 1 or 10 μmol/L DHEA for 24 h; and another population of macrophages were pre-treated with 10 μmol/L SB203580 for 1 h before treated with 0.1 μmol/L DHEA for another 24 h. Then, all that of cells were infected with *E. coli* O157:H7 in mid-exponential phase at a multiplicity of infection (MOI) of 10 for 3 h. After treatment, the total protein were extracted with RIPA lysis buff ;er containing protease inhibitor (Beyotime Institute of Biotechnology, China); and the nuclear protein were prepared using a nucleoprotein extraction kit (Sangon Biotechnology Co., Shanghai, China). The protein concentrations were determined using a BCA assay kit.

The extracted proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gel, and then the proteins were transferred onto PVDF membranes (Millipore, Bedford, MA, USA). Membranes were blocked with 5% BSA in TBST for 3 h and were incubated overnight with rabbit polyclonal antibodies against iNOS (1:500 dilution), COX-2 (1:1000 dilution), p38 MAPK (1:1000 dilution), phospho (p) -p38 MAPK (1:1000 dilution), p-IkB-α (1:500 dilution), IkB-α (1:1000 dilution) and p65 NF-κB (1:500 dilution). After washing with TBST, membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10000 dilution) at room temperature for 2 h. Protein bands were visualized using ECL SuperSignal TM West Pico substrate (Pierce, Rock-ford, IL, USA). A monoclonal antibody against β-actin (dilution 1:10000) or PCNA (dilution 1:10000) was used as a loading control, the nuclear protein level

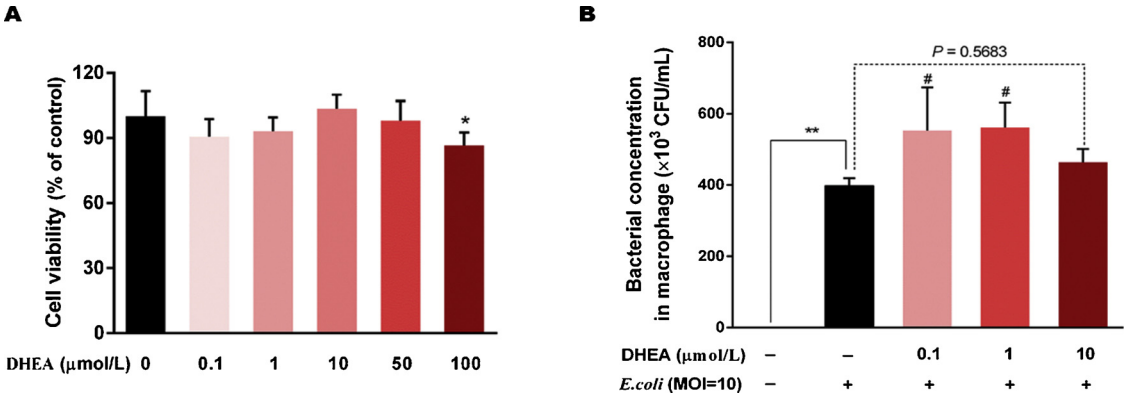

Fig. 1. Effects of DHEA on cell viability and phagocytic ability in mice peritoneal macrophages. A: Cell viability of macrophages treated with different dose DHEA; B: The bacterial concentration in *E. coli* O157:H7-infected macrophages treated with DHEA. Data are presented as mean ± SEM (n = 6). * $P < 0.05$, compared with the respective control group; # $P < 0.05$, compared with the *E. coli* O157:H7 alone infected group.

was normalized to PCNA and other protein level was normalized to β-actin.

2.8. Data analysis

All statistical analyses were estimated using two-way ANOVA followed by post hoc Dennett's test. Data were expressed as mean ± SEM, and p values less than 0.05 were considered significant. All statistical analyses were performed with SPSS 20.0 for Windows (StatSoft, Inc., Tulsa, USA).

3. Results

3.1. Effects of DHEA on the viability of mice peritoneal macrophages

No statistical difference was observed on the cell viability in mice peritoneal macrophages treated with 0.1–50 μmol/L ($P > 0.05$), while 100 μmol/L DHEA treatment decreased the macrophages viability than that of normal cells ($P < 0.05$) (Fig. 1A).

3.2. Effect of DHEA on peritoneal macrophages phagocytic ability

As shown in Fig. 1B, the bacterial concentration up to 3.98×10^5 CFU/mL in peritoneal macrophages after infected with *E. coli* O157:H7. Compared with *E. coli* O157:H7 alone infected group, 0.1 μmol/L and 1 μmol/L DHEA treatment significantly increased the bacterial concentration in *E. coli* O157:H7-infected peritoneal macrophages ($P < 0.05$) (Fig. 1B). Although no significant difference was observed, but the bacterial concentration in 10 μmol/L DHEA treatment group obviously higher than that of *E. coli* O157:H7 alone infected group ($P > 0.05$) (Fig. 1B).

3.3. Protective action of DHEA on the damage of peritoneal macrophages induced by E. Coli CO157:H7 infection

No damage was observed in peritoneal macrophages infected with *E. coli* O157:H7 for 1–2 h ($P > 0.05$); while the cells occurred obviously damage, which indicated by the increasing of AKP, NAG and LDH activity of cell supernatant, in peritoneal macrophages infected with *E. coli* O157:H7 for 3–4 h ($P < 0.01$) (Fig. 2). Compared with the group of *E. coli* O157:H7 alone infected for 3 h, 0.1 μmol/L and 10 μmol/L DHEA treatment decreased the AKP activity; 0.1 μmol/L and 1 μmol/L DHEA treatment decreased the NAG and LDH activity in cell supernatant of *E. coli* O157:H7-infected peritoneal macrophage ($P < 0.05$) (Fig. 2). However, different dose DHEA treatment almost not alerted the AKP, NAG and LDH activity in cell supernatant of peritoneal macrophage infected with *E. coli* O157:H7 for 4 h ($P > 0.05$) (Fig. 2).

3.4. Effect of DHEA on cytokines level in E. Coli O157:H7-infected peritoneal macrophages

As shown in Fig. 3, the content of TNF-α, IL-1β and IL-6 were significantly increased in *E. coli* O157:H7-infected peritoneal macrophages than that of normal cells ($P < 0.01$). 0.1μmol/L DHEA treatment markedly decreased the TNF-α and IL-1β content in *E. coli* O157:H7-infected peritoneal macrophages ($P < 0.01$) (Fig. 3A and 3B). Moreover, the IL-6 content was significantly decreased in 0.1 μmol/L and 1 μmol/L DHEA treatment group than that of *E. coli* O157:H7 alone infected group ($P < 0.05$) (Fig. 3C).

3.5. Eff ;ect of DHEA on iNOS and COX-2 protein level in E. Coli O157:H7-infected peritoneal macrophages

The iNOS and COX-2 protein level were significantly increased in peritoneal macrophages infected with *E. coli* O157:H7 than that of normal cells ($P < 0.01$) (Fig. 4). Compared with the *E. coli* O157:H7 alone infected group, 0.1–10 μmol/L DHEA treatment significantly reduced the iNOS and COX-2 protein levels in *E. coli* O157:H7-infected peritoneal macrophages ($P < 0.05$) (Fig. 4).

3.6. Impact of DHEA on p38 MAPK level in E. Coli O157:H7-infected peritoneal macrophages

E. coli O157:H7 infection significantly increased the phospho (p)-p38 MAPK protein level in peritoneal macrophages when compared to the normal cells ($P < 0.01$) (Fig. 5A and 5B). Compared with the *E. coli* O157:H7 alone infected group, 0.1 μmol/L and 10 μmol/L DHEA treatment significantly reduced the p-p38 MAPK protein level in *E. coli* O157:H7-infected peritoneal macrophages ($P < 0.05$) (Fig. 5A and 5B). Meanwhile, the decreasing of p-p38 MAPK protein level induced by 0.1 μmol/L DHEA treatment was obviously strengthened in *E. coli* O157:H7-infected peritoneal macrophages when the cells pre-treated with 10 μmol/L p38 MAPK inhibitor SB203580 ($P < 0.05$) (Fig. 5C and 5D).

3.7. Impact of DHEA on the activation of NF-κB in E. Coli O157:H7-infected peritoneal macrophages

Compared with normal cells, the cytosolic p- IκB-α protein level were significantly increased ($P < 0.01$); while the IκB-α protein levels were significantly decreased ($P < 0.01$) in peritoneal macrophages infected with *E. coli* O157:H7 (Fig. 6A - 6C). Compared with the *E. coli* O157:H7 alone infected group, 0.1–10 μmol/L DHEA treatment significantly reduced the p-IκB-α protein level ($P < 0.01$); but the IκB-α protein level in 0.1μmol/L and 10 μmol/L DHEA treatment group were

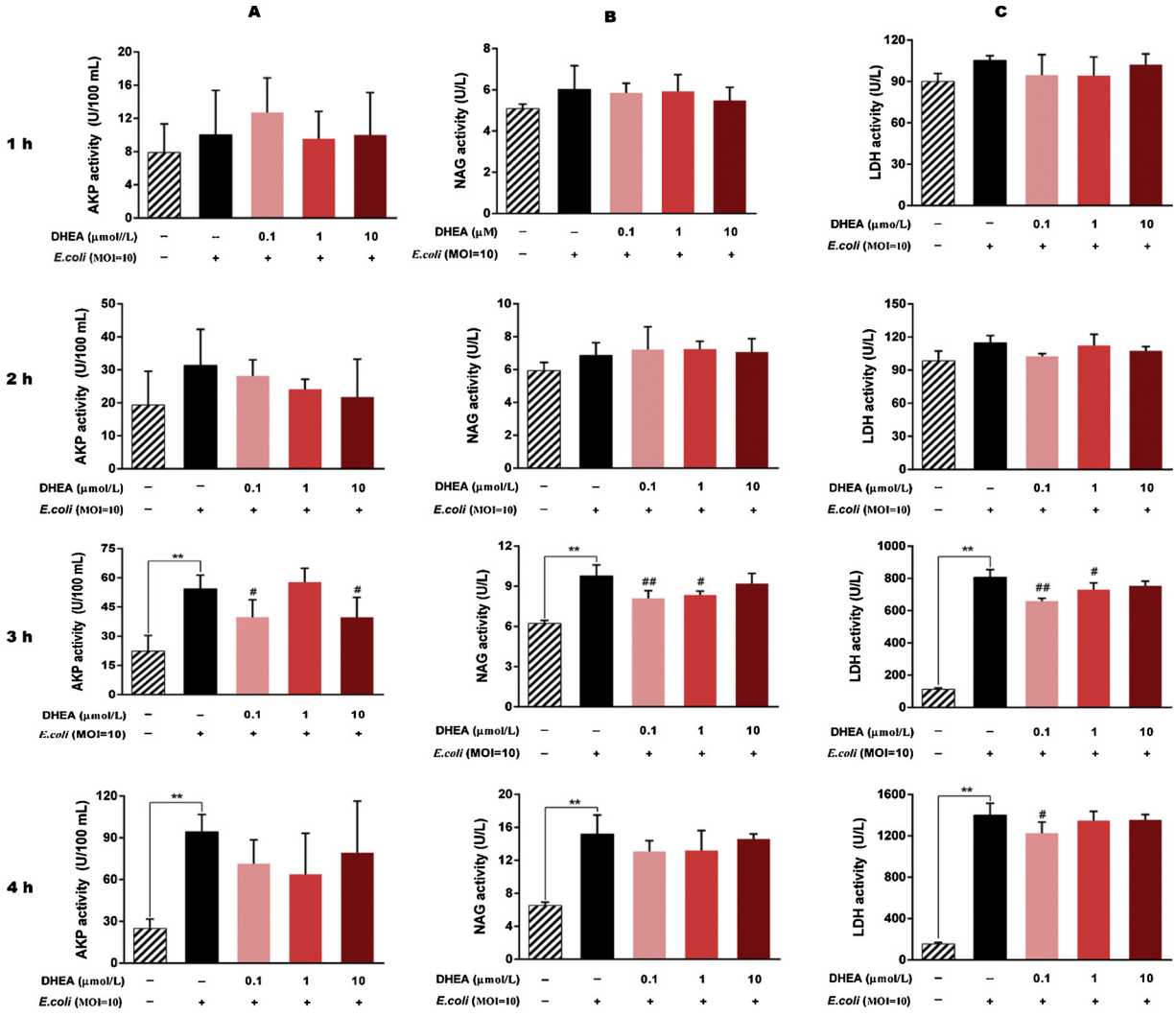

Fig. 2. Protective effect of DHEA on damage of peritoneal macrophages induced by *E. coli* O157:H7 infection. A: AKP activity in macrophages infected with *E. coli* O157:H7 for different time; B: NAG activity in macrophages infected with *E. coli* O157:H7 for different time; C: LDH activity in macrophages infected with *E. coli* O157:H7 for different time. Data are presented as mean ± SEM (n = 6). ** $P <$ 0.01, compared with the respective control group; $^{\#}$ $P < 0.05$ and $^{\#\#}$ $P < 0.01$, compared with the *E. coli* O157:H7 alone infected group.

increased in *E. coli* O157:H7-infected peritoneal macrophages ($P <$ 0.05) (Fig. 6A - 6C). In addition, the decreasing of p-IκB-α protein level and the increasing of IκB-α protein level induced by 0.1μmol/L DHEA treatment were heightened in *E. coli* O157:H7-infected peritoneal macrophages when the cells pre-treated with 10 μmol/L p38 MAPK inhibitor SB203580 ($P <$ 0.05) (Fig. 6F - 6H).

E. coli O157:H7 infection significantly increased the nuclear NF-κB protein level and decreased the cytosol NF-κB protein level than that of normal cells ($P <$ 0.01) (Fig. 6A, 6D-6E). Compared with the *E. coli* O157:H7 alone infected group, 0.1–10 μmol/L DHEA treatment significantly decreased the nuclear NF-κB protein level and increased the cytosol NF-κB protein level in *E. coli* O157:H7-infected peritoneal macrophages ($P <$ 0.05) (Fig. 6A, 6D-6E). Similar, the decreasing of nuclear NF-κB protein level induced by 0.1 μmol/L DHEA treatment was heightened in *E. coli* O157:H7-infected peritoneal macrophages when the cells pre-treated with 10 μmol/L p38 MAPK inhibitor SB203580 ($P <$ 0.05) (Fig. 6F and 6I).

4. Discussion

E. coli O157:H7 is a highly infectious pathogen that commonly causes gastrointestinal illness in animals and humans, and healthy rearing animals are the main reservoir (Branchu et al., 2014). With the incidence rate increasing, *E. coli* O157:H7 infection is be considered as a worldwide threat due to its virulence factors can improve the secretion of pro-inflammatory cytokines and chemokines (Tsai et al., 2010). Current, the treatment for *E. coli* O157:H7 infection is usually limited to supportive care as there is the problem of antibiotic resistance, which require a global revision of the approaches to treatment of bacterial infection. A significant clinical interest in DHEA due to there is a conjecture that the age-dependent reduction of DHEA level is associated with physical health in humans (Labrie et al., 1998). Previous studies found that low serum DHEA level is associated with inflammatory diseases; and it can regulate pro-inflammatory cytokines secretion and affect immune function (Alexaki et al., 2017; Hazeldine et al., 2010). It reported that DHEA up-regulate host immunity which resulting in a protective eff ;ect against *E. faecalis* infection (Loria and Ben-Nathan, 2011), while the underlying mechanism about DHEA in regulating pathogen infected remains unclear. Macrophages, highly specialized in the clearance of pathogens, play a pivotal role in host defense and immune function modulation. No difference was observed on cell viability in peritoneal macrophages after 0.1–50 μmol/L DHEA treatment, which implied that up to 50 μmol/L of DHEA treatment did not exhibit cytotoxicity to mice peritoneal macrophages. Importantly, DHEA obviously blocked the releasing of intracellular enzyme AKP, NAG and LDH in mice peritoneal macrophage infected with *E. coli* O157:H7 for

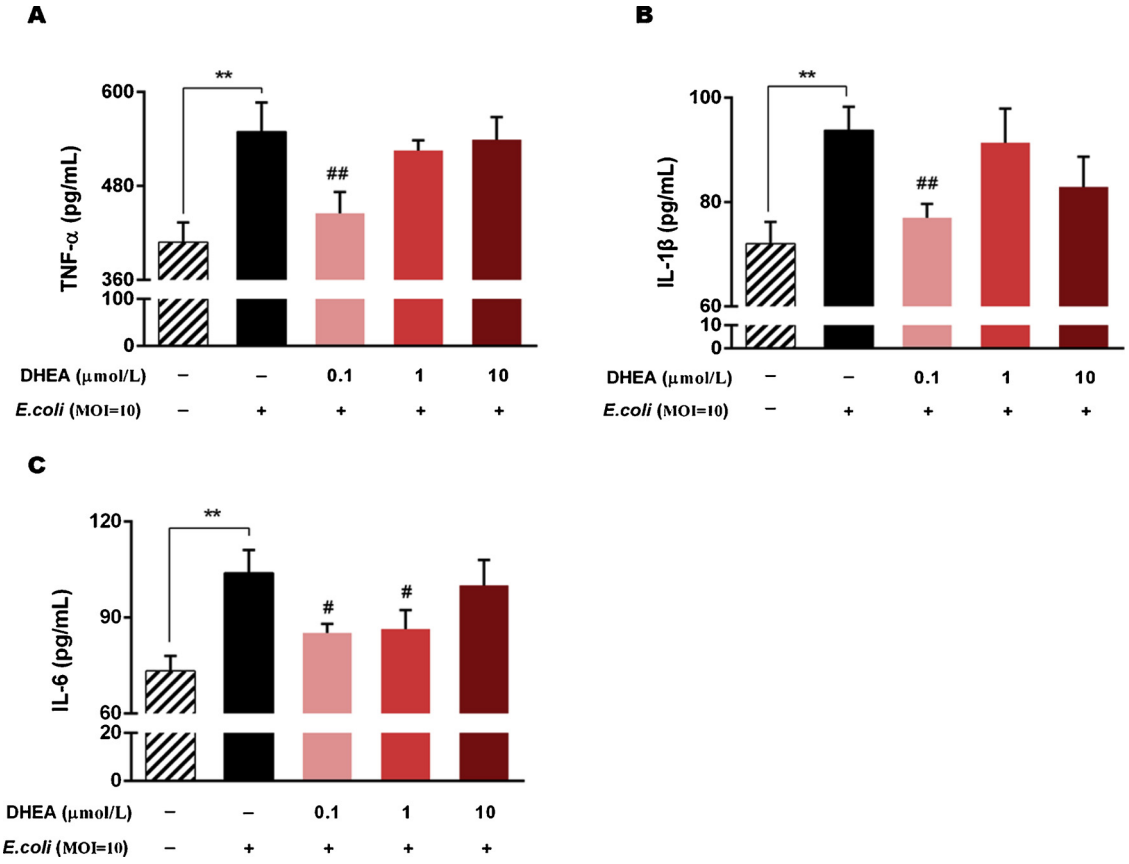

Fig. 3. Effects of DHEA on inflammatory mediator level in *E. coli* O157:H7-infected peritoneal macrophages. A: Tumor necrosis factor alpha (TNF-α) level; B: Interleukin-1β (IL-1β) level; C: Interleukin-6 (IL-6) level. Data are presented as mean ± SEM (n = 3). ** $P < 0.01$, compared with the respective control group; # $P < 0.05$ and ## $P < 0.01$, compared with the *E. coli* O157:H7 alone infected group.

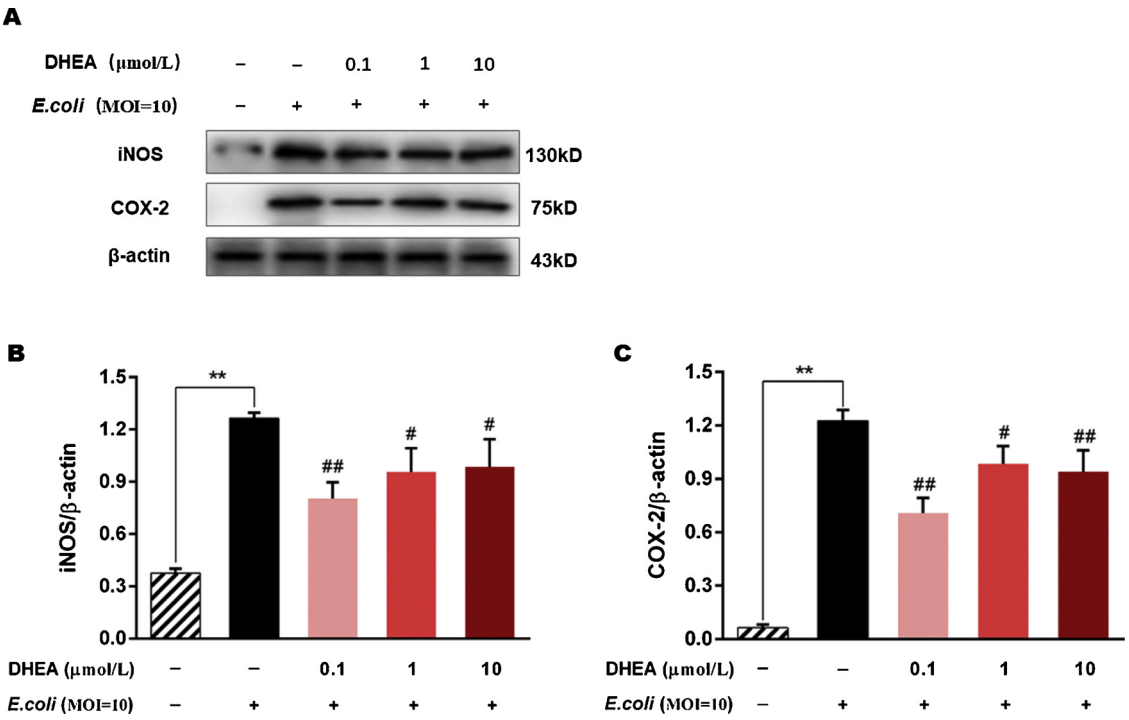

Fig. 4. Effect of DHEA on iNOS and COX-2 protein level in *E. coli* O157:H7-infected peritoneal macrophages. A: Immunoblot of iNOS and COX-2; B: iNOS protein level; C: COX-2 protein level. Data are presented as mean ± SEM (n = 3). ** $P < 0.01$, compared with the respective control group; # $P < 0.05$ and ## $P < 0.01$, compared with the *E. coli* O157:H7 alone infected group.

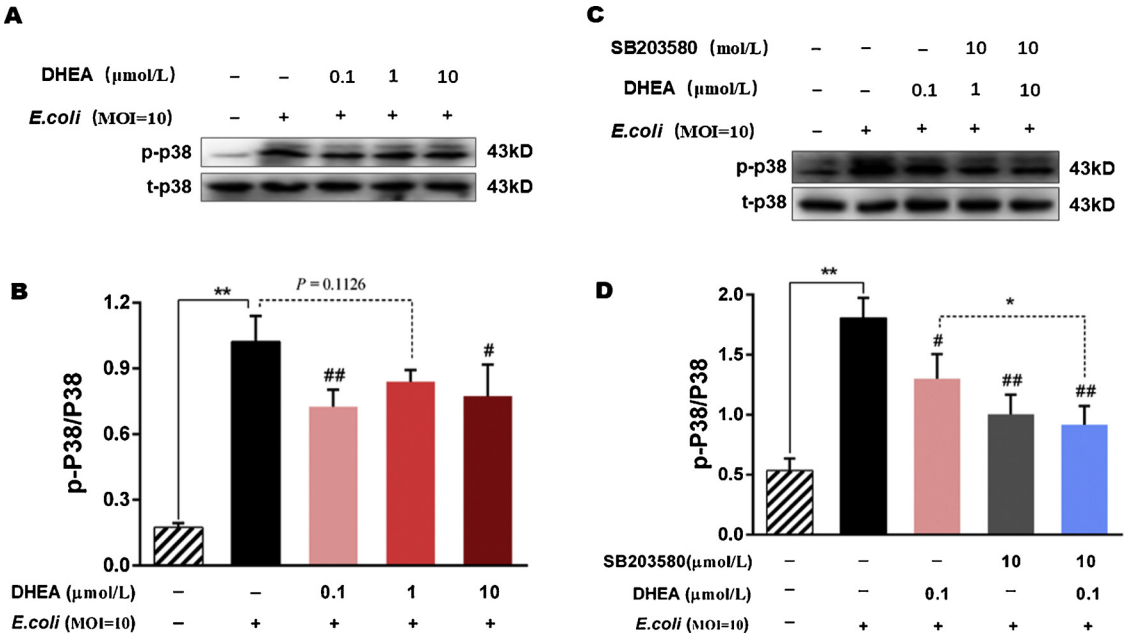

Fig. 5. Effect of DHEA on p38 MAPK protein level in *E. coli* O157:H7-infected peritoneal macrophages. A: Immunoblot of p-p38 in *E. coli* O157:H7-infected macrophages treated with DHEA; B: p-p38 protein level; C: Immunoblot of p-p38 in *E. coli* O157:H7-infected macrophages treated with DHEA and/or SB203580; D: p-p38 protein level. Data are presented as mean ± SEM (n = 3). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the respective control group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with the *E. coli* O157:H7 alone infected group.

3 h. Cytoplasmic enzymes, like LDH and NAG, are serve as indicators for the disturbances of cellular integrity induced by pathological conditions. Thus, these cytoplasmic enzymes are used as marker to determine the damage extent of cells (Drent et al., 1996; Zheng et al., 2016). These results indicated that DHEA partly alleviate the inflammatory damage in mice peritoneal macrophages caused by *E. coli* O157:H7 within 3 h after infection. In addition, we found that DHEA increased the bacterial concentration in *E. coli* O157:H7-infected peritoneal macrophages, which implied that DHEA improve the macrophages phagocytic ability. When bacteria bind to the surface of macrophages, the pathogens are rapidly internalized into phagosomes and then fuse with lysosomes, in which the enzymes and toxic peroxides digest the pathogens (Jeong et al., 2014). Thus, our results here suggested that DHEA against the bacterial infection may be associated with the increasing of mice peritoneal macrophages phagocytic ability.

In this study, DHEA markedly decreased the TNF-α, IL-1β and IL-6 level in *E. coli* O157:H7-infected mice peritoneal macrophages. The TNF-α, IL-1β and IL-6 are primary inflammatory cytokines in the acute-phase response which are secreted by macrophages (Shin et al., 2010). TNF-α can induce infiltration and activate the cascade of other pro-inflammatory cytokines or inflammatory mediators, while IL-6 plays a role in mediating the negative feedback on inflammatory responses (Wu et al., 2016). In general, the inhibition of TNF-α, IL-1β and IL-6 secretion is regarded as an effective strategy for controlling the inflammatory diseases (Chang et al., 2012). Moreover, present study also found that DHEA inhibited the increasing of iNOS and COX-2 protein expression levels induced by *E. coli* O157:H7 infection in mice peritoneal macrophages. It well known that nitric oxide (NO) is a pro-inflammatory mediator in the pathogenesis of inflammation (Pacher et al., 2007). In mammalian cells, NO is synthesized by three isoforms of NOS, and iNOS is expressed in macrophages in response to LPS or other various pro-inflammatory mediator (Rim et al., 2012). It reported that overproduction of NO is harmful to the host, and which is involved in the pathogenesis of various inflammatory diseases (Sharma et al., 2007). COX-1 and COX-2 can convert the arachidonic acid into prostaglandin (PG); and COX-2 expression is upregulated in an inflammatory background and which accounts for the excessive production of inflammatory PGs (Uto et al., 2010). Under normal conditions, COX-2 is undetectable in most tissues; whereas it is induced in cells after exposure to a noxious stimulus, such as bacterial lipopolysaccharides or cytokines (Shin et al., 2010). Excessive production of these inflammatory mediators and cytokines can cause inflammatory activities, even tissue necrosis (Zhai et al., 2016). Thus, these results implied that DHEA is capable of reducing the pro-inflammatory mediator production, which finally alleviate the inflammatory response in *E. coli* O157:H7-infected mice peritoneal macrophages.

Inflammation constitutes an essential part of the innate immune response to pathogens or the release of endogenous signal molecules (Rauch et al., 2013). Among the multitude transcription factors, NF-κB is the most important factor in regulating the pro-inflammatory production (Tak and Firestein, 2001). Recent studies reported that NF-κB plays a key role in regulation of pro-inflammatory mediators during the inflammatory response, including TNF-α, IL-1β, IL-6, iNOS and COX-2 (Fan et al., 2016; Zhai et al., 2016). In present study, DHEA increased the cytosol NF-κB protein level and decreased the nuclear NF-κB protein level, which implied that DHEA attenuate the nuclear translocation of NF-κB in *E. coli* O157:H7-infected mice peritoneal macrophages. Importantly, we found that DHEA blocked the degradation of cytosolic IκB-α which indicated as the decreasing of p-IκB-α protein level and increasing of IκB-α protein level in *E. coli* O157:H7-infected mice peritoneal macrophages. These results consist with the previous reported that DHEA reduce pro-inflammatory mediators production through inhibiting the NF-κB activation in human aortic endothelial cells or muscle cells (Altman et al., 2008; Jeon et al., 2015). These findings strongly suggested that DHEA alleviate the inflammatory response by blocking the activation of NF-κB in *E. coli* O157:H7-infected mice peritoneal macrophages.

NF-κB activation is triggered by several cellular kinases, including MAPKs (Lee et al., 2017). Among the members of the MAPKs, p38 MAPK plays an important role in inflammation (Saha et al., 2007). Our results showed that DHEA reduced the p-p38 AMPK protein level in *E. coli* O157:H7-infected peritoneal macrophages; and this decreasing in p-p38 protein AMPK level was obviously strengthened in the cells pretreated with p38 MAPK inhibitor SB203580. Upon stimulation with LPS, the phosphorylation of MAPK is involved in the NF-κB activation and which subsequently improve the cytokines production (Tian et al.,

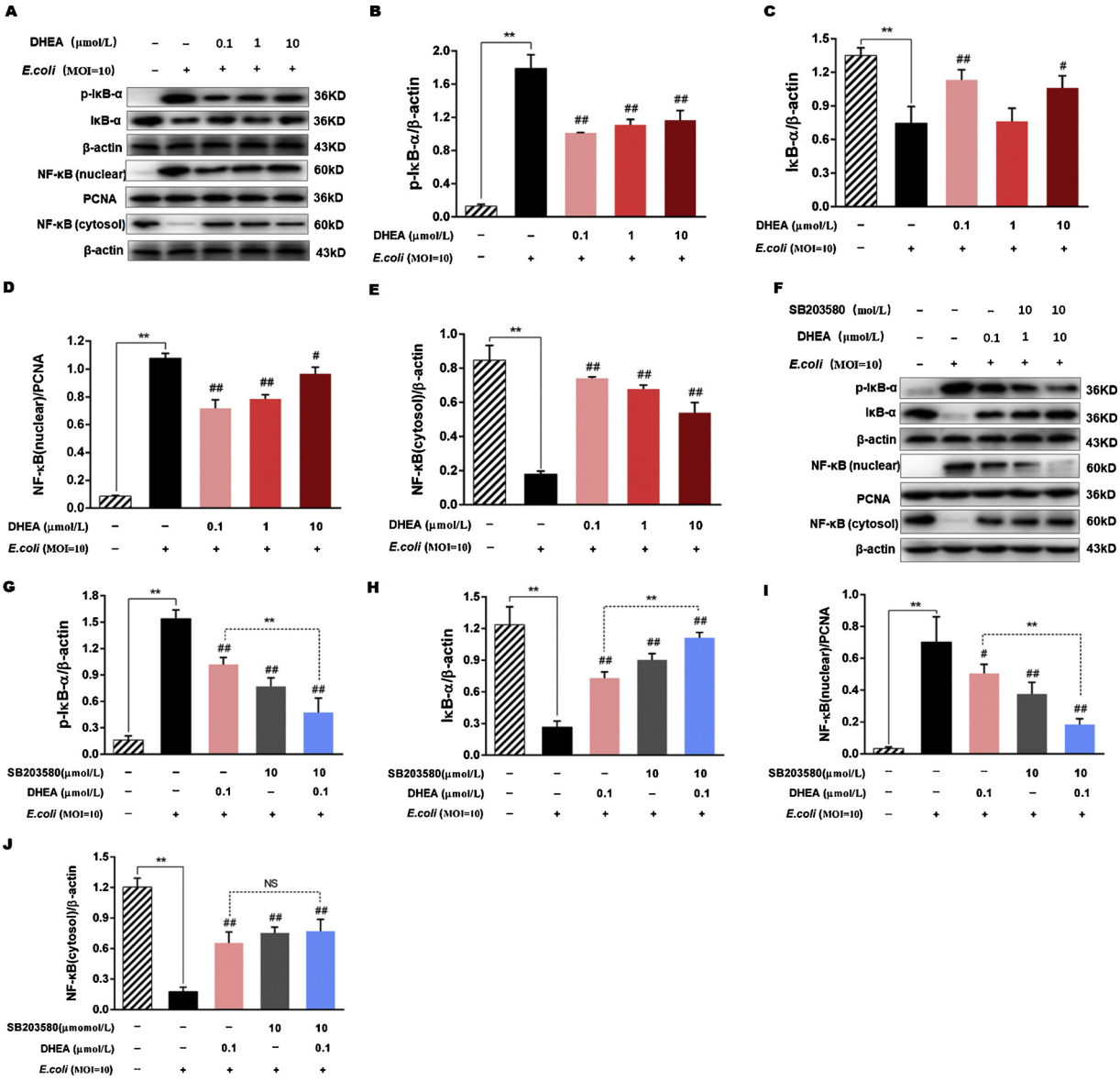

Fig. 6. Effect of DHEA on the NF-κB pathway in *E. coli* O157:H7-infected peritoneal macrophages.
A: Immunoblot of protein related to NF-κB pathway in *E. coli* O157:H7-infected macrophages treated with DHEA; B: p-IκB-α protein level; C: IκB-α level; D: Nuclear NF-κB protein level; E: Cytosol NF-κB protein level. F: immunoblot of protein related to NF-κB pathway in *E. coli* O157:H7-infected macrophages treated with DHEA and/or SB203580; G: p-IκB-α level; H: IκB-α protein level; I: Nuclear NF-κB protein level; J: Cytosol NF-κB protein level. Data are presented as mean ± SEM (n = 3). $^{**}P < 0.01$, compared with the respective control group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with the *E. coli* O157:H7 alone infected group; NS: no significance between the indicated groups.

2017). Several studies reported that inhibition of p38 MAPK could attenuate the transcriptional activity of NF-κB (Kumar and Abraham, 2017; Saha et al., 2007). Our study demonstrated that SB203580 alone treatment prominently repressed the NF-κB activation, which suggesting that p38 MAPK acts the upstream kinases of NF-κB signaling pathway in *E. coli* O157:H7-infected peritoneal macrophages. This results is consist with previous reported that phellinus linteus extract exerts anti-asthmatic effects through suppressing the activation of NF-κB and p38 MAPK in OVA-induced mouse model of asthma (Yan and Choi, 2014). Importantly, in agreement with the decreasing of p-p38 MAPK protein level induced by DHEA, we also found that DHEA decreased the cytosolic p-IκB-α and nuclear NF-κB protein level in *E. coli* O157:H7-infected mice peritoneal macrophages; and these inhibition effects of DHEA on cytosolic p-IκB-α and nuclear NF-κB protein level were heightened in *E. coli* O157:H7-infected mice peritoneal macrophages pre-treated with SB203580. Previous study revealed that DHEA regulated the pro-inflammatory mediator production, oxidative and apoptotic through down-regulation of MAPK and NF-κB pathway in muscle cells (Jeon et al., 2015). Taken above results, our present study certified that DHEA exert the anti-inflammatory effect in *E. coli* O157:H7-infected mice peritoneal macrophages by inhibiting the activation of p38 MAPK and NF-κB signaling pathways.

In conclusion, our results indicated that DHEA alleviate *E. coli* O157:H7-stimualted inflammation response by improving the phagocytic ability and inhibiting the pro-inflammatory mediator production in mice peritoneal macrophages. Meanwhile, the beneficial effect of DHEA on pro-inflammatory mediators might associated with its blocking the activation of p38 MAPK and NF-κB signaling pathways in *E. coli* O157:H7-infected mice peritoneal macrophages (Fig. 7). The results presented here detail elucidate the mechanisms underlying the anti-inflammatory action of DHEA, and shed light on the potential beneficial effects of DHEA in prevention of infectious and inflammatory

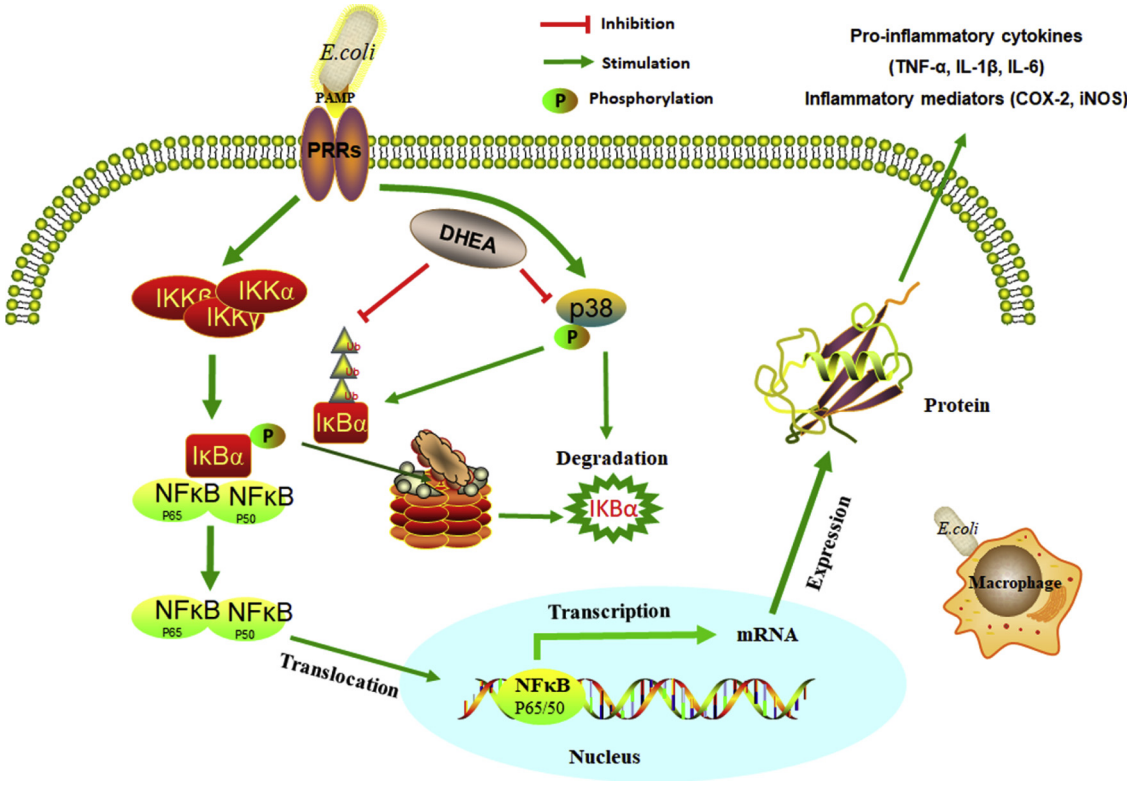

Fig. 7. Possible mechanism of DHEA alleviate *E. coli* O157:H7-stimualted inflammation response in peritoneal macrophages.
DHEA inhibited the production of pro-inflammatory mediator, reduced the p-p38 MAPK protein level and blocked the nuclear translocation of NF-κB by inhibiting the degradation of cytosolic IκB-α in *E. coli* O157:H7-infected mice peritoneal macrophages. These results implied that DHEA alleviate *E. coli* O157:H7-stimualted inflammation response through inhibiting the activation of p38 MAPK and NF-κB signaling pathways in *E. coli* O157:H7-infected mice peritoneal macrophages.

diseases.

Declaration of Competing Interest

The authors declare no competing interests.

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References

Alexaki, V.I., Fodelianaki, G., Neuwirth, A., Mund, C., Kourgiantaki, A., Ieronimaki, E., Lyroni, K., Troullinaki, M., Fujii, C., Kanczkowski, W., Ziogas, A., Peitzsch, M., Grosskklaus, S., Sonnichsen, B., Gravanis, A., Bornstein, S.R., Charalampopoulos, I., Tsatsanis, C., Chavakis, T., 2017. DHEA inhibits acute microglia-mediated inflammation through activation of the TrkA-Akt1/2-CREB-Jmjd3 pathway. Mol. Psychiatry 23, 1410–1420.

Altman, R., Motton, D.D., Kota, R.S., Rutledge, J.C., 2008. Inhibition of vascular inflammation by dehydroepiandrosterone sulfate in human aortic endothelial cells: roles of PPARalpha and NF-κB. Vascul. Pharmacol. 48, 76–84.

Alves, V.B., Basso, P.J., Nardini, V., Silva, A., Chica, J.E., Cardoso, C.R., 2016. Dehydroepiandrosterone (DHEA) restrains intestinal inflammation by rendering leukocytes hyporesponsive and balancing colitogenic inflammatory responses. Immunobiology 221, 934–943.

Arthur, J.S., Ley, S.C., 2013. Mitogen-activated protein kinases in innate immunity. Nat. Rev. Immunol. 13, 679–692.

Bak, M.J., Hong, S.G., Lee, J.W., Jeong, W.S., 2012. Red ginseng marc oil inhibits iNOS and COX-2 via NFkappaB and p38 pathways in LPS-stimulated RAW 264.7 macrophages. Molecules 17, 13769–13786.

Branchu, P., Matrat, S., Vareille, M., Garrivier, A., Durand, A., Crepin, S., Harel, J., Jubelin, G., Gobert, A.P., 2014. NsrR, GadE, and GadX interplay in repressing expression of the *Escherichia coli* O157:H7 LEE pathogenicity island in response to nitric oxide. PLoS Pathog. 10, 1–14.

Brazão, V., Santello, F.H., Caetano, L.C., Filipin, M.D.V., Toldo Jr, M.P.A., J.C.D.P, 2010. Immunomodulatory effects of zinc and DHEA on the Th-1 immune response in rats infected with Trypanosoma cruzi. Immunobiology 215, 427–434.

Brazao, V., Santello, F.H., Caetano, L.C., Del Vecchio Filipin, M., Toldo, M.P., do Prado Jr, J.C., 2010. Immunomodulatory effects of zinc and DHEA on the Th-1 immune response in rats infected with *Trypanosoma cruzi*. Immunobiology 215, 427–434.

Chang, W.T., Huang, W.C., Liou, C.J., 2012. Evaluation of the anti-inflammatory effects of phloretin and phlorizin in lipopolysaccharide-stimulated mouse macrophages. Food Chem. 134, 972–979.

Drent, M., Cobben, N.A.M., Henderson, R.F., Wouters, E.F.M., van Dieijen-Visser, M., 1996. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur. Respir. J. 9, 1736–1742.

Fan, G., Jiang, X., Wu, X., Fordjour, P.A., Miao, L., Zhang, H., Zhu, Y., Gao, X., 2016. Anti-Inflammatory Activity of Tanshinone IIA in LPS-Stimulated RAW264.7 Macrophages via miRNAs and TLR4-NF-κB Pathway. Inflammation 39, 375–384.

Gentilini, M.V., Velasquez, L.N., Barrionuevo, P., Arriola Benitez, P.C., Giambartolomei, G.H., Delpino, M.V., 2015. Adrenal steroids modulate the immune response during *Brucella abortus* infection by a mechanism that depends on the regulation of cytokine production. Infect. Immun. 83, 1973–1982.

Hazeldine, J., Arlt, W., Lord, J.M., 2010. Dehydroepiandrosterone as a regulator of immune cell function. J. Steroid Biochem. Mol. Biol. 120, 127–136.

Jeon, S., Hur, J., Kim, J., 2015. DHEA alleviates oxidative stress of muscle cells via activation of Nrf2 pathway. Appl. Biochem. Biotechnol. 176, 22–32.

Jeong, K.-M., Choi, J.-I., Lee, S.-H., Lee, H.-J., Son, J.-K., Seo, C.-S., Song, S.-W., Kwak, S.-H., Bae, H.-B., 2014. Effect of sauchinone, a lignan from Saururus chinensis, on bacterial phagocytosis by macrophages. Eur. J. Pharmacol. 728, 176–182.

Kiemer, A.K., Hartung, T., Huber, C., Vollmar, A.M., 2003. Phyllanthus amarus has anti-inflammatory potential by inhibition of iNOS, COX-2, and cytokines via the NF-κB pathway. J. Hepatol. 38, 289–297.

Kumar, R.P., Abraham, A., 2017. Inhibition of LPS induced pro-inflammatory responses in RAW 264.7 macrophage cells by PVP-coated naringenin nanoparticle via down regulation of NF-kB/P38MAPK mediated stress signaling. Pharmacol. Rep. 69, 908–915.

Labrie, F., Bélanger, A., Luu-The, V., Labrie, C., Simard, J., Cusan, L., Gomez, J.L., Candas, B., 1998. DHEA and the Intracrine Formation of Androgens and Estrogens in Peripheral Target Tissues: Its Role during Aging. Steroids 63, 322–328.

Lee, S.C., Kwon, Y.W., Park, J.Y., Park, S.Y., Lee, J.H., Park, S.D., 2017. Antioxidant and anti-inflammatory effects of herbal formula SC-E3 in lipopolysaccharide-stimulated RAW 264.7 macrophages. Evid. Complement. Alternat. Med. 2017, 1–13.

Loria, R.M., Ben-Nathan, D., 2011. Protective eff ;ects of DHEA and AED against viral, bacterial and parasitic infections. Israel Journal of Veterinary Medicine 4, 119–129.

Namazi, M.R., 2009. The Th1-promoting effects of dehydroepiandrosterone can provide

an explanation for the stronger Th1-immune response of women. Iran. J. Allergy Asthma Immunol. 8, 65–69.
Neurath, M.F., 2014. Cytokines in inflammatory bowel disease. Nature reviews. Immunology 14, 329–342.
Pacher, P., Beckman, J.S., Liaudet, L., 2007. Nitric oxide and peroxynitrite in health and disease. Physiol. Rev. 87, 315–424.
Park, T., Chen, H., Kevala, K., Lee, J.W., Kim, H.Y., 2016. N-Docosahexaenoylethanolamine ameliorates LPS-induced neuroinflammation via cAMP/PKA-dependent signaling. J. Neuroinflammation 13, 284.
Powell, J.M., Sonnenfeld, G., 2006. The effects of dehydroepiandrosterone (DHEA) on in vitro spleen cell proliferation and cytokine production. J. Interferon Cytokine Res. 26, 34–39.
Prall, S.P., Muehlenbein, M.P., 2018. DHEA modulates immune function: a review of evidence. Vitam. Horm. 108, 125–144.
Rauch, I., Muller, M., Decker, T., 2013. The regulation of inflammation by interferons and their STATs. JakStat 2, e23820.
Rim, H.K., Cho, W., Sung, S.H., Lee, K.T., 2012. Nodakenin suppresses lipopolysaccharide-induced inflammatory responses in macrophage cells by inhibiting tumor necrosis factor receptor-associated factor 6 and nuclear factor-kappaB pathways and protects mice from lethal endotoxin shock. J. Pharmacol. Exp. Ther. 342, 654–664.
Ruland, J., 2011. Return to homeostasis: downregulation of NF-κB responses. Nat. Immunol. 12, 709–714.
Saha, R.N., Jana, M., Pahan, K., 2007. MAPK p38 Regulates Transcriptional Activity of NF-κB in Primary Human Astrocytes via Acetylation of p65. J. Immunol. 10, 7101–7109.
Sharma, J.N., Al-Omran, A., Parvathy, S.S., 2007. Role of nitric oxide in inflammatory diseases. Inflammopharmacology 15, 252–259.
Shin, J.S., Park, Y.M., Choi, J.H., Park, H.J., Shin, M.C., Lee, Y.S., Lee, K.T., 2010. Sulfuretin isolated from heartwood of Rhus verniciflua inhibits LPS-induced inducible nitric oxide synthase, cyclooxygenase-2, and pro-inflammatory cytokines expression via the down-regulation of NF-κB in RAW 264.7 murine macrophage cells. Int. Immunopharmacol. 10, 943–950.
Tak, P.P., Firestein, G.S., 2001. NF-kB: a key role in inflammatory diseases. J. Clin. Invest. 107, 7–11.
Tian, L., Zhou, X.Q., Jiang, W.D., Liu, Y., Wu, P., Jiang, J., Kuang, S.Y., Tang, L., Tang, W.N., Zhang, Y.A., Xie, F., Feng, L., 2017. Sodium butyrate improved intestinal immune function associated with NF-kB and p38MAPK signalling pathways in young grass carp (*Ctenopharyngodon idella*). Fish Shellfish Immunol. 66, 548–563.
Tsai, Y.T., Cheng, P.C., Pan, T.M., 2010. Immunomodulating activity of*Lactobacillus paracasei subsp. paracasei* NTU 101 in enterohemorrhagic Escherichia coli O157H7-infected mice. J. Agric. Food Chem. 58, 11265–11272.
Uto, T., Suangkaew, N., Morinaga, O., Kariyazono, H., Oiso, S., Shoyama, Y., 2010. Eriobotryae folium extract suppresses LPS-induced iNOS and COX-2 expression by inhibition of NF-kappaB and MAPK activation in murine macrophages. Am. J. Chin. Med. (Gard City N Y) 38, 985–994.
Wu, H., Zhao, G., Jiang, K., Chen, X., Rui, G., Qiu, C., Guo, M., Deng, G., 2016. IFN-τ Alleviates Lipopolysaccharide-Induced Inflammation by Suppressing NF-κB and MAPKs Pathway Activation in Mice. Inflammation 39, 1141–1150.
Yan, G.H., Choi, Y.H., 2014. Phellinus linteus Extract Exerts Anti-asthmatic Effects by Suppressing NF-κB and p38 MAPK Activity in an OVA-induced Mouse Model of Asthma. Immune Netw. 14, 107–115.
Yang, Q., Mao, Q., Liu, M., Wang, K., Wu, Z., Fang, W., Yang, Z., Luo, P., Ke, S., Shi, L., 2016. The inhibitory effect of dehydroepiandrosterone and its derivatives against influenza A virus in vitro and in vivo. Arch. Virol. 161, 3061–3072.
Zhai, X.T., Zhang, Z.Y., Jiang, C.H., Chen, J.Q., Ye, J.Q., Jia, X.B., Yang, Y., Ni, Q., Wang, S.X., Song, J., Zhu, F.X., 2016. *Nauclea officinalis* inhibits inflammation in LPS-mediated RAW 264.7 macrophages by suppressing the NF-κB signaling pathway. J. Ethnopharmacol. 183, 159–165.
Zheng, L., Xu, Y., Lu, J., Liu, M., Bin, D., Miao, J., Yin, Y., 2016. Variant innate immune responses of mammary epithelial cells to challenge by *Staphylococcus aureus*, *Escherichia coli* and the regulating effect of taurine on these bioprocesses. Free Radic. Biol. Med. 96, 166–180.