Oleocanthal alleviated lipopolysaccharide-induced acute lung injury in chickens by inhibiting TLR4/NF-κB pathway activation

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ABSTRACT This study aimed to investigate the ameliorative effect of oleocanthal (**OC**) on lipopolysaccharide (**LPS**)-induced acute lung injury (**ALI**) in chickens and its possible mechanisms. In total, 20 chickens were randomly divided into 4 groups: control (**CON**) group, LPS group, LPS + OC group, and OC group. LPS + OC and OC groups were intragastrically administered a 5 mg/kg·d OC dose for 7 d. On d 8, the LPS group and LPS + OC group were intratracheally administered 2 mg/kg LPS for 12 h. It was found that OC ameliorated the pathological morphology and significantly suppressed apoptosis after OC treatment in LPS-induced ALI chicken ($P < 0.01$). Antioxidant capacity was higher in the LPS + OC group compared with the LPS group ($P < 0.01$). OC downregulated the related genes and proteins expression of toll-like receptor 4/nuclear factor-κB (**TLR4/NF-κB**) pathway in LPS group ($P < 0.01$). In conclusion, OC supplementation can alleviate LPS-induced ALI in chickens by suppressing apoptosis, enhancing lung antioxidant capacities and inhibiting TLR4/NF-κB pathway activation.

Key words: oleocanthal, chicken, acute lung injury, lipopolysaccharide, toll-like receptor 4/nuclear factor-κB

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INTRODUCTION

Innate immunity is the first line of defence against microbial infections (Kursa et al., 2022). Bacterial pneumonia is a leading cause of mortality in poultry (Dreyfuss and Ricard, 2005). Poultry is susceptible to mixed and secondary *Escherichia coli* diseases when infected with viral, bacterial, and parasitic diaeases and environmental, nutritional, and other stress conditions, leading to *E. coli* proliferation (Sid et al., 2015; El-Ghany, 2021). Studies have revealed that diseases caused by *E. coli* infections are a severe problem in poultry farming. *E. coli* is a resident bacterium in poultry and a Gram-negative bacterium whose lipopolysaccharide (**LPS**) in the cell wall membrane is the primary substance that stimulates the host immune response and causes multitissue damage (Kim et al., 2020; Kursa et al., 2022).

Respiratory diseases in poultry infected by *E. coli* are rising, leading to higher mortality rates, reduced production performance, and increased treatment costs for poultry, resulting in significant economic losses to the poultry farming industry (Fancher et al., 2021). Mitigating this tissue damage and reducing the harm caused by the inflammatory response to the body has become a hot topic of current research. Although new drugs are being developed to treat *E. coli* infections, pathogenic microorganism resistance is increasing, making it critical to develop safe and effective natural drugs to treat this disease (Li et al., 2015; Ibrahim et al., 2019).

Endotoxaemia due to severe Gram-negative bacterial infections predisposes to acute lung injury (**ALI**) (Ansari et al., 2016). LPS defined as endotoxins, is potent stimulants of immune responses (Peri et al., 2010). LPS/toll-like receptor 4 (**TLR4**) signaling has been intensively studied in the past few years. LPS mainly attacks the epithelial cells, and vascular endothelial cells of the lung bind to TLR4 ligands and activate the TLR4/nuclear factor-κB (**NF-κB**) signaling pathway, leading to the secretion of cellular inflammatory factors and inflammatory response of the body (Miller et al., 2005; Devaney et al., 2013). Therefore, TLR4 has been recognized as an important pharmacological target. Molecules with activity as endotoxin antagonists that are able to inhibit the process of TLR4 activation are interesting hit or lead compounds for antiseptic drug development (Miller et al., 2005).

The Mediterranean diet, which consists mainly of virgin olive oil (**VOO**), has been associated with health

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benefits (Yubero-Serrano et al., 2019). A high intake of VOO exerts a protective effect on the pathology of chronic diseases by reducing pro-inflammatory mediators (Nocella et al., 2018). VOO contains high phenolic compounds with powerful anti-inflammatory effects (Tripoli et al., 2005; Parkinson and Cicerale, 2016). Oleocanthal (**OC**), a phenolic compound found in VOO, has anti-inflammatory properties similar to ibuprofen. This pharmacological similarity has sparked interest in OC, and few studies have confirmed its anti-inflammatory and potentially therapeutic properies (Lucas et al., 2011; Parkinson and Keast, 2014; Scotece et al., 2015). In animal farming, olive oil related by-products a good source of feed ingredients. In recent years, significant studies have shown the possible use of olive oil and derivatives to improve animal performance and product quality (Bilal et al., 2021).

OC has emerged as a compound with natural pharmacological properties and been shown to play a critical regulatory role in inflammatory diseases. However, whether it has anti-inflammatory and antioxidant effects on LPS-induced ALI in chickens has not been reported. This study investigated the ameliorative effects and mechanisms of lung injury in chickens by measuring the effects of OC treatment on lung tissue morphological changes, apoptosis levels, oxidative indicators, inflammatory pathways, and cytokines and pointed out its potential medicinal value.

MATERIALS AND METHODS

Chickens and Study Design

Seven-day-old White Leghorn healthy chickens were provided by Guizhou Xindali Biological Technology Co., Ltd (Guiyang, China). All chickens were fed and watered ad libitum with a 12/12 h light/dark cycle. After a week of acclimation, 20 chickens were randomly assigned to 4 groups, including the control group (**CON**), lipopolysaccharide group (LPS), LPS + oleocanthal group (**LPS + OC**), and oleocanthal group (OC). Each group included 5 chickens (Figure 1A). OC (Yuanye Biotechnology Co., Ltd., Shanghai, China) dissolved in normal saline containing 5% dimethyl sulfoxide (**DMSO**). LPS + OC and OC groups were intragastrically administered a 5 mg/kg·d OC dose for 7 d (Qosa et al., 2015; Siddique et al., 2020). For 7 d, CON and LPS groups received the same dose of saline containing 5% DMSO intragastrically. On d 8, the LPS group and LPS + OC group were intratracheally administered 2 mg/kg LPS (Sigma-Aldrich Co., Ltd., St. Louis, MO) for 12 h (Chapman et al., 2005; Ishii et al., 2015). CON and OC groups received the same saline dose intratracheally for 12 h. This study was approved by the Animal Protection and Utilization Committee of Guizhou University, Guizhou, China (NO. EAE-GZU-2021-T096).

Detection of Serum TNF-α and IL-6

Blood was collected from under the wings of each treatment group of chickens and centrifuged at 3,000 r/min (15 min) for obtain the serum. First, the serum levels of tumor necrosis factor-α (**TNF-α**) and interleukin-6 (**IL-6**) were detected by enzyme-linked immunoassay (**ELISA**) kits (Sangon Biotech Co., Ltd., Shanghai, China). Then, 100 μL of TNF-α or IL-6 antibody was added at 37°C for 60 min. Next, 100 μL of working solution (**HRP**) was added at 37°C for 30 min, and 90 μL of the color developer was added at 37°C for 15 min. Finally, OD was measured at 450 nm.

Histological Structure

Hematoxylin and Eosin (H&E) Staining Fresh right-sided lung tissue was fixed in 4% paraformaldehyde for 24 h. The fixed tissues were blocked, dehydrated, and paraffin-embedded in turn. The lung samples were cut into 3 to 5-μm thick sections using a sled microtome

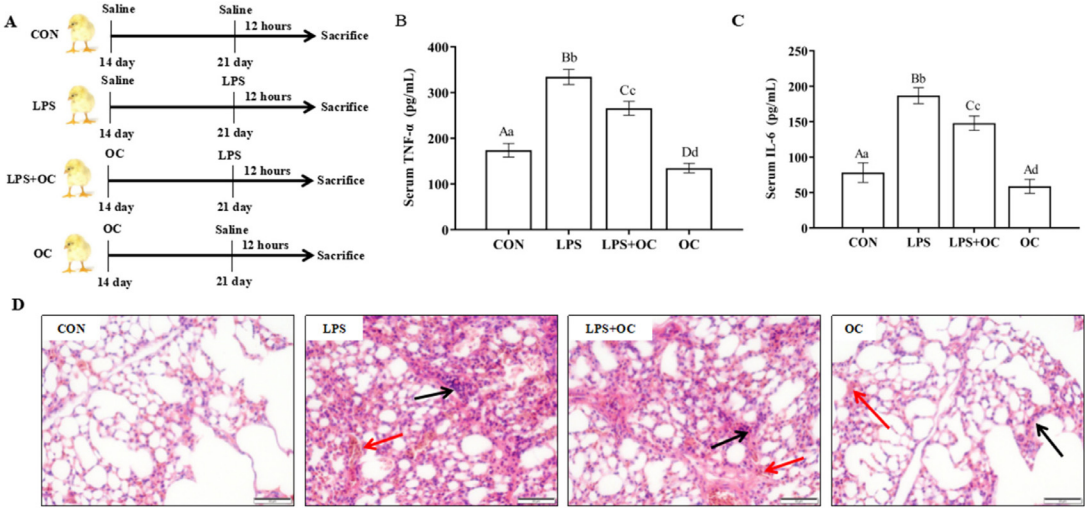

Figure 1. Effect of OC on serum inflammatory cytokines and pathological lung changes. (A) Experimental design of this study. (B) Levels of serum TNF-α with ELISA. (C) Levels of serum IL-6 with ELISA. (D) Morphological changes in the chicken lungs with H&E staining (Scale bar = 50 μm). Black arrow: inflammatory cells; Red arrow: Red blood cells.

(Typ RM 2235, Leica Microsystems, Wetzlar, Germany). Sections were stained using the H&E method (hematoxylin staining for 10 to 15 min; eosin re-staining for 20 s; Servicebio Co., Ltd., Wuhan, China). After sealing the sections with neutral gum, lung histopathological changes were observed using a light microscope to compare each treatment group (Olympus CX43 microscope, Tokyo, Japan).

Transmission Electron Microscope (TEM) Each treatment group's proper lung tissue was intercepted for 0.5 to 1 cm and fixed with glutaraldehyde and osmium acid. The tissue was dehydrated using gradient ethanol, and an embedding agent was used with acetone. The tissue was sectioned to a thickness of 50 to 60 nm, and the sections were stained with 3% uranyl acetate and lead citrate. Transmission electron microscopy (JEM-100 CXII, JEOL Ltd., Tokyo, Japan) was used to observe the ultrastructural changes of chicken lung tissue.

TUNEL Staining

Transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (**TUNEL**) staining kit (Servicebio Co., Ltd., Wuhan, China) was used to detect apoptosis in lung tissues of each treatment group. Paraffin embedding and sectioning of lung tissue were performed. Dewaxing of paraffin slices was performed using a xylene solution. The sections were stained in TUNEL solution for 8 min. 4′,6-diamidino-2-phenylindole (**DAPI**) solution was used for nuclear staining. The sections were sealed using an anti-fluorescence quencher and photographed under a fluorescent microscope (Eclipse C1, Nikon, Tokyo, Japan).

Detection of Oxidative Stress Index

Oxidative stress indicators in the lung tissue of each treatment group were measured, including superoxide dismutase (**SOD**), glutathione peroxidase (**GSH-pX**), catalase (**CAT**), and malondialdehyde (**MDA**). In addition, saline was used to prepare a 10% lung tissue homogenate. The levels of oxidative stress index were detected according to the kits' instructions (Jiancheng Bioengineering Institute, Nanjing, China).

Detection of mRNA Transcription Levels

Real-time quantitative polymerase chain reaction (**qRT-PCR**) was used to detect the mRNA transcription levels of toll-like receptor/nuclear factor-κB (TLR4/NF-κB) signaling pathway. Total RNA from lung tissues was extracted according to the Trizol method, and the concentration of total RNA was measured at 260/280 nm using a spectrophotometer. cDNA synthesis was performed according to the manufacturer's instructions (Tiangen Biotech Co., Ltd., Beijing, China). The primer sequences of TLR4/NF-κB pathway-related genes [TLR4, myeloid differentiation factor-88 (**MyD88**), NF-κB, TNF-α, IL-6, and interleukin-1β (**IL-1β**)] were identified according to the gene sequences in GeneBank, which Tsingke, Beijing, China synthesized. The qRT-PCR reaction condition was as follows: 95°C, 1 min; (95°C, 5 s; Tm, 20 s; 72°C, 30 s), 35 cycles. β-actin gene was chosen as the internal reference gene.

TLR4: 5'AGTCACCGCTTTCACTTCCC3', 5'CTGAGAGAGGTCAGGTTGGC3';
MyD88: 5'CTGCTGGAAGAGTGGCAGAG3', 5'TACGGATCATCCCTCGTGGT3';
NF-κB: 5'TCAACGCAGGACCTAAAGACAT3', 5'GCAGATAGCCAAGTTCAGGATG3';
TNF-α: 5'AGCCTTTGTGCCCACTTACA3', 5'GTTGGCATAGGCTGTCCTGA3';
IL-6: 5'CGACGAGGAGAAATGCCTGA3', 5'CTTTCGGAGCGGCCTTCATA3';
IL-1β: 5'TCTTCTACCGCCTGGACAGC3', 5'TAGGTGGCGATGTTGACCTG3';
β-actin: 5'CCCATCTATGAGGGCTACGCT3', 5'TCCTTGATGTCACGGACGATT3'.

Detection of TLR4 and p-p65 Proteins Expression

TLR4 and p-p65 protein expression levels in lung tissues were measured by Western blot (**WB**). Protein extraction from lung tissues was performed and quantified using the BCA method (Bio-Rad, Hercules, CA). Protein electrophoresis in sodium dodecyl sulfate-polyacrylamide gel and polyvinylidene fluoride (**PVDF**) membrane was transferred. After 2 h of room temperature closure, the corresponding primary antibodies TLR4 (1: 400), p-p65 (1: 600), p65 (1: 700), β-actin (1: 500) were added (Sangon Biotech Co., Ltd., Shanghai, China) and incubated overnight at 4°C. The membranes were washed with TBST buffer and incubated with the corresponding secondary antibodies at room temperature for 1 h. The membranes were washed with TBST buffer. The gel imaging system (Universal Hood II, Bio-Rad Laboratories) was used to image the luminescence generated by the dropwise addition of chemiluminescent solution.

Detection of Inflammatory Factors Expression

- *TNF-α*. TNF-α expression levels in lung tissues of each treatment group were detected by immunofluorescence (**IF**). The lung tissues were paraffin-embedded and sectioned. After paraffin slices were dewaxed, repaired, and closed using xylene solution, TNF-α primary antibody (1: 500, Abcam Inc., Cambridge, England) was added and incubated at 37°C for 1 h. Next, fluorescently labeled IgG (1: 200) secondary antibody was added dropwise and incubated at room temperature for 1 h. DAPI was used to restain the cell nuclei for 5 min. Finally, anti-quenching

glycerol was used to seal the slices, and fluorescent microscopy was used to observe and collect images (Eclipse C1, Nikon, Tokyo, Japan).

- *IL-6*. IL-6 expression levels in the lung tissues of each treatment group were measured by immunohistochemistry (**IHC**). Paraffin embedding and sectioning of lung tissues were performed. Paraffin slices were dewaxed using a xylene solution. The sections were rehydrated with ethanol, repaired with citric acid antigen solution, incubated with catalase, and then incubated at 37°C for 1 h with IL-6 antibody (1: 300, Abcam Inc., Cambridge, England). Next, 50 μL of DAB reagent was added for 3 to 5 min. Hematoxylin was re-stained for 10 min, followed by gradient ethanol dehydration and xylene transparency. The slices were sealed with neutral gum, observed, and images were acquired under a light microscope (Olympus CX43 microscope, Tokyo, Japan).
- *IL-1β and IL-18*. The lung tissues of each treatment group were prepared into 10% homogenate solution, centrifuged at 5,000 r/min for 10 min, and the supernatant was extracted. IL-1β and IL-18 in lung tissues were measured by ELISA kits (Sangon Biotech Co., Ltd., Shanghai, China). Then, 100 μL of IL-1β and IL-18 antibodies were added at 37°C for 60 min. Next, 100 μL of working solution (HRP) was added at 37°C for 30 min, and 90 μL of the color developer was added at 37°C for 15 min. Finally, OD was measured at 450 nm.

Statistical Analysis

SPSS (23.0, SPSS Inc., Chicago, IL) statistical software was used to analyze the test results. Graphpad prism (8.0, GraphPad Software Inc., San Diego, CA) was used for graphing. Comparisons between multiple groups were made using one-way ANOVA, expressed as mean ± standard deviation (Mean ± SD). The uppercase letters in the histogram indicate a significant difference ($P < 0.01$). The lowercase letters in the histogram indicate a significant difference ($P < 0.05$).

RESULTS

Effect of OC on Serum Inflammatory Cytokines and Lung Histopathology

First, the expression levels of serum inflammatory cytokines TNF-α (Figure 1B) and IL-6 (Figure 1C) were measured using ELISA. LPS induced TNF-α and IL-6 overexpression, but OC significantly reduced the expression of inflammatory cytokines of LPS-induced ALI in chicken ($P < 0.01$). Figure 1D depicts the lung histopathology using H&E staining. The lung tissue structure in the CON and OC groups were normal. After LPS induction, the chicken lung tissue structure had typical inflammatory features: blurred lung lobule borders, narrowing of the atrial cavity, congested lung tissue, and infiltration of inflammatory cells. The histopathological damage to the lung could be reduced after pre-gavage with OC.

Effect of OC on Apoptosis and Ultrastructure of Lung Cells

The ultrastructural changes in chicken lung tissue are displayed in Figure 2B. The nuclei of CON and OC groups were elliptical, the nuclear membrane pores were evenly distributed, the mitochondrial cristae were structured, and there were no apparent pathological phenomena such as edema in the cells. The LPS model group showed typical features of apoptosis, such as mitochondrial swelling, endoplasmic reticulum expansion, nuclear consolidation, and chromatin condensation. The

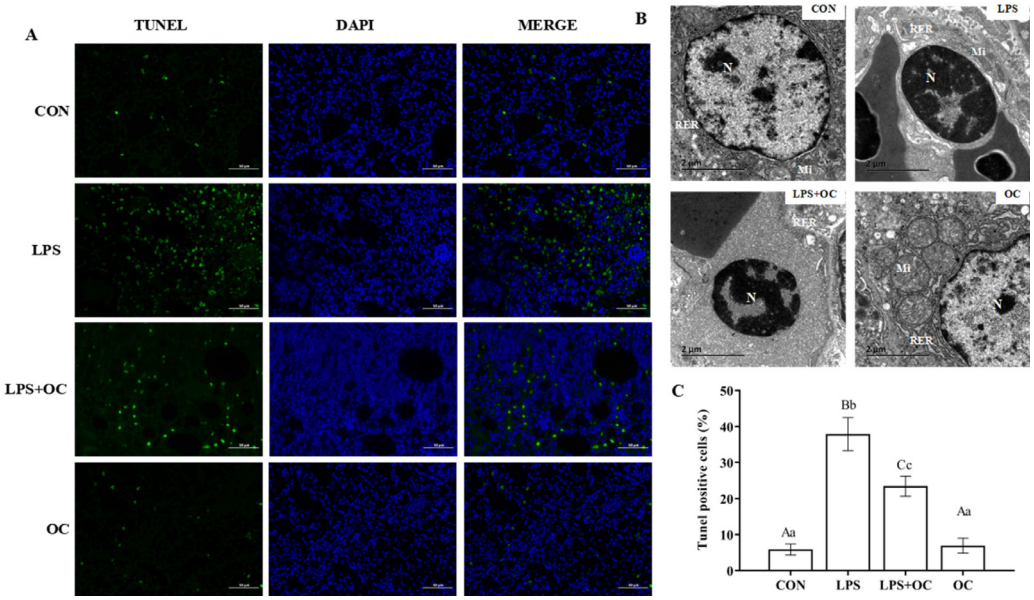

Figure 2. Effect of OC on apoptosis and ultrastructure of lung cells. (A) Apoptosis with TUNEL staining (Scale bar = 50 μm). (B) Ultrastructure with TEM. (C) Positive cells of apoptosis. Abbreviations: Mi, Mitochondria; N, Nucleus; RER, Rough endoplasmic reticulum.

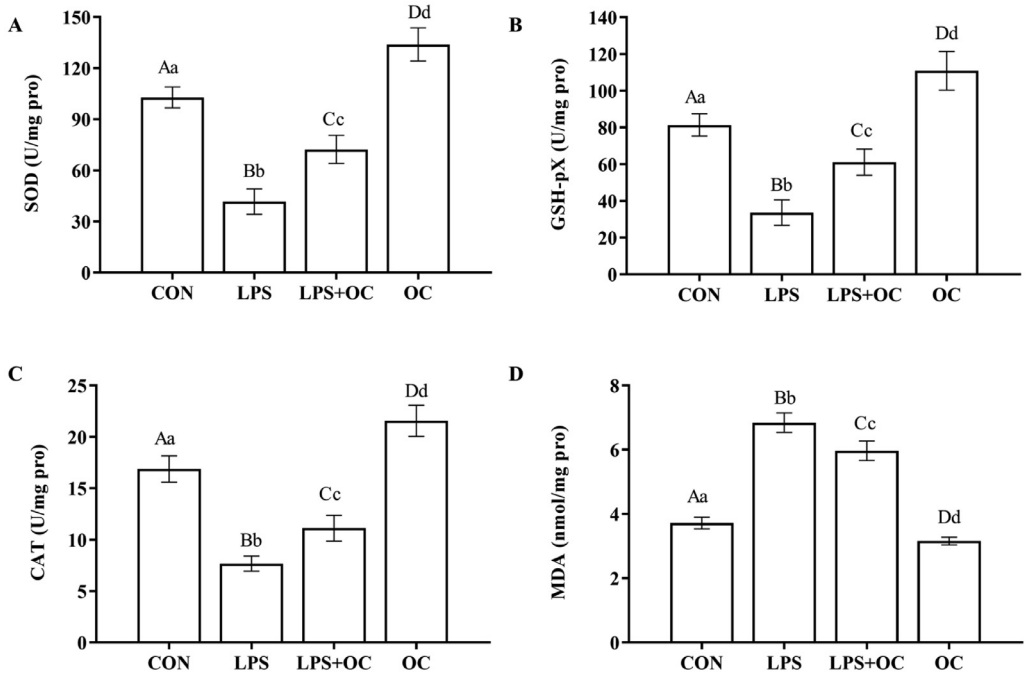

Figure 3. Effect of OC on expression levels of oxidative stress products in lung tissues of LPS-induced ALI chickens. (A) SOD. (B) GSH-pX. (C) CAT. (D) MDA.

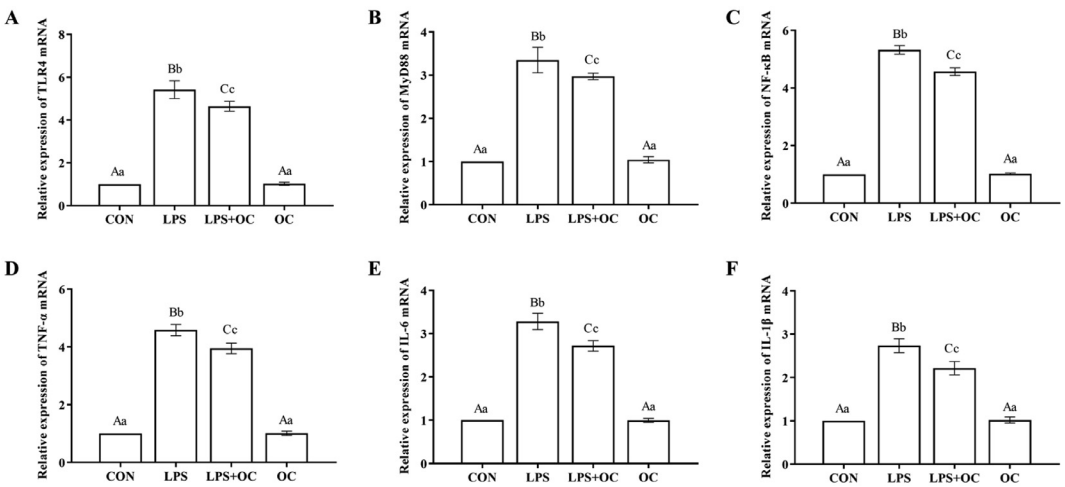

Figure 4. Effect of OC on mRNA transcript of TLR4/NF-κB in lung tissues of LPS-induced ALI chickens. (A) TLR4 mRNA transcript with qRT-PCR. (B) MyD88 mRNA transcript with qRT-PCR. (C) NF-κB mRNA transcript with qRT-PCR. (D) TNF-α mRNA transcript with qRT-PCR. (E) IL-6 mRNA transcript with qRT-PCR. (F) IL-1β mRNA transcript with qRT-PCR.

LPS + OC group showed a lesser degree of ultramicropathological damage. In addition, lung apoptosis was detected using the TUNEL method (Figure 2A). The LPS model group had more apoptosis-positive cells than the control group ($P < 0.01$; Figure 2C). However, apoptosis-positive cells were significantly suppressed after OC treatment in LPS-induced ALI chicken ($P < 0.01$). These findings indicated that OC suppressed apoptosis in the lung of LPS-induced ALI in chickens.

Effects of OC on the Levels of Oxidative Stress Products

The levels of oxidative stress products after OC treatment are shown in Figure 3A-3D. SOD, GSH-pX, and MDA levels in the LPS group were significantly reduced, and MDA content was increased ($P < 0.01$ vs. CON group). Conversely, compared with the LPS group, the levels of SOD, GSH-pX, and MDA in the LPS + OC group were significantly increased, and MDA content was reduced ($P < 0.01$). These findings imply that OC improves chickens' lung antioxidant levels of LPS-induced ALI.

Effects of OC on the mRNA Transcription of TLR4/NF-κB Pathway

TLR4/NF-κB pathway plays a critical regulatory role in LPS-induced ALI gene expression and participates in inflammatory responses. The mRNA transcription levels

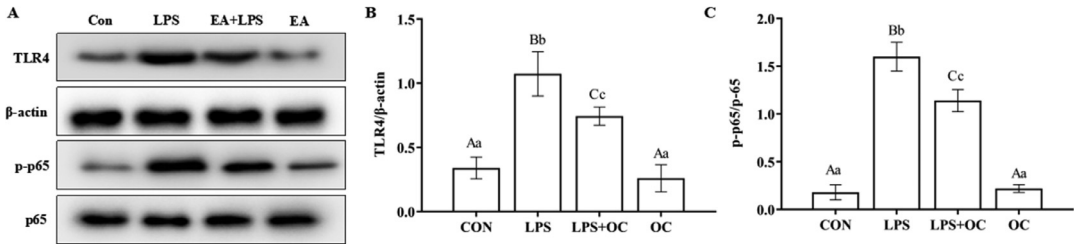

Figure 5. Effect of OC on the expression of TLR4 and p-p65 proteins in lung tissues of LPS-induced ALI chickens. (A) Protein expression of TLR4 and p-p65 with WB. (B) TLR4/β-actin. (C) p-p65/p65.

of key factors (including TLR4, MyD88, NF-κB, TNF-α, IL-6, and IL-1β) in lung tissues were determined using qRT-PCR (Figures 4A−4F). LPS infection significantly upregulated these gene mRNA transcription levels compared to the CON group ($P < 0.01$). In contrast, these mRNA transcription levels were significantly downregulated in the LPS + OC group compared to the LPS group ($P < 0.01$). These findings indicate that OC inhibits the TLR4/NF-κB pathway of LPS-induced ALI in chickens.

Effects of OC on the Expression of TLR4 and p-p65 Protein

In addition, the expression levels of TLR4 and p-p65 proteins in lung tissues were detected using WB (Figure 5). The expression levels of TLR4 and p-p65 in the LPS group were significantly high compared to the CON group ($P < 0.01$). However, the levels of TLR4 and p-p65 proteins in the LPS + OC group were reduced compared with the LPS group ($P < 0.01$).

Effect of OC on the Expression of Pro-inflammatory Cytokines

Lastly, the expression levels of TLR4 and NF-κB pathway-related inflammatory factors in lung tissues were detected by IF, IHC, and ELISA methods (Figures 6A−6F). LPS induced the high expression of TNF-α, IL-6, IL-1β, and IL-18 in lung tissues ($P <$ 0.01 vs. CON group). The expression of TNF-α, IL-6, IL-1β, and IL-18 were significantly downregulated after OC pretreatment ($P < 0.01$). It suggests that OC can inhibit the expression of pro-inflammatory cytokines in chickens with LPS-induced ALI.

DISCUSSION

OC is a polyphenolic compound characteristic of the Oleaceae family and is mainly found in VOO (Parkinson and Keast, 2014). The positive effects of OC on various antioxidant, anti-inflammatory, antibacterial, and neuroprotective activities have been widely demonstrated (Scotece et al., 2015). The objective of

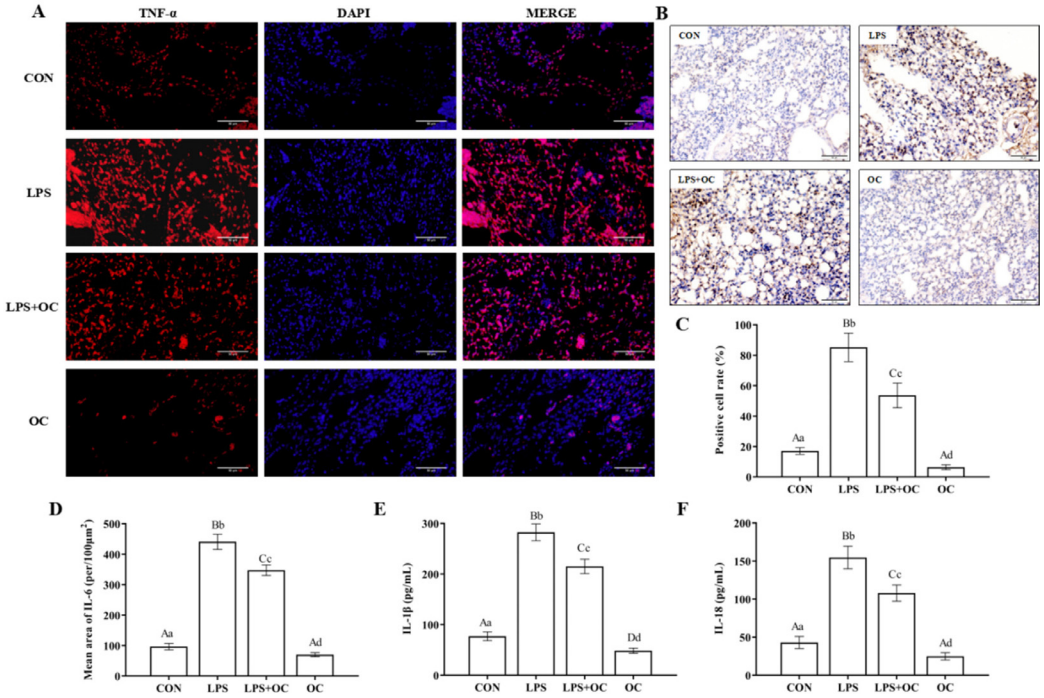

Figure 6. Effect of OC on the expression of pro-inflammatory cytokines in lung tissues of LPS-induced ALI chickens. (A) Protein expression of TNF-α with IF (Scale bar = 50 μm). (B) Protein expression of IL-6 with IHC (Scale bar = 50 μm). (C) Positive cell rate of TNF-α. (D) Mean area of IL-6. (E) Levels of IL-1β with ELISA. (F) Levels of IL-18 with ELISA.

this experiment was to investigate the ameliorative mechanisms of LPS-induced ALI in chickens by measuring the effects of OC treatment on lung tissue morphological changes, apoptosis levels, oxidative indicators, inflammatory pathways, and cytokines and point out its potential medicinal value. In the current study, OC alleviated LPS-induced ALI inflammatory pathological responses in chickens and reduced lung apoptosis and inflammatory factor levels. Furthermore, OC inhibited TLR4/NF-κB signaling activation and enhanced antioxidant capacity.

Bacterial pneumonia is one of the leading causes of poultry mortality, and endotoxemia caused by bacterial infection increases the risk of ALI (Dreyfuss and Ricard, 2005). Previous studies have shown that the main feature of ALI was increased lung inflammation. Pathologically, the lung lobules are blurred, the alveolar walls are thickened, and the interstitium is widened and infiltrated by inflammatory cells. In addition, the lung tissue's epithelial cells, endothelial cells, and macrophages show nuclear consolidation and lysis into fragments, producing apoptotic vesicles (Chapman et al., 2005; Chen et al., 2014; Yuan et al., 2020). Apoptosis is an inflammatory form of programmed cell death (**PCD**) that plays a vital role in ALI pathology.

In this study, intranasal LPS induced pathological responses in chicken lung tissue, with significant increases in serum levels of inflammatory factors TNF-α and IL-6, nuclei consolidation in lung tissue, and increased apoptosis rates, indicating that the test animals had entered a state of lung injury. However, OC gavage attenuated the pathological changes of LPS-induced lung injury in chickens and reduced the apoptosis rate in lung tissues. Mete et al. demonstrated that OC reduces oxidative stress and apoptosis in a rat model of traumatic brain injury (Mete et al., 2018). It can be speculated therefore that the OC partly alleviated LPS-induced lung injury.

Recent studies have shown that oxidative stress plays a vital role in the development of ALI. In ALI animal experiments, SOD, GSH-pX, and CAT levels were decreased, and MDA levels were increased in lung tissue (Zhang et al., 2021). Antioxidant enzymes can eliminate oxygen free radicals produced by the body's cellular respiration, reduce the content of lipid peroxides such as MDA, protect the integrity of the cellular membrane function and structure of lung tissue, and thus improve the resistance of the body's lung tissue to disease (Metnitz et al., 1999; Zheng et al., 2019). This study examined oxidative stress-related indicators in lung tissue to investigate the role of oxidative stress in LPS-induced ALI in chickens. This study suggested that LPS induction significantly reduced SOD, GSH-pX, and CAT levels and increased MDA levels. However, OC pretreatment significantly increased SOD, GSH-pX, and CAT levels and decreased MDA levels, reducing oxidative stress. Similar observations were found in previous studies. OC was demonstrated to against H_2O_2-induced oxidative stress by increasing reduced glutathione (**GSH**), reducing reactive oxygen species (**ROS**) production and upregulating the expression of antioxidant enzyme peroxiredoxin 1 (**Prdx1**) in neuron-like SH-SY5Y cells (Giusti et al., 2018).

In the pathogenesis of bacterial pneumonia, TLR4 plays a vital role in recognizing LPS and activating the inflammatory cascade (Seki et al., 2010). LPS activates the TRL4 receptor inflammatory downstream signaling pathway, of which NF-κB is a major target (He et al., 2014). NF-κB targets several inflammatory factor genes and does not typically exhibit its activity. However, when cells are stimulated, NF-κB dissociates and activates in the nucleus, regulating the expression of downstream factors and releasing large amounts of inflammatory factors (Song and Li, 2021). TLR4, NF-κB, TNF-α, IL-1β, IL-6, and other proteins were overexpressed in bacterial pneumonia, and lung inflammation in bacterial infections could be reduced or treated by inhibiting the expression of these genes and proteins. (Jeyaseelan et al., 2007; Song et al., 2017). Studies have confirmed that inhibition of the TLR4/NF-κB pathway can effectively inhibit the release of inflammatory cytokines in ALI (Wang et al., 2017; Cao et al., 2018).

Previous studies have shown that OC plays a positive role in LPS-induced inflammation. Iacono et al. (2010) found that OC and its derivatives decreased LPS-induced nitric oxide synthase (**NOS2**) synthesis in a murine chondrocyte cell line without significantly affecting cell viability at lower concentrations. Scotece et al. (2018) demonstrated that OC downregulates pro-inflammatory factors, such as IL-6, IL-8, and TNF-α induced by LPS in human primary osteoarthritis (**OA**) chondrocytes through mitogen-activated protein kinases (MAPKs)/NF-κB pathways. Furthermore, the anti-inflammatory actions of OC in LPS-stimulated macrophages were related to the inhibition of NO production, via iNOS downregulation, and also to a decrease of relevant pro-inflammatory cytokines, such as IL-1β, IL-6, TNF-α, macrophage inflammatory protein-1α (**MIP-1α**), and granulocyte/macrophage colony-stimulating factor (**GM-CSF**) (Scotece et al., 2012).

In this study, OC gavage anticipation treatment significantly inhibited the expression levels of TLR4, NF-κB, MyD88, TNF-α, IL-6, and IL-1β genes and proteins in LPS-induced ALI in chickens. This result suggests that OC may improve the pathology of LPS-induced acute lung injury in chickens by inhibiting TLR4/NF-κB signaling pathway activation. Furthermore, OC is a potent anti-inflammatory compound. Montoya et al. (2021) demonstrated that adding OC to the diet significantly reduced pro-inflammatory factors [(IL-6, IL-1β, TNF-α, interleukin-17 (**IL-17**), and interferon γ (**IFN-γ**)] and inhibited signaling pathways associated with janus kinase-signal transducer and activator of transcription (**JAK-STAT**), MAPKs, and NF-κB, thereby controlling the production of inflammatory mediators in mice with collagen-induced arthritis. The anti-inflammatory effect of OC in the brains of

Alzheimer's disease (**AD**) mice was evident, where it reduced astrocytes activation and IL-1β levels (Qosa et al., 2015). Furthermore, OC exerted a promising antifibrotic effect via a combined reduction of oxidative stress and inflammation involving putative miRNAs, reducing hepatic stellate cell activation and liver fibrosis (Gabbia et al., 2021). Taken together, these data suggest that OC has a significant anti-inflammatory effect and could be an interesting therapeutic agent for the treatment of ALI diseases. However, further research is needed to confirm itsmechanism.

CONCLUSIONS

Our findings reveal that OC alleviates LPS-induced ALI inflammatory pathological responses in chickens and reduces lung apoptosis and inflammatory factor levels. Furthermore, the anti-inflammatory mechanism of OC may inhibit TLR4/NF-κB signaling activation and enhance antioxidant capacity. OC may be a prominent functional food candidate for ALI treatment.

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Data availability: All materials, data, and associated protocols promptly available to readers without undue qualifications in material transfer agreements.

DISCLOSURES

The authors declare no conflict of interest.

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