Heliyon 10 (2024) e25004

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

CGA alleviates LPS-induced inflammation and milk fat reduction in BMECs through the NF-κB signaling pathway

Chen-Chen Lyu [1], Xing-Yu Ji [1], Hao-Yu Che, Yu Meng, Hong-Yu Wu, Jia-Bao Zhang, Yong-Hong Zhang [**,2], Bao Yuan [*,2]

College of Animal Science, College of Animal Sciences, Jilin Provincial Key Laboratory of Animal Model, Jilin University, Changchun, Jilin, PR China

ARTICLE INFO

Keywords:
Chlorogenic acid
Mastitis
BMECs
Oxidative stress
NF-κB

ABSTRACT

Mastitis is an easy clinical disease in dairy cows, which seriously affects the milk yield and quality of dairy cows. Chlorogenic acid (CGA), a polyphenolic substance, is abundant in Eucommia ulmoides leaves and has anti-inflammatory and anti-oxidative stress effects. Here, we explore whether CGA attenuated lipopolysaccharide (LPS)-induced inflammation and decreased milk fat in bovine mammary epithelial cells (BMECs). 10 μg/mL LPS was used to induce mastitis in BMECs. QRT-PCR, Western blotting, oil red O staining, and triglyceride (TG) assay were used to examine the effects of CGA on BMECs, including inflammatory response, oxidative stress response, and milk fat synthesis. The results showed that CGA repaired LPS-induced inflammation in BMECs. The expression of IL-6, IL-8, TNF-α, IL-1β, and iNOS was decreased, and the expression levels of CHOP, XCT, NRF2, and HO-1 were increased, which reduced the oxidative stress level of cells and alleviated the reduction of milk fat synthesis. In addition, the regulation of P65 phosphorylation by CGA suggests that CGA may exert its anti-inflammatory and anti-oxidative effects through the NF-κB signaling pathway. Our study showed that CGA attenuated LPS-induced inflammation and oxidative stress, and restored the decrease in milk fat content in BMECs by regulating the NF-κB signaling pathway.

1. Introduction

As one of the most common mammary gland diseases in dairy cows, mastitis seriously affects animal health and is closely related to the decline in milk yield and quality [1–4]. It is related to the important economic production factors of dairy cows and has attracted extensive attention from production and scientific researchers [5,6]. The dairy cow mammary gland is very susceptible to various bacterial pathogens, and bacterial infection further leads to the inflammatory response [7]. The main component of the cell wall of Gram-negative bacteria is LPS, after the death and lysis of Gram-negative bacteria, they act on the mammary epithelial cells of dairy cows, which can induce an inflammatory response [8,9]. At present, intramammary injection of antibiotics is often used to treat

* Corresponding author.
** Corresponding author.
E-mail addresses: cclv20@mails.jlu.edu.cn (C.-C. Lyu), jixingyu0410@163.com (X.-Y. Ji), chehy20@mails.jlu.edu.cn (H.-Y. Che), mengy21@mails.jlu.edu.cn (Y. Meng), wuhy21@mails.jlu.edu.cn (H.-Y. Wu), zjb@jlu.edu.cn (J.-B. Zhang), yonghong@jlu.edu.cn (Y.-H. Zhang), yuan_bao@jlu.edu.cn (B. Yuan).
[1] These authors contributed equally.
[2] Permanent address: Xi'an road 5333, Changchun 130062, Jilin, P.R. China.

https://doi.org/10.1016/j.heliyon.2024.e25004
Received 9 May 2023; Received in revised form 6 January 2024; Accepted 18 January 2024
Available online 21 January 2024

mastitis in dairy cows. However, with the increase in public concerns about antibiotics, especially the emergence of drug resistance and antibiotic residues, it is urgent to use safer and more reliable alternative drugs [3,10].

Traditional Chinese medicine and its extracts are good alternatives to antibiotics, and some plants and their extracts have antibacterial and anti-inflammatory effects [10]. Chlorogenic acid (CGA) is a polyphenolic substance that is abundant in nature and widely exists in traditional Chinese medicinal herbs such as honeysuckle, Eucommia ulmoides leaves, and chrysanthemum [11–13]. It has been reported that chlorogenic acid, one of the most studied phenolic compounds in the past decade, has a variety of functions, including anti-inflammatory, antibacterial, and antioxidative stress functions [14,15]. Studies have shown that CGA protects jejunal cell integrity by alleviating inflammation and apoptosis induced by DON in IPEC-J2 cells [12]; mice show inhibited inflammation and apoptosis after oral administration of CGA to alleviate colitis inflammation [14]. CGA can also inhibit the occurrence of neuroinflammation and oxidative stress, thereby protecting neurons and improving posttraumatic stress disorder [16]. The addition of CGA to the basal diet of weanling pigs can effectively improve the intestinal barrier, which is achieved by inhibiting the TLR4/NF-κB signaling pathway and activating the NRF2/HO-1 signaling pathway to inhibit inflammation and oxidative stress [11]. At present, CGA has been proven to be able to regulate intestinal homeostasis, alleviate renal inflammation, improve posttraumatic stress disorder, and improve the antioxidant capacity of liver tissue [16–19].

Previous studies have shown that CGA can act on mammary epithelial cells of dairy cows, alleviate the inflammatory response caused by *Staphylococcus aureus*, and reduce the growth rate and invasion rate of *Staphylococcus aureus* [20]. This study suggests that CGA may be an effective drug for the treatment of bovine mastitis. However, the specific role and mechanism of CGA in LPS-induced bovine mammary epithelial cell inflammation still needs to be explored. Here, an inflammation model of LPS-induced BMECs was established. After adding CGA, the expression levels of related inflammatory factors, milk fat synthesis-related genes, and oxidative stress-related genes were measured, and the changes in related signaling pathways were further analyzed to explore the effects of CGA on inflammation, and milk fat synthesis in LPS-induced BMECs.

2. Materials and methods

2.1. Cell culture

BMEC cells were purchased from Shanghai Qingqi Biological Co., LTD (Shanghai, China). The composition of the culture medium consisted of DMEM/F12 medium (BI, Kibbutz Beit Haemek, Israel), 10 % FBS (LONSERA, Shanghai, China) and 1 % penicillium-streptomycin solution (HyClone, South Logan, UT, USA). During cell culture, the carbon dioxide concentration was maintained at 5 % in constant temperature incubator at 37 °C. LPS (Sigma, St. Louis, Missouri, USA) was prepared using sterile PBS (Solarbio, Beijing, China) to concentration of 1 mg/mL. According to our previous study, the concentration of LPS was set at 10 μg/mL for 24 h [21]. A 50 mg/mL CGA solution was prepared by dissolving 20 mg CGA (Solarbio, Beijing, China) in 400 μL DMSO. After the cells were treated with LPS, CGA (0, 10, 20, 30, 40, and 50 μg/mL) was added to the medium and cultured for 24 h. The final concentration of CGA was set at 10 μg/mL.

2.2. The CCK-8 assay of cell viability

Using a 96-well plate, 5000 cells/well were seeded, covered with 100 μL of medium, and incubated for 24 h at 37 °C. Then, the medium was converted to CGA containing medium. After 24 h of culture, 10 μl CCK-8 (Beyotime, Shanghai, China) was added and treated for 1 h. Absorbance values at 450 nm were determined with a microplate reader (Tecan, Safire, Austria). Each concentration had three replicate wells.

2.3. RNA extraction and QRT–PCR

Total RNA was extracted from BMECs cultured in 12-well plates using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA concentration was determined and quality was evaluated using NanoDrop2000 spectrophotometer (Thermo, Waltham, MA, USA). The extracted total RNA was reverse transcribed into cDNA by a reverse transcription kit (Tiangen, Beijing, China). According to the concentration of RNA, different volumes were added to synthesize an equal amount of cDNA. QRT–PCR was performed using a 20 μL system with 1 μL cDNA, 1 μL primer mixture, and SYBR Green (Tiangen, Beijing, China). The relative expression levels of all genes were determined by the $2^{-\Delta\Delta Ct}$ method. The sequences were shown in Supplementary Table 1.

2.4. Western blotting

BMECs were seeded on a six-well plate and cultured. RIPA cell lysate (Solarbio, Beijing, China) was mixed with trypsin inhibitor 100:1 to prepare the lysate. The total protein concentration of BMECs was determined using the BCA Protein Assay kit (Beyotime, Shanghai, China). Equal amounts of protein were loaded into each well, and protein separation was performed using 10 % SDS-PAGE electrophoresis. Proteins were subsequently transferred to PVDF (Sigma Aldrich, St. Louis, Missouri, USA) membranes. PVDF membranes were blocked for 1 h at room temperature in a shaker using 5 % BSA and TBST buffer. The primary antibodies with membranes were diluted at a ratio of 1:1000 and incubated overnight at 4 °C. Next, the PVDF membrane was washed with TBST buffer for 5 min for a total of four times. The 1:3000 dilution of anti-rabbit IgG (ab205718, Abcam, Cambridge, UK) was incubated with PVDF membrane for 1 h at room temperature and washed again four times. Finally, the protein bands were visualized by chemiluminescence substrate

(Yamei, Shanghai, China) and analyzed by ImageJ.

NRF2 (Bioworld, Beijing, China), FASN (3180, CST, MA, USA), P–P65 (WL02169, Wanleibio, Shenyang, China), PPARγ (2443, CST, MA, USA), SREBP (ab28481, Abcam, Cambridge, UK), P65 (8242, CST, MA, USA), and α-tubulin (ab52866, Abcam, Cambridge, UK) were used.

2.5. Oil red O staining

Cells were cultured in 12-well plates, washed twice with PBS after the medium was discarded, fixed, permeabilized with 60 % isopropanol, washed, and then oil red O dye was added to soak the cells for 20 min. Distilled water was used to remove the dye solution, and hematoxylin dye solution was added to dye the nucleus for 1 min. Distilled water was added again to wash away the multisuccinic dye solution, and the cells were then fixed. A distilled water coating cover was added, observed, and photographed under an inverted microscope (Nikon, Tokyo, Japan), and the results were collected.

2.6. Determination of triglycerides

The mammary epithelial cells of dairy cows were lysed by a triglyceride enzyme assay kit (Pulilia, Beijing, China) to measure the triglyceride content in the cells. The cells were washed twice with PBS before lysis. After adding lysis buffer, the cells were shaken thoroughly and incubated for 10 min. The supernatant was split in half, half of which was used to determine triglyceride content, and the other half was used to determine total protein content by a BCA kit. Finally, the triglyceride content was normalized to protein concentration per mg.

2.7. Statistical analysis

At least three replicates were performed for each experiment. The mean ± SD was used to represent all the experimental data. GraphPad Prism 9.0 software was used to perform the multiple comparisons of one-way ANOVA. $P < 0.05$ was considered statistically significant; $P < 0.01$, the difference was considered significant; $P > 0.05$ was considered that there was no statistical significance.

3. Results

3.1. Concentration screening of CGA

To determine whether CGA affected the viability of BMECs, we stimulated cells at different CGA concentrations (0, 10, 20, 30, 40, 50 μg/ml) for 24 h, and detected by CCK-8 (Fig. 1). Compared with the control group, 10 μg/mL CGA increased cell viability significantly ($P = 0.0012$). In contrast, 50 μg/mL CGA inhibited cell viability ($P = 0.0473$).

3.2. CGA reduces LPS-induced inflammation

To explore the effect of CGA on LPS-induced inflammation in BMECs, we measured changes in inflammatory factors in cells after LPS stimulation and LPS + CGA treatment. Compared with the control group, the mRNA expression levels of the inflammatory factors

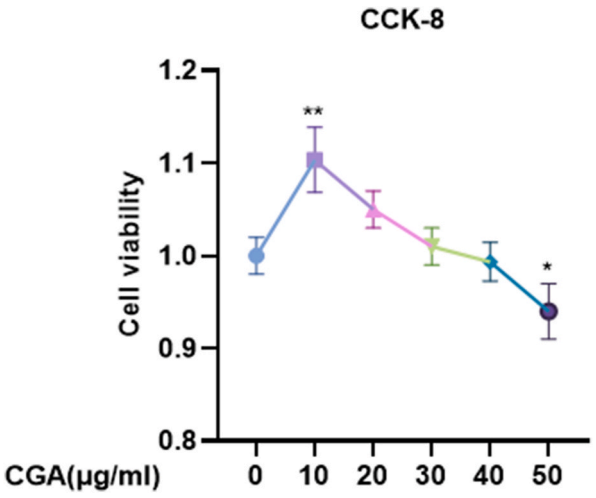

Fig. 1. Effect of CGA on the viability of Bovine mammary epithelial cells. All experiments were performed in triplicate. The data are shown as the mean ± SD. $P = 0.0012$ between 10 vs 0 μg/mL, $P = 0.0473$ between 50 vs 0 μg/mL.

IL-6 ($P < 0.0001$) (Fig. 2A), *IL-8* ($P < 0.0001$) (Fig. 2B), *TNF-α* ($P < 0.0001$) (Fig. 2C), *IL-1β* ($P = 0.0001$) (Fig. 2D), and *iNOS* ($P = 0.0022$) (Fig. 2F)were significantly increased after LPS treatment. Compared with the LPS alone group, LPS + CGA treatment was performed. The expression of the inflammation-related genes *IL-6* ($P = 0.0012$), *IL-8* ($P < 0.0001$), *TNF-α* ($P < 0.0001$), *IL-1-β* ($P = 0.0021$) and *iNOS* ($P = 0.0003$) was decreased(Fig. 2A–F).

3.3. CGA reduces oxidative stress

To investigate the effect of CGA on oxidative stress in cells by detecting oxidative stress factors in cells. Compared with the control group, the expression levels of *CHOP* ($P = 0.1831$) (Fig. 3B) and *XCT* ($P = 0.2492$) (Fig. 3C) were decreased after LPS treatment, and the expression levels of *NRF2* ($P = 0.0070$) (Fig. 3A), *HO-1* ($P = 0.0007$) (Fig. 3D) and *GPR78* ($P = 0.0006$) (Fig. 3E) were significantly decreased. The expression levels of *CHOP* ($P < 0.0001$) (Fig. 3B), *XCT* ($P = 0.0005$) (Fig. 3C), *NRF2* ($P = 0.0005$) (Fig. 3A), *HO-1* ($P = 0.0087$) (Fig. 3D) and *GPR78* ($P = 0.1125$) (Fig. 3E) in the BMECs treated with CGA were increased. At the same time, the results of Western blotting also further verified the results at the gene level. As shown in Fig. 3F, LPS induced significantly reduced expression of NRF2 in cells ($P = 0.0472$). Co-treatment with CGA and LPS significantly increased NRF2 expression compared with LPS alone ($P = 0.0345$) (Fig. 3F).

3.4. The reduction in the synthesis of milk fat was reversed by CGA

Further experiments showed that CGA could alleviate the decrease in milk fat in BMECs caused by LPS to a certain extent. The TG concentration were used for determination. LPS stimulation caused a decrease in TG secretion in BMECs ($P = 0.0047$), while CGA could significantly save the phenomenon of reduced TG synthesis in cells ($P = 0.0033$) (Fig. 4A). The lipid droplets in BMECs were significantly reduced after LPS addition, and this situation was alleviated after CGA addition (Fig. 4 B). The results of QRT-PCR showed that the mRNA expression levels of *SREBP* ($P = 0.0360$), *FASN* ($P = 0.0328$), and *PPARγ* ($P = 0.0468$) in cells treated with LPS were significantly decreased. However, CGA could restore this decrease. The expression levels of *SREBP* ($P = 0.4450$), *FASN* ($P = 0.0006$), and *PPARγ* ($P = 0.0224$) were significantly increased (Fig. 4C–E). LPS treatment significantly reduced the protein levels of SREBP ($P =$

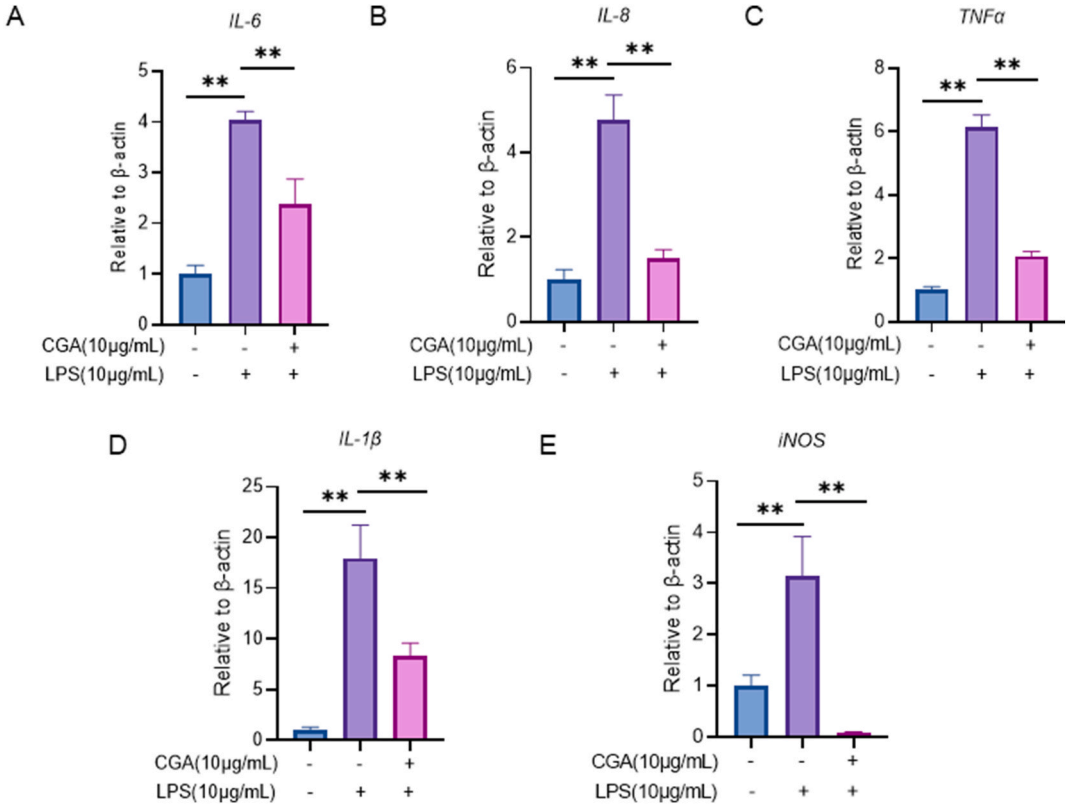

Fig. 2. CGA can alleviate LPS induced inflammatory response of Bovine mammary epithelial cells. (A) *IL*-6 mRNA level. $P < 0.0001$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0012$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (B) *IL*-8 mRNA level. $P < 0.0001$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P < 0.0001$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (C) *TNFα* mRNA level. $P < 0.0001$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P < 0.0001$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (D) *IL 1-β* mRNA level. $P = 0.0001$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0021$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (E) *iNOS* mRNA level. $P = 0.0022$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0003$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. The data are shown as the mean ± SD.

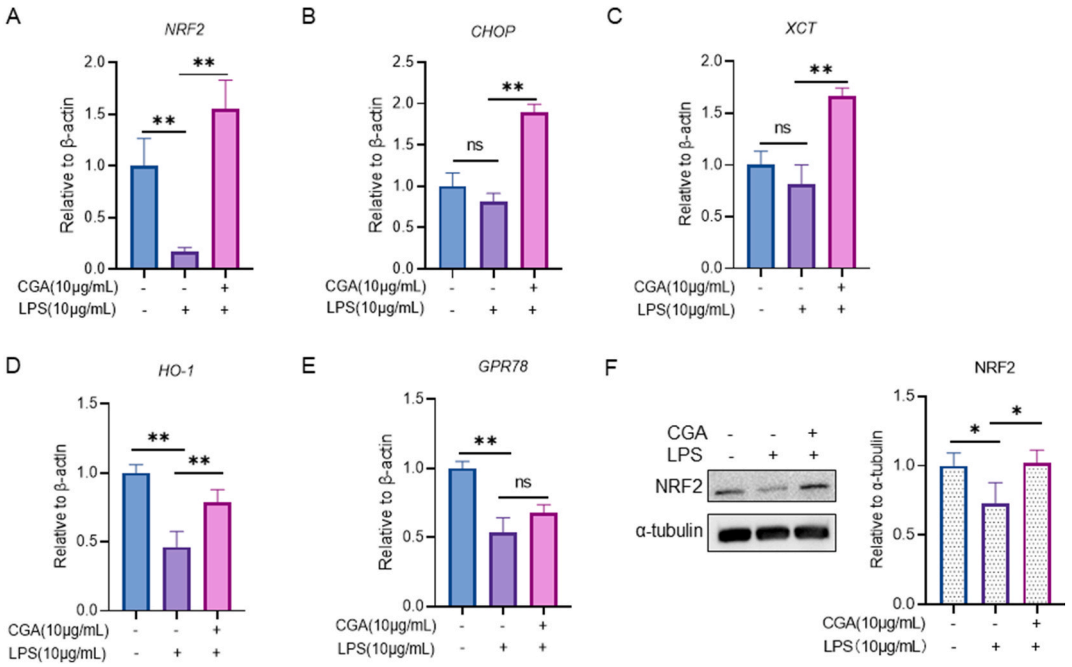

Fig. 3. CGA can improve oxidative stress caused by LPS. (A) *NRF2* mRNA level. $P = 0.0070$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0005$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (B) *CHOP* mRNA level. P = 0.1831 between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P < 0.0001$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (C) *XCT* mRNA level. $P = 0.2492$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0005$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (D) *HO*-1 mRNA level. $P = 0.0007$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0087$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (E) *GPR7*8 mRNA level. $P = 0.0006$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.1125$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (F) Detection of NRF2 levels by Western blotting. Protein grayscale analysis. α-tubulin was used as the loading control. $P = 0.0472$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0345$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. The data are shown as the mean ± SD.

0.0499) and FASN ($P = 0.0102$) in mammary epithelial cells of dairy cows, while CGA could reverse the decrease in the protein levels of SREBP ($P = 0.0197$) and FASN ($P = 0.0409$), which was consistent with our QRT−PCR results (Fig. 4F).

3.5. Chlorogenic acid inhibits inflammation and restores milk fat synthesis through NF-κB

We further explored whether CGA exerted its effects through the NF-κB pathway. Western blot was used to measure the phosphorylation of NF-κB/P65 (Fig. 5A). In BMECs, LPS significantly increased the phosphorylation of P65 ($P = 0.0088$), and this increase was significantly reduced by CGA ($P = 0.0478$) (Fig. 5A and B). Similarly, Toll-like receptor 4 (TLR4), a major molecule downstream of NF-κB, was also altered, and CGA treatment attenuated TLR4 protein increased by LPS (Fig. 5A–C).

4. Discussion

LPS is an endotoxin that acts as an important pathogenic component of the cell wall of Gram-negative bacteria [8]. Previous studies have shown that after the bacterial cell wall is damaged, LPS is released from the bacterial membrane, leading to the release of proinflammatory cytokines [22]. When it directly acts on the mammary epithelial cells of dairy cows, mastitis can be induced [7,9,23]. Previous studies have shown that monomeric components of traditional Chinese herbal medicines, such as vanillin, can alleviate LPS-induced mastitis [24]. Vanillin inhibits the LPS-induced inflammatory response by inhibiting the MAPK/NF-κB signaling pathways to protect breast cells. CGA is also a rich monomer in nature and has anti-inflammatory and antioxidant effects [25–27]. CGA can inhibit the activation of TLR4/NF-κB signaling pathway and reduce inflammation in *E. coli* endometritis [28]. Studies have also shown that CGA can protect the integrity of the intestine and reduce mucosal damage and the inflammatory response caused by indomethacin [29]. Previous studies have also shown that CGA can alleviate mastitis caused by *Staphylococcus aureus* and reverse the decline in milk yield and quality [20].

It is generally accepted that cytokines are important players in the inflammatory response [12]. On the one hand, the synthesis and release of pro-inflammatory cytokines (TNF-α, IL-6, and iNOS) mark the occurrence of inflammation, which is a kind of self-protection response [30]. On the other hand, overexpression of inflammatory cytokines (IL-6, TNF-α, and IL-1β) can also cause mammary inflammation [31]. For example, IL-1β induces an inflammatory response, often by activating neutrophils, macrophages, and monocytes [32]. Therefore, inhibiting these factors alleviates LPS-induced breast inflammation [33]. According to previous studies, LPS activate the MyD88/NF-κB pathway by binding to the TLR4 receptor to increase the release of proinflammatory cytokines in cells

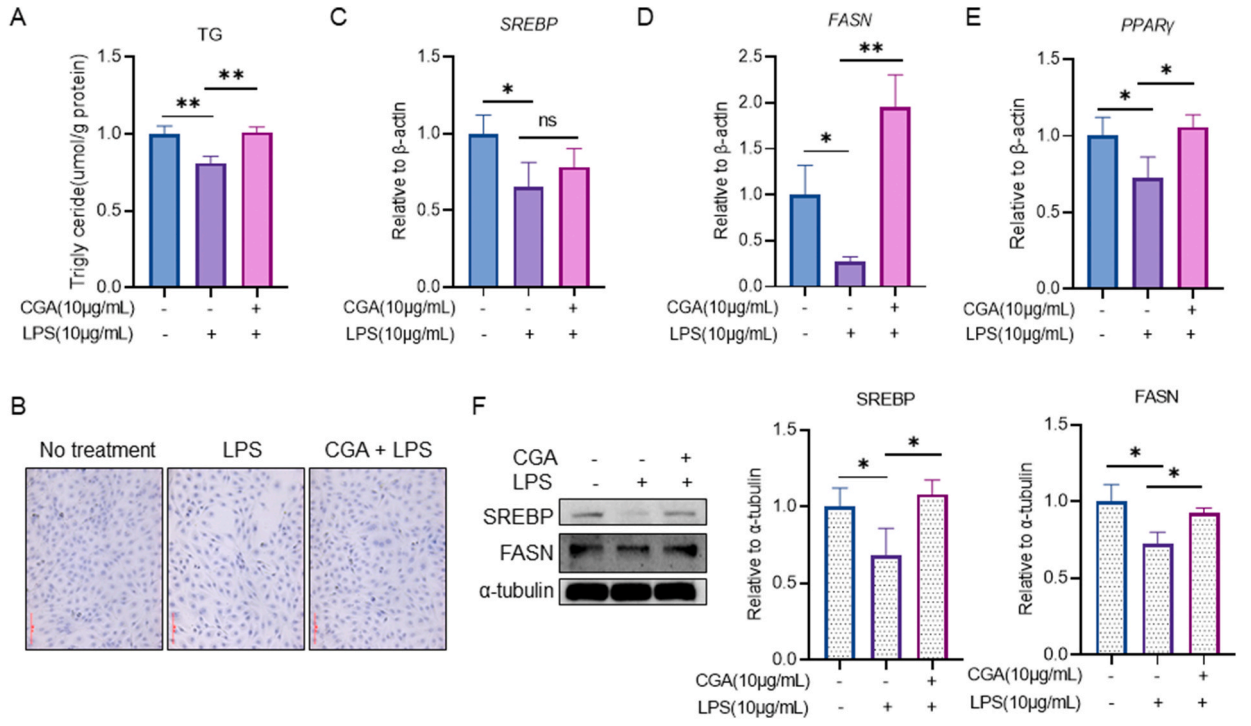

Fig. 4. CGA can improve oxidative stress caused by LPS. (A) Results of the measurement of triglyceride content. $P = 0.0047$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0033$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (B) Oil red O staining results, scale bar = 100 μm. (C) *SREBP* mRNA level. $P = 0.0360$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.4450$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (D) *FASN* mRNA level. $P = 0.0328$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0006$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (E) *PPARγ* mRNA level. $P = 0.0468$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0224$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. (F) Detection of SREBP and FASN levels by Western blotting. Protein grayscale analysis. α-tubulin was used as the loading control. SREBP: $P = 0.0499$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0197$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. FASN: $P = 0.0102$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0409$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. The data are shown as the mean ± SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

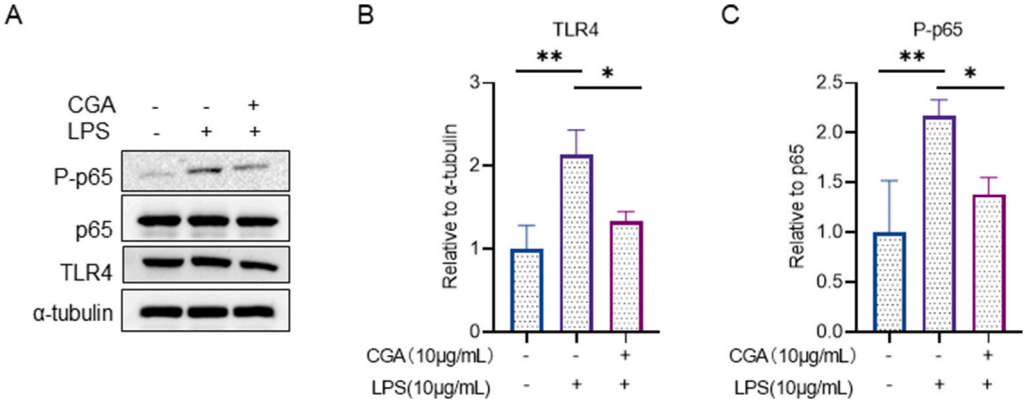

Fig. 5. CGA inhibited LPS-induced activation of NF-κB signaling pathway. (A) Western blot of P-p65, p65, and TLR4. (B) P-p65/p65 protein abundance analysis. $P = 0.0088$ between CGA-LPS- vs CGA-LPS+, $P = 0.0478$ between CGA-LPS + vs CGA + LPS+. (C)TLR4 relative protein abundance analysis. $P = 0.0011$ between $CGA^{-}LPS^{-}$ vs $CGA^{-}LPS^{+}$, $P = 0.0243$ between $CGA^{-}LPS^{+}$ vs $CGA^{+}LPS^{+}$. Data are presented as mean ± SD deviation.

[34]. Therefore, in our study, CGA reduced TLR4 expression in LPS-stimulated BMECs by inhibiting the activation of MyD88/NF-κB signaling pathway, and ultimately reduce the expression of proinflammatory cytokines, thereby alleviating breast inflammation.

Before and after calving, the feed consumption and energy demand of dairy cows will change dramatically [35]. In a high metabolic period, the mismatch between free radicals and antioxidant capacity in dairy cows will lead to oxidative stress [36,37]. Continuous oxidative stress can damage the breast, affect the production and quality of milk, and even cause mastitis [38,39]. The reduction of

cellular oxidative stress can reduce the activation of NF-κB pathway, further reducing the production of proinflammatory cytokines and thereby alleviating the inflammatory response [30]. Studies have shown that CGA can reduce the oxidative stress and mitochondrial dysfunction of hepatocytes induced by a high-fat diet by activating SIRT1 [40]. Previous studies have also shown that CGA can inhibit apoptosis, activate the NRF2/HO-1 signaling pathway, and alleviate the pathological progression of osteoporosis [41]. It has also been reported that CGA plays a role in scavenging reactive oxygen species and reactive nitrogen species, reducing oxidative stress between cells, and inhibiting the NF-κB signaling pathway, to produce anti-inflammatory effects [30]. NRF2 is an antioxidant transcription factor that regulates the expression of multiple protective genes in cells, such as HO-1 [42]. The HO-1 gene is closely related to oxidative stress, and its upregulation can inhibit the expression of inflammatory cytokines to a certain extent, thereby protecting tissue cells [43]. LPS can inhibit NRF2 and HO-1 in the brain, further inducing oxidative stress and acute neuroinflammation [44]. Therefore, we hypothesized that CGA may attenuate LPS-induced oxidative stress by increasing the expression of NRF2 and HO-1.

After inflammation in the mammary gland of dairy cows, intracellular lipase activity increases, and the membrane of milk fat globules is destroyed, which induces milk fat to be degraded by lipoprotein lipase, thereby reducing the milk fat content [45]. Studies have shown that fats and fatty acids in breast milk can be used as important regulators of immunity and metabolism to reduce the occurrence of inflammation to a certain extent [46]. In addition, lactating cows are prone to lipid oxidation, accumulate a large number of lipid peroxides, and regulate inflammation by affecting the development of oxidative stress [37]. Thus, lipid metabolism is closely related to inflammation; some lipid metabolism enzymes, such as P450 and COX2, are regulated in the process of inflammation, and the lack of certain oxygen lipids also promotes oxidative stress [37]. Studies have shown that LPS can be used to induce breast inflammation, downregulate the expression of PPARγ and FASN genes related to milk fat synthesis, reduce intracellular TG content and reduce milk fat synthesis [47]. Our study shows that CGA can rescue the LPS-induced reduction in milk fat synthesis, which may be by upregulating the expression of the milk fat synthesis genes SREBP, FASN, and PPARγ, thereby promoting the synthesis of fatty acids and increasing the content of TG.

As a transcription factor, NF-κB exists in the cytoplasm and can activate the transcription of the proinflammatory factors (IL-6, IL-1β, and TNF-α) after being activated and transferred to the nucleus [27,48]. In the liver, inflammatory signaling factors can also aggravate inflammation and oxidative stress by activating the downstream NF-κB signaling pathway [25]. It has been reported that LPS can further activate IKK by activating the TLR4 receptor on the membrane of endometrial epithelial cells so that IκB is phosphorylated, releasing and activating NF-κB, the phosphorylation and nuclear translocation of P65, which initiates the transcription of inflammatory cytokines [49]. CGA can reduce the inflammatory response by reducing the expression of TLR4 [28,50]. Therefore, we speculated that CGA may inhibit the activation of P65/NF-κB by downregulating the expression of the TLR4 receptor in mammary epithelial cells of dairy cows, thereby protecting against LPS-induced mastitis.

In summary, CGA can repair LPS-induced mammary inflammation, alleviates oxidative stress, and restores milk fat synthesis by inhibiting the NF-κB signaling pathway. CGA can be used as a potential therapeutic drug to alleviate LPS-induced injury of bovine mammary gland cells and decreased milk fat synthesis by improving oxidative stress and alleviating inflammation.

Data availability statement

Data will be made available on request.

Funding

The Chinese Agricultural Science Research System (Ministry of Agriculture; CARS-37).

CRediT authorship contribution statement

Chen-Chen Lyu: Writing – original draft, Software, Methodology, Data curation, Conceptualization. **Xing-Yu Ji:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Hao-Yu Che:** Formal analysis. **Yu Meng:** Formal analysis. **Hong-Yu Wu:** Formal analysis. **Jia-Bao Zhang:** Writing – review & editing, Resources. **Yong-Hong Zhang:** Writing – review & editing. **Bao Yuan:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Bao Yuan reports financial support was provided by The Chinese Agricultural Science Research System. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the earmarked fund for CARS (Item No. CARS37). Yuan Bao.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25004.

References

[1] Sharun Khan, Dhama Kuldeep, Ruchi Tiwari, et al., Advances in therapeutic and managemental approaches of bovine mastitis: a comprehensive review, Vet. Q. 41 (1) (2021) 107–136, https://doi.org/10.1080/01652176.2021.1882713.

[2] W. Petzl, H. Zerbe, J. Günther, et al., Pathogen-specific responses in the bovine udder. Models and immunoprophylactic concepts, Res. Vet. Sci. 116 (2018) 55–61, https://doi.org/10.1016/j.rvsc.2017.12.012.

[3] S. Mushtaq, A.M. Shah, A. Shah, et al., Bovine mastitis: an appraisal of its alternative herbal cure, Microb. Pathog. 114 (2018) 357–361, https://doi.org/10.1016/j.micpath.2017.12.024.

[4] Narender Kumar, A. Manimaran, A. Kumaresan, et al., Mastitis effects on reproductive performance in dairy cattle: a review, Trop. Anim. Health Prod. 49 (4) (2017) 663–673, https://doi.org/10.1007/s11250-017-1253-4.

[5] P.L.A. Ruegg, 100-Year Review: mastitis detection, management, and prevention, J. Dairy Sci. 100 (12) (2017) 10381–10397, https://doi.org/10.3168/jds.2017-13023.

[6] E. Motaung Thabiso, R. Petrovski Kiro, Petzer Inge-Marie, et al. Importance of bovine mastitis in Africa, Anim. Health Res. Rev. 18 (1) (2017) 58–69, https://doi.org/10.1017/s1466252317000032.

[7] Yongjiang Wu, Yawang Sun, Zhu Zhang, et al., Effects of peptidoglycan, lipoteichoic acid and lipopolysaccharide on inflammation, proliferation and milk fat synthesis in bovine mammary epithelial cells, Toxins 12 (8) (2020) 497, https://doi.org/10.3390/toxins12080497.

[8] Ruihua Li, Hengtong Fang, Jinglin Shen, et al., Curcumin alleviates LPS-induced oxidative stress, inflammation and apoptosis in bovine mammary epithelial cells via the NFE2L2 signaling pathway, Toxins 13 (3) (2021) 208, https://doi.org/10.3390/toxins13030208.

[9] C. Li, L. Li, K. Chen, et al., UFL1 alleviates lipopolysaccharide-induced cell damage and inflammation via regulation of the TLR4/NF-κB pathway in bovine mammary epithelial cells, Oxid. Med. Cell. Longev. 2019 (2019) 6505373, https://doi.org/10.1155/2019/6505373.

[10] A. El-Sayed, M. Kamel, Bovine mastitis prevention and control in the post-antibiotic era, Trop. Anim. Health Prod. 53 (2) (2021) 236, https://doi.org/10.1007/s11250-021-02680-9.

[11] J. Chen, B. Yu, D. Chen, et al., Chlorogenic acid improves intestinal barrier functions by suppressing mucosa inflammation and improving antioxidant capacity in weaned pigs, J. Nutr. Biochem. 59 (2018) 84–92, https://doi.org/10.1016/j.jnutbio.2018.06.005.

[12] X. Xu, J. Chang, P. Wang, et al., Effect of chlorogenic acid on alleviating inflammation and apoptosis of IPEC-J2 cells induced by deoxyniyalenol, Ecotoxicol. Environ. Saf. 205 (2020) 111376, https://doi.org/10.1016/j.ecoenv.2020.111376.

[13] J. Zeng, D. Zhang, X. Wan, et al., Chlorogenic acid suppresses miR-155 and ameliorates ulcerative colitis through the NF-κB/NLRP3 inflammasome pathway, Mol. Nutr. Food Res. (2020) e2000452, https://doi.org/10.1002/mnfr.202000452.

[14] I. Vukelić, D. Detel, L.B. Pučar, et al., Chlorogenic acid ameliorates experimental colitis in mice by suppressing signaling pathways involved in inflammatory response and apoptosis, Food Chem. Toxicol. 121 (2018) 140–150, https://doi.org/10.1016/j.fct.2018.08.061.

[15] D. Bagdas, Z. Gul, J.A. Meade, et al., Pharmacologic overview of chlorogenic acid and its metabolites in chronic pain and inflammation, Curr. Neuropharmacol. 18 (3) (2020) 216–228, https://doi.org/10.2174/1570159x17666191021111809.

[16] X.D. Chen, J.J. Tang, S. Feng, et al., Chlorogenic acid improves PTSD-like symptoms and associated mechanisms, Curr. Neuropharmacol. 19 (12) (2021) 2180–2187, https://doi.org/10.2174/1570159x19666210111155110.

[17] X. Zhou, B. Zhang, X. Zhao, et al., Chlorogenic acid supplementation ameliorates hyperuricemia, relieves renal inflammation, and modulates intestinal homeostasis, Food Funct. 12 (12) (2021) 5637–5649, https://doi.org/10.1039/d0fo03199b.

[18] N. Ali, S. Rashid, S. Nafees, et al., Protective effect of Chlorogenic acid against methotrexate induced oxidative stress, inflammation and apoptosis in rat liver: an experimental approach, Chem. Biol. Interact. 272 (2017) 80–91, https://doi.org/10.1016/j.cbi.2017.05.002.

[19] W.A. Al-Megrin, D.M. Metwally, O.A. Habotta, et al., Nephroprotective effects of chlorogenic acid against sodium arsenite-induced oxidative stress, inflammation, and apoptosis, J. Sci. Food Agric. 100 (14) (2020) 5162–5170, https://doi.org/10.1002/jsfa.10565.

[20] X.X. Gong, X.S. Su, K. Zhan, et al., The protective effect of chlorogenic acid on bovine mammary epithelial cells and neutrophil function, J. Dairy Sci. 101 (11) (2018) 10089–10097, https://doi.org/10.3168/jds.2017-14328.

[21] M.Q. Wang, C.H. Zhou, S. Cong, et al., Lipopolysaccharide inhibits triglyceride synthesis in dairy cow mammary epithelial cells by upregulating miR-27a-3p, which targets the PPARG gene, J. Dairy Sci. 104 (1) (2021) 989–1001, https://doi.org/10.3168/jds.2020-18270.

[22] F. Zhu, J. Ma, W. Li, et al., The orphan receptor Nur77 binds cytoplasmic LPS to activate the non-canonical NLRP3 inflammasome, Immunity 56 (4) (2023), https://doi.org/10.1016/j.immuni.2023.03.003, 753-767.e758.

[23] J.K. Ryu, S.J. Kim, S.H. Rah, et al., Reconstruction of LPS transfer cascade reveals structural determinants within LBP, CD14, and TLR4-MD2 for efficient LPS recognition and transfer, Immunity 46 (1) (2017) 38–50, https://doi.org/10.1016/j.immuni.2016.11.007.

[24] W. Guo, B. Liu, G. Hu, et al., Vanillin protects the blood-milk barrier and inhibits the inflammatory response in LPS-induced mastitis in mice, Toxicol. Appl. Pharmacol. 365 (2019) 9–18, https://doi.org/10.1016/j.taap.2018.12.022.

[25] A. Shi, T. Li, Y. Zheng, et al., Chlorogenic acid improves NAFLD by regulating gut microbiota and GLP-1, Front. Pharmacol. 12 (2021) 693048, https://doi.org/10.3389/fphar.2021.693048.

[26] Jiali Chen, Yuheng Luo, Li Yan, et al., Chlorogenic acid attenuates oxidative stress-induced intestinal epithelium injury by Co-regulating the PI3K/akt and ικβαnf-κb signaling, Antioxidants 10 (12) (2021) 1915, https://doi.org/10.3390/antiox10121915.

[27] Buko Vyacheslav, Zavodnik Ilya, Budryn Grażyna, et al., Chlorogenic acid protects against advanced alcoholic steatohepatitis in rats via modulation of redox homeostasis, inflammation, and lipogenesis, Nutrients 13 (11) (2021) 4155, https://doi.org/10.3390/nu13114155.

[28] X. Hu, M. Wang, Y. Pan, et al., Anti-inflammatory effect of astragalin and chlorogenic acid on Escherichia coli-induced inflammation of sheep endometrial epithelium cells, Front. Vet. Sci. 7 (2020) 201, https://doi.org/10.3389/fvets.2020.00201.

[29] Y. Yan, X. Zhou, K. Guo, et al., Chlorogenic acid protects against indomethacin-induced inflammation and mucosa damage by decreasing bacteroides-derived LPS, Front. Immunol. 11 (2020) 1125, https://doi.org/10.3389/fimmu.2020.01125.

[30] Bisht Akshay, Dickens Martin, Rutherfurd-Markwick Kay, et al., Chlorogenic acid potentiates the anti-inflammatory activity of curcumin in LPS-stimulated THP-1 cells, Nutrients 12 (9) (2020) 2706, https://doi.org/10.3390/nu12092706.

[31] D. Bhattarai, T. Worku, R. Dad, et al., Mechanism of pattern recognition receptors (PRRs) and host pathogen interplay in bovine mastitis, Microb. Pathog. 120 (2018) 64–70, https://doi.org/10.1016/j.micpath.2018.04.010.

[32] D. Nowicka, E. Grywalska, Staphylococcus aureus and host immunity in recurrent furunculosis, Dermatology 235 (4) (2019) 295–305, https://doi.org/10.1159/000499184.

[33] M. Meng, R. Huo, Y. Wang, et al., Lentinan inhibits oxidative stress and alleviates LPS-induced inflammation and apoptosis of BMECs by activating the Nrf2 signaling pathway, Int. J. Biol. Macromol. 222 (Pt B) (2022) 2375–2391, https://doi.org/10.1016/j.ijbiomac.2022.10.024.

[34] Y.J. Fu, B. Xu, S.W. Huang, et al., Baicalin prevents LPS-induced activation of TLR4/NF-κB p65 pathway and inflammation in mice via inhibiting the expression of CD14, Acta Pharmacol. Sin. 42 (1) (2021) 88–96, https://doi.org/10.1038/s41401-020-0411-9.

[35] L.A. Wagner, D. Fritsche, J.J. Gross, et al., Effects of different nutrient supply on metabolism and mammary immune response to an LPS challenge in early lactation of dairy cows, J. Dairy Sci. 106 (4) (2023) 2948–2962, https://doi.org/10.3168/jds.2022-22641.
[36] M.J. Kuhn, V. Mavangira, J.C. Gandy, et al., Production of 15-F(2t)-isoprostane as an assessment of oxidative stress in dairy cows at different stages of lactation, J. Dairy Sci. 101 (10) (2018) 9287–9295, https://doi.org/10.3168/jds.2018-14669.
[37] V. Mavangira, L.M. Sordillo, Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle, Res. Vet. Sci. 116 (2018) 4–14, https://doi.org/10.1016/j.rvsc.2017.08.002.
[38] W. Guo, J. Liu, J. Sun, et al., Butyrate alleviates oxidative stress by regulating NRF2 nuclear accumulation and H3K9/14 acetylation via GPR109A in bovine mammary epithelial cells and mammary glands, Free Radic. Biol. Med. 152 (2020) 728–742, https://doi.org/10.1016/j.freeradbiomed.2020.01.016.
[39] S. Qu, C. Dai, Z. Hao, et al., Chlorogenic acid prevents vancomycin-induced nephrotoxicity without compromising vancomycin antibacterial properties, Phytother Res. 34 (12) (2020) 3189–3199, https://doi.org/10.1002/ptr.6765.
[40] L. Yang, J. Wei, F. Sheng, et al., Attenuation of palmitic acid-induced lipotoxicity by chlorogenic acid through activation of SIRT1 in hepatocytes, Mol. Nutr. Food Res. 63 (14) (2019) e1801432, https://doi.org/10.1002/mnfr.201801432.
[41] D. Han, X. Gu, J. Gao, et al., Chlorogenic acid promotes the Nrf2/HO-1 anti-oxidative pathway by activating p21(Waf1/Cip1) to resist dexamethasone-induced apoptosis in osteoblastic cells, Free Radic. Biol. Med. 137 (2019) 1–12, https://doi.org/10.1016/j.freeradbiomed.2019.04.014.
[42] C.Y. Huang, J.S. Deng, W.C. Huang, et al., Attenuation of lipopolysaccharide-induced acute lung injury by hispolon in mice, through regulating the TLR4/PI3K/Akt/mTOR and keap1/Nrf2/HO-1 pathways, and suppressing oxidative stress-Mediated ER stress-induced apoptosis and autophagy, Nutrients 12 (6) (2020), https://doi.org/10.3390/nu12061742.
[43] K. Shen, Y. Jia, X. Wang, et al., Exosomes from adipose-derived stem cells alleviate the inflammation and oxidative stress via regulating Nrf2/HO-1 axis in macrophages, Free Radic. Biol. Med. 165 (2021) 54–66, https://doi.org/10.1016/j.freeradbiomed.2021.01.023.
[44] S.A. Shah, M. Khan, M.H. Jo, et al., Melatonin stimulates the SIRT1/nrf2 signaling pathway counteracting lipopolysaccharide (LPS)-Induced oxidative stress to rescue postnatal rat brain, CNS Neurosci. Ther. 23 (1) (2017) 33–44, https://doi.org/10.1111/cns.12588.
[45] J.L. Gonçalves, C. Kamphuis, H. Vernooij, et al., Pathogen effects on milk yield and composition in chronic subclinical mastitis in dairy cows, Vet. J. 262 (2020) 105473, https://doi.org/10.1016/j.tvjl.2020.105473.
[46] D. Ramiro-Cortijo, P. Singh, Y. Liu, et al., Breast milk lipids and fatty acids in regulating neonatal intestinal development and protecting against intestinal injury, Nutrients 12 (2) (2020), https://doi.org/10.3390/nu12020534.
[47] Y. Wu, Y. Sun, Z. Zhang, et al., Effects of peptidoglycan, lipoteichoic acid and lipopolysaccharide on inflammation, proliferation and milk fat synthesis in bovine mammary epithelial cells, Toxins 12 (8) (2020), https://doi.org/10.3390/toxins12080497.
[48] S.S. Singh, S.N. Rai, H. Birla, et al., Effect of chlorogenic acid supplementation in MPTP-intoxicated mouse, Front. Pharmacol. 9 (2018) 757, https://doi.org/10.3389/fphar.2018.00757.
[49] Amgk Athapaththu, K.T. Lee, M.H.D. Kavinda, et al., Pinostrobin ameliorates lipopolysaccharide (LPS)-induced inflammation and endotoxemia by inhibiting LPS binding to the TLR4/MD2 complex, Biomed. Pharmacother. 156 (2022) 113874, https://doi.org/10.1016/j.biopha.2022.113874.
[50] Ran Xin, Yan Zhang, Yuanxi Yang, et al., Dioscin improves pyroptosis in LPS-induced mice mastitis by activating AMPK/Nrf2 and inhibiting the NF-κB signaling pathway, Oxid. Med. Cell. Longev. 2020 (2020) 1–25, https://doi.org/10.1155/2020/8845521.