The protective effect of icariin and phosphorylated icariin against LPS-induced intestinal goblet cell dysfunction

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Abstract
In this study, we used LS174T cells as a model to investigate the protective effects of icariin and phosphorylated icariin on LPS-induced goblet cell dysfunction. Our results indicated that icariin and phosphorylated icariin increased the cell viability and decreased lactate dehydrogenase activity in LPS-treated LS174T cells. Icariin and phosphorylated icariin attenuated LPS-induced changes in mucin 2 synthesis and secretion. Besides, Icariin and phosphorylated icariin reduced the levels of ROS, MDA, and H2O2 and increased the activity of SOD, GPx, CAT, and T-AOC in LPS-treated LS174T cells. Moreover, the levels of IL-1β, IL-6, IL-8, and TNF-α were reduced in the Icariin and phosphorylated icariin group. Furthermore, Icariin and phosphorylated icariin decreased gene abundance or enzyme activity of Bip, XBP1, GRP78, CHOP, caspase-3, and caspase-4 in LPS-treated LS174T cells. Our data suggest that Icariin and phosphorylated icariin effectively attenuate LPS-induced intestinal goblet cell function damage through regulating oxidative stress, inflammation, apoptosis, and mucin expression.

Keywords
Icariin, phosphorylated icariin, mucus barrier, LPS, LS174T cells

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Introduction
The intestine is the main site of human metabolism and the largest immune organ in the human body. The intestinal mucosal surface is covered with intestinal epithelial cells, forming an intestinal epithelial barrier between the intestinal tract and the external environment.1,2 The surface of intestinal epithelial cells is lined with a thick mucus layer to form the mucus barrier of the intestine, which is a major component of the intestinal epithelial barrier. The mucus layer is the first line of defense against invading microorganisms and pathogenic Ags.3,4 The mucus layer of the large intestine consists of two layers with different structures: one is a dense layer directly attached to the intestinal epithelial cells, which is almost sterile and prevents bacteria from entering the epithelial cells; the other layer loosely covers the outer surface of the dense layer, which allows the colonization of certain specific bacteria.5,6 Under pathological conditions, pathogenic bacteria and other harmful substances in the intestinal lumen penetrate into the mucus layer, destroying the intestinal epithelial cell barrier, leading to intestinal diseases.7 The intestinal mucus layer is formed by mucin, which is synthesized and secreted by intestinal goblet cells.8 Increasing research has shown that various exogenous stimuli, such as microbial products, hormones, signaling mediators, and filtrating bacteria, induce abnormal

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expression of mucins, causing outbreaks of related diseases.\textsuperscript{9–11} The synthesis of mucin begins in the endoplasmic reticulum (ER), and a certain proportion of mucins are misfolded during biosynthesis. When intestinal goblet cells are exposed to ER stress, the probability of misfolding of mucins is greatly increased, and the synthesis and secretions of mucins are reduced, thereby affecting the formation of mucus layers.\textsuperscript{12}

Intestinal bacteria can usually be divided into commensal bacteria and pathological bacteria. Symbiotic bacteria can help host cells to degrade nutrients and participate in metabolic reactions, whereas pathological bacteria can affect host cell function in many different ways.\textsuperscript{13} Under normal physiological conditions, commensal bacteria and pathological bacteria are in a delicate balance, which can lead to different adverse consequences when this balance is broken.\textsuperscript{14,15} One of the main pathways by which pathological bacteria affect host cells is through the secretion of harmful bacterial toxins. Among them, bacterial LPS is a toxin that is currently attracting wide attention among researchers.\textsuperscript{16} LPS is the main component of the cell envelope of most Gram-negative bacteria, usually composed of lipid A (which is toxic depending on its structure and is then called endotoxin), a non-repetitive core of oligosaccharide, and distal polysaccharide (O-Ag).\textsuperscript{17} Toxic LPS activates downstream signaling pathways through specific lipid transfer proteins (LPS-binding proteins) to induce intestinal diseases such as necrotizing enterocolitis and inflammatory bowel disease (IBD).\textsuperscript{18,19} The intestinal mucus barrier plays an irreplaceable role in maintaining human intestinal health. However, the effects of LPS on intestinal goblet cells and the intestinal mucus barrier have not been reported.

Icariin (ICA) is a natural product of flavonoids derived from \textit{Epimedium}. Previous studies have shown that ICA has many biological activities and pharmacological effects, such as anti-oxidation, anti-aging, anti-tumor, and anti-osteoporosis,\textsuperscript{20,21} As our understanding of the anti-disease potential of natural products increases, scholars have attempted to alter the molecular structure of natural products to obtain more potent derivatives.\textsuperscript{22,23} In our previous study, phosphorylated ICA (pICA) was obtained by altering the covalent modification of ICA, and we found that pICA is not only more soluble in water than ICA, but also has stronger antioxidant, anti-inflammatory, and anti-duck hepatitis virus effects.\textsuperscript{24,25} However, whether ICA and pICA can antagonize LPS-induced changes in intestinal goblet cell function remains unclear. Therefore, in the present study, human intestinal goblet LS174T cells were used as a model to study the effects of LPS on the function of intestinal goblet cells. In addition, we also explored the protective effects of ICA and pICA on intestinal goblet cells after LPS infection, and its underlying mechanisms. We systematically examined the effects of LPS on LS174T cell proliferation, mucin synthesis and secretion, oxidative-antioxidant balance, inflammatory response, ER stress, and apoptosis, and evaluated the efficacy of ICA and pICA in antagonizing LPS.

Material and methods

Cell culture and treatment

The LS174T cell line is a well-differentiated human colonic goblet cell line.\textsuperscript{12} LS174 T cells were cultured at 37°C in a humid environment containing 5% CO\textsubscript{2} in RPMI 1640 supplemented with 10% FBS and antibiotics (10 U/ml penicillin G and 10 mg/ml streptomycin). The cells were divided into four groups: control (CON group), 50 μg/ml LPS (LPS group), 50 μg/ml LPS + 20 μg/ml ICA (LPS + ICA group), 50 μg/ml LPS + 80 μg/ml pICA (LPS + pICA group). LPS (\textit{Escherichia coli} 055:B5) was purchased from Sigma (lot no. L2630). ICA (lot no. 20171125, net content 90.00%) was bought from Xi’an Grassroot Chemical Engineering Co. Ltd. (Xian, China). pICA was synthesized using the method used in our previous study.\textsuperscript{24}

Cell proliferation assay

We used two methods (CCK-8 and LDH) to measure cell viability and to further evaluate cell proliferation. Cell viability was tested as described previously.\textsuperscript{26} Briefly, cells (~2 × 10\textsuperscript{5} cells) were cultured in 96-well cell culture plates with treatment. After 24 h, 10 μl of the CCK-8 assay solution was added to each well and incubated for another 1 h. The optical densities were then read on a microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm. LDH measurement was also used to assess cell viability.\textsuperscript{27} Cells were cultured in 96-well cell culture plates with treatment, after which the amount of LDH released into the medium was determined using an assay kit. The theory is based on the NAD + /NADH + H conversion induced by the enzyme; NADH + H converts a tetrazolium salt into a red product. The absorbance at 450 nm can then be measured with a microplate reader. Cell viability is presented relative to the control group.

Determination of mucin 2 content

The content of mucin 2 in the intracellular and cell supernatants was measured using the periodic acid-Schiff (PAS) assay. The PAS assay in LS174T cells used a previously reported method.\textsuperscript{28} In brief, LS174T cells (~2 × 10\textsuperscript{6} cells) were disrupted in PBS using sonication (VCX105; Sonics, Newtown, CT,
USA) to obtain soluble proteins. Protein concentration was determined using a BCA protein assay kit. Cellular soluble fractions and culture medium were incubated with 0.1% periodic acid (Sigma-Aldrich) for 2 h at 37°C. Next, the Schiff reagent (Sigma-Aldrich) was added and incubated for 30 min at 37°C. The OD of the resulting solution at 550 nm wavelength was taken as a measure of the amount of PAS-positive product present. The PAS OD value was expressed as the fold change relative to the mean value of the control sample.

Flow cytometry
Intracellular reactive oxygen species (ROS) in LS174T cells were measured with 2',7'-dichlorofluorescein diacetate (DCFH-DA; Sigma) as previously reported. In brief, after dilution to a final concentration of 10 μM with serum-free DMEM, DCFH-DA was added to the cells after the culture medium was removed and incubated for 30 min at 37°C. Next, the cells were washed three times with PBS. The cells were re-suspended in PBS, and the fluorescence intensity was measured for more than 10,000 cells of each sample using a FACSVerse flow cytometer (excitation/emission wavelength = 504/529 nm). The level of total intracellular ROS, paralleled by an increase in fluorescence intensity, was calculated as the percentage of control cells.

Mitochondrial ROS in LS174T cells were measured with MitoSox red mitochondrial superoxide indicator (Invitrogen, Carlsbad, CA, USA) as described previously. Briefly, after removing the culture medium, the cells were washed three times with PBS. MitoSox red mitochondrial superoxide indicator, diluted to a final concentration of 4 mM with serum-free DMEM, was added to the cells and incubated for 20 min at 37°C in the dark. The cells were then washed three times with PBS. The cells were re-suspended in PBS and the fluorescence was measured immediately using a FACsVerse flow cytometer (excitation/emission wavelength = 510/580 nm). The level of mitochondrial ROS corresponded with an increase in fluorescence and was calculated as the percentage of the measured signals for control cells.

Determination of oxidative status
LS174T cells (∼2 × 10^6 cells) were homogenized by sonication (VCX105; Sonics, Newtown, CT, USA) in ice-cold PBS containing the protease inhibitor cocktail Complete EDTA-free (Roche, Penzberg, Germany). The homogenates were centrifuged at 12745.2 g for 20 min at 4°C and then collected the supernatant fraction. Protein concentration was determined using a BCA Protein Assay kit (Pierce, Rockford, IL, USA). The malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) content were determined according to the manufacturer’s instructions using commercially available kits (Shanghai Enzyme-linked Biotechnology Co. Ltd, Shanghai, China).

Determination of anti-oxidative status
The activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and total antioxidant capacity (T-AOC) were determined by using ELISA kits (Shanghai Enzyme-linked Biotechnology Co. Ltd, Shanghai, China), according to the manufacturer’s instructions.

Determination of pro-inflammatory cytokines
The levels of IL-1β, IL-6, IL-8, and TNF-α were determined by ELISA kits (Shanghai Enzyme-linked Biotechnology Co. Ltd, Shanghai, China), according to the manufacturer’s instructions.

Determination of caspase activity
The activities of caspase-3 and 4 were determined by using ELISA kits (Shanghai Enzyme-linked Biotechnology Co. Ltd, Shanghai, China), according to the manufacturer’s instructions.

RNA isolation, cDNA synthesis, and real-time quantitative PCR
Total RNA was extracted from 100 mg of each colonic mucosa and liver specimen with Trizol Reagent. Concentration and quality of the RNA were measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (2 μg) was then treated with RNase-free DNase and reverse transcribed according to the manufacturer’s instructions. Diluted cDNA (2 μl, diluted 1:20, vol/vol) was used for real-time PCR, which was performed in a Mx3000P qPCR machine (Stratagene, La Jolla, CA, USA). GAPDH, which is not affected by the experimental factors, was chosen as the reference gene. All primers used in this study (listed in Table 1) were synthesized by Generay Company (Shanghai, China). The 2^(-ΔΔCt) method was used to analyze real-time PCR results, and gene mRNA levels were expressed as the fold change relative to the mean value of the control group.

Statistical analysis
Data are presented as mean ± SEM. Statistical significance was assessed by the independent sample t-test using SPSS (SPSS v. 20.0, SPSS Inc., Chicago, IL, USA) software packages. Data was considered
statistically significant when \( P < 0.05 \). Numbers of replicates used for statistics are noted in the figures.

**Results**

**ICA and pICA regulate proliferation of LS174T cells**

To investigate the potential protective effects of ICA and pICA on LS174T cells proliferation, we used 20 \( \mu \)g/ml ICA or 80 \( \mu \)g/ml pICA to process cells together with 50 \( \mu \)g/ml LPS for 12 h. The results showed that the viability of LS174T cells in the LPS group was significantly higher than that in the control group, while the cell viability in the ICA and pICA groups was markedly lower than that in the control group (\( P < 0.05 \), Figure 1a). Moreover, the LDH activity of LS174T cells in the LPS group was significantly lower than that in the control group, while the LDH activity in the ICA and pICA groups was markedly higher than that in the LPS group (\( P < 0.05 \), Figure 1b).

**ICA and pICA mediate mucin 2 synthesis and secretion in LS174T cells**

To investigate the potential protective effects of ICA and pICA on mucin 2 synthesis and secretion, we examined the content of mucin 2 intracellularly and in cell supernatants after incubation with ICA or pICA and LPS. The results showed that mucin 2 gene expression in LS174T cells in the LPS group was significantly higher than that in the control group, while mucin 2 gene expression in the ICA and pICA groups was markedly lower than that in the LPS group (\( P < 0.05 \), Figure 2a). Moreover, the content of mucin 2 intracellularly and in supernatants of LS174T cells in the LPS group was also significantly higher than that in the control group, while the content of mucin 2 intracellularly and in cell supernatants in the ICA and pICA groups was markedly lower than that in the LPS group (\( P < 0.05 \), Figure 2b and c).

**ICA and pICA suppress the oxidative status of LS174T cells**

To investigate the potential protective effects of ICA and pICA on LS174T cells oxidative status, we examined mitochondrial ROS, intracellular ROS, and intracellular MDA and H\( _2 \)O\( _2 \) levels after incubation with ICA or pICA and LPS. As shown in Figure 3, compared with the control cells, LPS significantly increased mitochondrial ROS, intracellular ROS, and intracellular MDA and H\( _2 \)O\( _2 \) levels in LS174T cells (\( P < 0.05 \), Figure 3a–d). Meanwhile, compared with cells in the LPS group, both ICA and pICA significantly reduced mitochondrial ROS, intracellular ROS, and intracellular MDA and H\( _2 \)O\( _2 \) levels in LS174T cells (\( P < 0.05 \), Figure 3a–d).
ICA and pICA promote the anti-oxidative status of LS174T cells

To investigate the potential protective effects of ICA and pICA on LS174T cells anti-oxidative status, we examined the activity of SOD, GPx, CAT, and T-AOC after incubation with ICA or pICA and LPS. As shown in Figure 4, compared with control cells, LPS significantly reduced the activity of SOD, GPx, CAT, and T-AOC in LS174T cells (\(P < 0.05\), Figure 4a–d). Meanwhile, compared with cells in the LPS group, both ICA and pICA significantly enhanced the SOD, GPx, CAT, and T-AOC activity in LS174T cells (\(P < 0.05\), Figure 4a–d).

ICA and pICA suppress the inflammatory status of LS174T cells

To investigate the potential protective effects of ICA and pICA on LS174T cells inflammatory status, we examined the levels of IL-1\(\beta\), IL-6, IL-8, and TNF-\(\alpha\) after incubation with ICA or pICA and LPS. As shown in Figure 5, compared with the control cells, LPS significantly increased the levels of IL-1\(\beta\), IL-6, IL-8, and TNF-\(\alpha\) in LS174T cells (\(P < 0.05\), Figure 5a–d). Meanwhile, compared with cells in the LPS group, both ICA and pICA significantly reduced the IL-1\(\beta\), IL-6, IL-8, and TNF-\(\alpha\) levels in LS174T cells (\(P < 0.05\), Figure 5a–d).
ICA and pICA suppress the ER stress-mediated apoptosis of LS174T cells

To investigate the potential protective effects of ICA and pICA on LS174T cells apoptotic status, we examined the gene expression and enzyme activity of ER stress-mediated apoptosis-related indicators after incubation with ICA or pICA and LPS. The results showed that gene expression of Bip, XBP1, GRP78, and CHOP in the LPS group was significantly higher than that in the control group, while Bip, XBP1, GRP78 and CHOP gene transcript abundance in the ICA and pICA groups was markedly lower than that in the LPS group (P < 0.05, Figure 6a–d).

Figure 4. Protective effects of ICA and pICA on the anti-oxidative status of LS174T cells after LPS treatment. Changes in the activity of (a) SOD, (b) GPx, (c) CAT, and (d) T-AOC in LS174T cells treated with LPS (50 μg/ml), ICA (20 μg/ml), and pICA (80 μg/ml) for 12 h. Values are mean ± standard error (n = 6). *P < 0.05 vs. CON group; #P < 0.05 vs. LPS group. T-AOC, Total antioxidant capacity.

Figure 5. Protective effects of ICA and pICA on the inflammatory status of LS174T cells after LPS treatment. Changes in the levels of (a) IL-1β, (b) IL-6, (c) IL-8, and (d) TNF-α in LS174T cells treated with LPS (50 μg/ml), ICA (20 μg/ml) and pICA (80 μg/ml) for 12 h. Values are mean ± standard error (n = 6). *P < 0.05 vs. CON group; #P < 0.05 vs. LPS group.

ICA and pICA suppress the ER stress-mediated apoptosis of LS174T cells

To investigate the potential protective effects of ICA and pICA on LS174T cells apoptotic status, we examined the gene expression and enzyme activity of ER stress-mediated apoptosis-related indicators after incubation with ICA or pICA and LPS. The results showed that gene expression of Bip, XBP1, GRP78, and CHOP in the LPS group was significantly higher than that in the control group, while Bip, XBP1, GRP78 and CHOP gene transcript abundance in the ICA and pICA groups was markedly lower than that in the LPS group (P < 0.05, Figure 6a–d).
Moreover, gene expression and enzyme activity of caspase-3 and 4 in the LPS group was significantly higher than that in the control group, while caspase-3 and 4 transcript abundance and enzyme activity in the ICA and pICA groups was markedly lower than that in the LPS group ($P < 0.05$, Figure 6e–h).

**Discussion**

As a strong stimulator of innate immunity and inflammation, bacterial endotoxin (toxic LPS) is found in almost all outer membranes of Gram-negative bacteria. Mucin is produced and secreted by specific human intestinal cells (goblet cells). Goblet cells are scattered amongst epithelial cells, especially in the respiratory and gastrointestinal tracts, and secrete high molecular mass mucins to form viscous gels that trap microbes and irritants and limit their spread to the epithelium. Previous studies have shown that excessive secretion of mucin is associated with inflammation caused by bacterial infection. Due to their high safety and low cost, natural products and their derivatives are increasingly used in combating human diseases. In this study, we used human intestinal goblet LS174T cells as a model. It was found that LPS induced abnormal proliferation of intestinal goblet cells, excessive mucin secretion, imbalance of oxidative-antioxidative status, expression of pro-inflammatory cytokines, and cell apoptosis. Importantly, ICA and pICA strongly attenuated the adverse biological effects seen in LPS-treated LS174T cells.

Goblet cells can release mucin in response to a variety of stimuli, including irritating gases, nerve activation, ROS, inflammatory mediators, changes in normal microbiota, bacterial infections, and changes in the physical microbial environment. Previous studies have shown that LPS can induce morphological changes in goblet cells and goblet cell proliferation in different types of mucosa. In the current study, we found, by detecting CCK-8 and LDH activity, that LPS caused abnormal proliferation of LS174T cells; meanwhile, ICA and pICA significantly attenuated this effect. Previous *in vivo* and *in vitro* studies have shown that LPS stimulates mucin secretion and up-regulates the mucin gene. Consistently, our data revealed that LPS induces excessive synthesis and secretion of mucin by LS174T cells. Besides, ICA and pICA also restore mucin synthesis and secretion to normal levels. These results indicate that LPS induces pathological proliferation of intestinal goblet cells and leads to overexpression and hypersecretion of mucin, while ICA and pICA effectively alleviate these effects to physiological levels.

Oxidative stress is a very important mechanism among the many potential mechanisms by which toxic substances affect mammalian cells. Under physiological conditions, some intermediate products, such as ROS produced during oxidative phosphorylation, are important signaling molecules that regulate cellular biochemical processes. However, when the body is in oxidative stress, the ROS content is significantly increased, exceeding the body’s ability to effectively scavenge free radicals, leading to DNA oxidative damage and abnormal protein expression, ultimately leading to physical damage.

Previous studies have demonstrated many potential mechanisms by which LPS affect mammalian cells. Among these mechanisms, the accumulation of intermediates of oxidative

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*Figure 6. Protective effects of ICA and pICA on the apoptotic status of LS174T cells after LPS treatment. Changes in the gene expression of (a) Bip, (b) XBP1, (c) GRP78, (d) CHOP, (e) caspase-4, and (f) caspase-3, and enzyme activity of (g) caspase-4 and (h) caspase-3 in LS174T cells treated with LPS (50 μg/ml), ICA (20 μg/ml) and pICA (80 μg/ml) for 12 h. Values are mean ± standard error ($n = 6$). *$P < 0.05$ vs. CON group; **$P < 0.05$ vs. LPS group.*
stress has received great attention.\textsuperscript{41,42} Our data clearly indicate that LPS causes a change in oxidative status, characterized by elevated levels of mitochondrial ROS, intracellular ROS, and intracellular MDA and H$_2$O$_2$ content in LS174T cells. The body has an antioxidant defense system that scavenges free radicals, including enzymes and non-enzymatic antioxidant defense mechanisms. For example, SOD converts O$_2$ to H$_2$O$_2$ by disproportionation, and GPx and CAT convert H$_2$O$_2$ to H$_2$O and O$_2$.\textsuperscript{43} In the present study, the antioxidant capacity of LS174T was inhibited in the LPS group, mainly due to a significant decrease in SOD, GPx, CAT, and T-AOC enzyme activities. At present, the exact mechanism underlying the effects of ICA and pICA is not fully understood, but it may be related to the antioxidant properties of ICA and pICA.\textsuperscript{24}

Regarding antioxidant activity, data from current studies showed that ICA and pICA significantly reduce mitochondrial ROS, intracellular ROS, and intracellular MDA and H$_2$O$_2$ levels in LS174T cells. Meanwhile, the enzyme activities of SOD, GPx, CAT, and T-AOC after ICA and pICA treatment were also significantly increased. These findings indicate that ICA and pICA exert antioxidant activity to modulate the oxidative-antioxidant balance of LPS-treated LS174T cells.

Previous studies have shown that LPS activates the nuclear transcription factor NF-κB by regulating downstream signaling pathways, inducing the expression of pro-inflammatory cytokines, and ultimately leading to local inflammation.\textsuperscript{44} In the present study, we found that ICA and pICA significantly inhibit the expression of LPS-induced pro-inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α). IL-8 is a key mediator associated with inflammation, where it plays a key role in neutrophil recruitment and degranulation.\textsuperscript{45} Moreover, previous studies have shown that LPS up-regulates mucin gene expression in an IL-8-dependent manner.\textsuperscript{46} Such evidence suggests that ICA and pICA alleviate LPS-induced mucin overexpression and hypersecretion by modulating inflammatory responses.

The ER is the starting site for mucin synthesis. Due to the large molecular mass and complex structure of mucin, there is a certain probability of misfolding during biosynthesis.\textsuperscript{11} ER stress is emerging as an important contributor to the pathology observed in IBD.\textsuperscript{12,47} Some key proteins, such as Bip, XBP1, GRP78, and CHOP, are often used to reflect the status of ER stress.\textsuperscript{12} In this study, our data suggest that LPS causes a significant increase in important biological indicators of ER stress (Bip, XBP1, GRP78, and CHOP). Apoptosis refers to the ordered death of cells controlled by genes to maintain homeostasis. The mechanism of apoptosis is very complex, and the apoptotic pathway induced by ER stress is one of the classical mechanisms involved.\textsuperscript{48} Previous study has shown that some members of the caspase family (caspase-3 and -4) can act as liaisons for ER stress and apoptosis, mediating apoptosis in the ER stress pathway.\textsuperscript{49} The present study also found that the expression and activity of key proteins responsible for apoptosis induced by ER stress (caspase-3 and -4) were significantly increased after LPS treatment. Furthermore, ICA and pICA significantly reduced ER stress and apoptosis in LS174T cells induced by LPS. These results indicated that LPS alters mucin biosynthesis by modulating ER stress-induced apoptosis, while ICA and pICA have superior anti-LPS effects.

In conclusion, our results highlight that ICA and pICA are effective natural products that can protect the health of intestinal goblet cells. Depending on their excellent antioxidant, anti-inflammatory, and anti-ER stress capacity, ICA and pICA were effective against LPS-induced overexpression and hypersecretion of mucin. These results suggest that ICA and pICA may be effective adjuvants for the prevention of intestinal mucus barrier dysfunction caused by LPS.

**Declaration of conflicting interests**

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