



Fisetin inhibits the generation of inflammatory mediators in interleukin-1 β -induced human lung epithelial cells by suppressing the NF- κ B and ERK1/2 pathways

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ABSTRACT

Fisetin, a flavone that can be isolated from fruits and vegetables, has anti-tumor and anti-oxidative properties and ameliorates airway hyperresponsiveness in asthmatic mice. This study investigated whether fisetin can suppress the expression of inflammatory mediators and intercellular adhesion molecule 1 (ICAM-1) in A549 human lung epithelial cells that were stimulated with interleukin-1 β (IL-1 β) to induce inflammatory responses. A549 cells were treated with fisetin (3–30 μ M) and then with IL-1 β . Fisetin significantly inhibited COX-2 expression and reduced prostaglandin E₂ production, and it suppressed the levels of IL-8, CCL5, monocyte chemoattractant protein 1, tumor necrosis factor α , and IL-6. Fisetin also significantly attenuated the expression of chemokine and inflammatory cytokine genes and decreased the expression of ICAM-1, which mediates THP-1 monocyte adhesion to inflammatory A549 cells. Fisetin decreased the translocation of nuclear transcription factor kappa-B (NF- κ B) subunit p65 into the nucleus and inhibited the phosphorylation of proteins in the ERK1/2 pathway. Co-treatment of IL-1 β -stimulated A549 cells with ERK1/2 inhibitors plus fisetin reduced ICAM-1 expression. Furthermore, fisetin significantly increased the effects of the protective antioxidant pathway by promoting the expression of nuclear factor erythroid-2-related factor-2 and heme oxygenase 1. Taken together, these data suggest that fisetin has anti-inflammatory effects and that it suppresses the expression of chemokines, inflammatory cytokines, and ICAM-1 by suppressing the NF- κ B and ERK1/2 signaling pathways in IL-1 β -stimulated human lung epithelial A549 cells.

1. Introduction

Asthma is a common disorder that involves airway inflammation. Allergens, as well as bacterial and viral infections, can induce asthma attacks in patients [1]. Acute asthma attacks cause wheezing and coughing and involve mucus hypersecretion that plugs the airways and makes breathing difficult [2]. At the cellular level, a large number of inflammatory cells infiltrate into the airways and lungs of patients with chronic asthma, and these cells release inflammatory cytokines and

chemokines that trigger more serious inflammation and damage cells. This exacerbates the symptoms of asthma and makes treatment difficult [1]. Research shows that activated macrophages in the lungs of asthmatic patients secrete IL-1 β that stimulates airways and lung epithelial cells, which then release more proinflammatory cytokines that cause inflammation and release additional chemokines that attract immune cells and exacerbate inflammatory damage in the lungs [3,4]. Hence, many studies suggest that reducing the inflammatory response and inhibiting the activation of lung epithelial cells can ameliorate asthma

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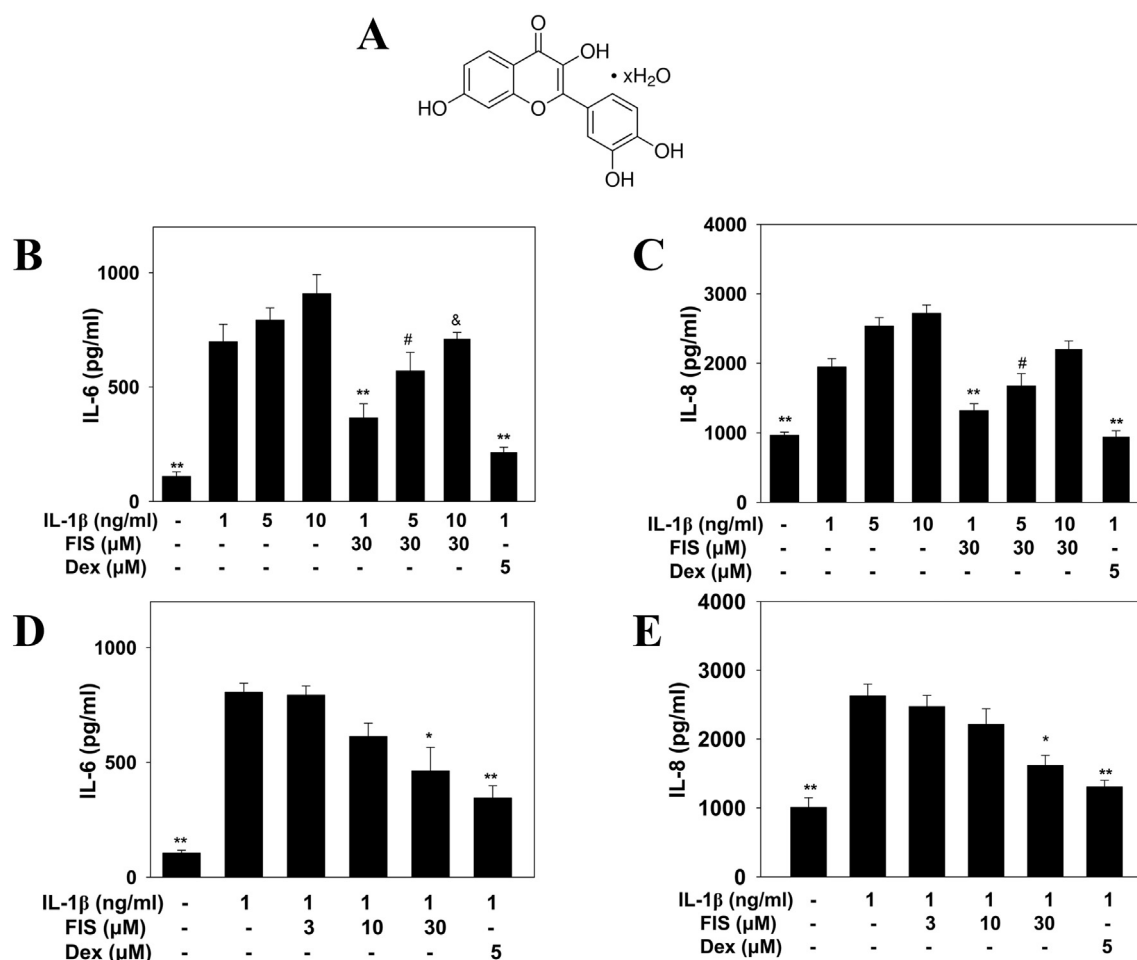


Fig. 1. The effects of fisetin (FIS) on IL-1 β -induced IL-6 and IL-8 production. (A) The chemical structure of fisetin. A549 cells were treated with fisetin or dexamethasone (Dex) for 1 h and then stimulated with the indicated doses of IL-1 β (1–10 ng/ml) for 24 h. IL-6 (B) and IL-8 (C) levels were measured by ELISA. We also treated A549 cells with fisetin plus IL-1 β at the same time and then cultured the cells for 24 h. IL-6 (D) and IL-8 (E) levels were measured by ELISA. The data are presented as means $\pm$ SDs of three independent experiments ($n = 6$); * $p < 0.05$, ** $p < 0.01$ compared with the (1 ng/ml) IL-1 β -treated group. # $p < 0.05$, compared with the (5 ng/ml) IL-1 β -treated group, & $p < 0.05$, compared with the (10 ng/ml) IL-1 β -treated group.

symptoms, reduce cell damage, and improve respiratory function [5,6].

Steroids are anti-inflammatory drugs that are often used to prevent or treat asthma attacks [7]. Previous studies showed that steroids have a remarkable ability to inhibit the inflammatory response of airway epithelial cells in asthmatic patients and to reduce the secretion of pro-inflammatory cytokine into bronchoalveolar lavage fluid [8]. Experiments in which lung epithelial cells were stimulated with IL-1 β and treated with dexamethasone found that dexamethasone inhibited inflammatory cytokine and IL-8 expression and reduced cyclooxygenase (COX)-2 expression by decreasing the activity of nuclear transcription factor kappa-B (NF- κ B) or mitogen-activated protein kinase (MAPK) pathways [9]. Previous studies also found that glucocorticoids reduce the expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), in lung epithelial cells, which increase inflammatory cell infiltration into lung tissue [10]. Unfortunately, treatment with steroids does not improve the asthma symptoms of patients with steroid-resistant asthma [8,11]. One study found that patients with steroid-resistant asthma have more neutrophils and macrophages, but not more eosinophils, infiltrating their airways and lung tissue [11]. Inflammatory neutrophils and macrophages also secrete more IL-1 β , which causes severe pulmonary inflammatory reactions in the lungs of patients with asthma [12]. Patients with steroid-resistant asthma cannot use steroids to prevent or treat their asthma. Therefore, researchers are looking for other drugs or natural compounds that can improve airway inflammation in asthmatic patients.

Fisetin is a flavone that can be isolated from fruits and vegetables [13]. Previous work showed that fisetin reduces the inflammatory response in lipopolysaccharide-treated RAW264.7 cells by suppressing the NF- κ B and JNK pathways [14]. Fisetin also improves acute lung injury in rats by regulating Toll-like receptor 4 and the NF- κ B pathway [15]. In addition, fisetin attenuates airway inflammation in asthmatic mice by suppressing the NF- κ B pathway [16]. However, it is not clear how fisetin regulates the inflammatory response of lung epithelial cells. This study investigated the anti-inflammatory effects of fisetin and its effects on MAPK and NF- κ B pathway regulation in IL-1 β -stimulated A549 human lung epithelial cells.

2. Materials and methods

2.1. Materials

Fig. 1A shows the chemical structures of fisetin ($\geq 98\%$ purity by HPLC; Sigma-Aldrich, St. Louis, MO, USA) (Fig. 1A). A stock solution of 100 mM fisetin was prepared in DMSO. The final DMSO concentration did not exceed 0.1% in the culture medium.

2.2. Cell line and the cell viability assay

A549 human lung epithelial cells were obtained from the Bioresource Collection and Research Center (BCRC, Taiwan) and grown

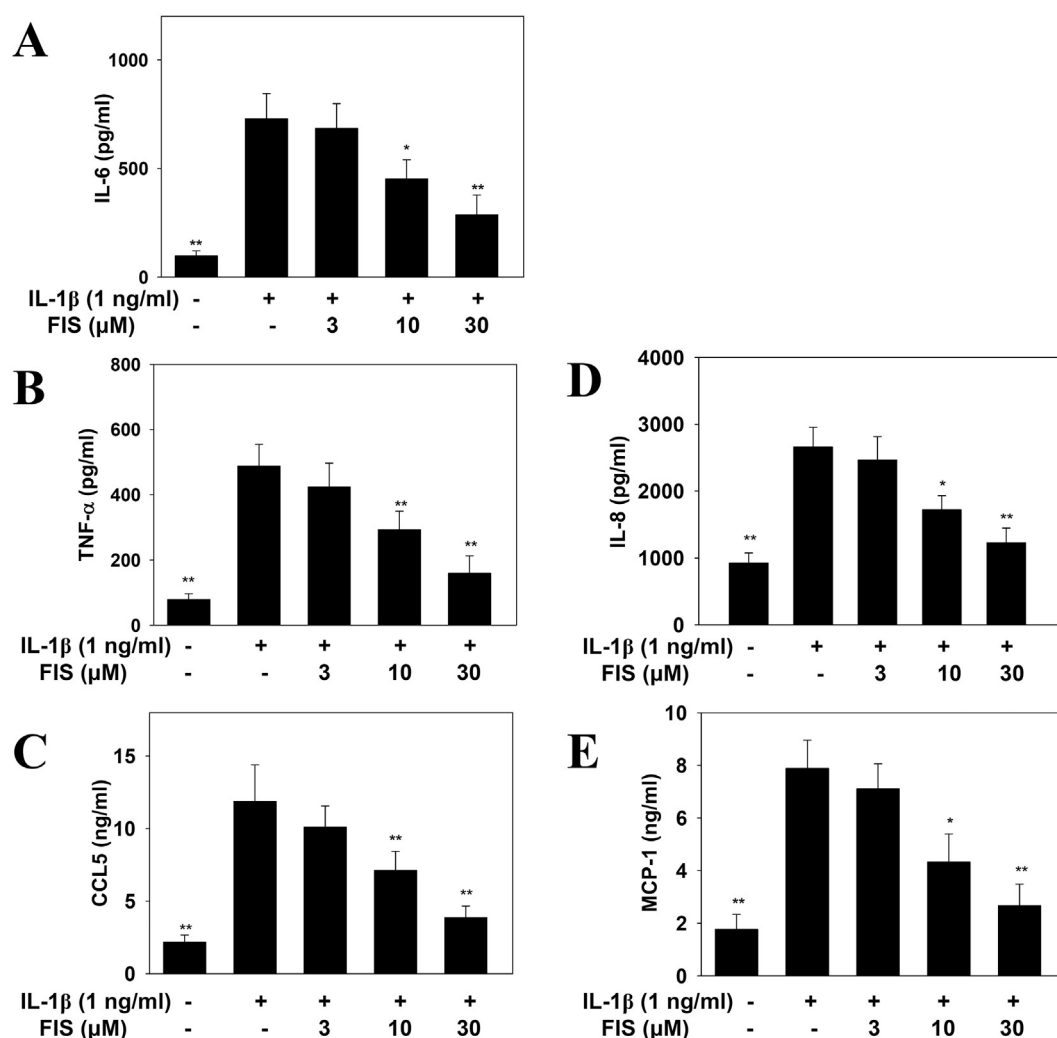


Fig. 2. The effects of fisetin (FIS) on IL-1β-induced production of IL-6, TNF-α, IL-8, CCL5, and MCP-1. A549 cells were treated with fisetin for 1 h and then stimulated with IL-1β for 24 h. The data are presented as means $\pm$ SDs of three independent experiments ($n = 12$); * $p < 0.05$, ** $p < 0.01$ compared with the IL-1β-treated group.

in a humidified 5% CO₂ atmosphere at 37 °C in F12 medium (Invitrogen-Gibco™, Paisley, Scotland) supplemented with 10% FBS and 100 U/ml penicillin and streptomycin. Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) assay, as described previously [17]. In brief, A549 cells were seeded in 96-well culture plates and treated with the indicated concentration of fisetin for 24 h. After one day of treatment with fisetin, MTT solution (5 mg/ml) was added and the cells were incubated at 37 °C for 4 h. Isopropanol was used to dissolve the formazan crystals, and a microplate reader (Multiskan FC, Thermo Fisher Scientific, Waltham, MA, USA) was used to record absorbance at 570 nm.

2.3. RNA extraction and real-time PCR

Total RNA was extracted from A549 cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription was performed on the RNA samples using a cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA) as described previously [18]. Gene expression was quantified by real-time PCR with SYBR Green using Real-Time PCR Supermixes Kits (Bio-Rad Laboratories) and an iCycler Real-Time PCR detection system (Bio-Rad Laboratories).

2.4. ELISAs for detecting cytokine and chemokine expression

A549 cells were pretreated with or without fisetin for 1 h and then stimulated with IL-1β (1 ng/ml) for 24 h. The cells were then pelleted by centrifugation, and the supernatant was assayed to detect cytokine, chemokine, and prostaglandin E2 (PGE₂) levels using specific ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The absorbance in each well was measured using a microplate reader (Multiskan FC) at 450 nm.

2.5. Preparation of total and nuclear proteins

A549 cells (5×10^5 cells/ml) were pretreated with or without fisetin in 6-well plates for 1 h and then stimulated with IL-1β (1 ng/ml) for 30 min to detect protein phosphorylation, for 1 h to detect NF-κB translocation into the nucleus, or for 24 h to evaluate total protein expression. Cellular proteins were extracted using protein lysis buffer, and nuclear proteins were isolated using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Fisher Scientific, Rockford, IL, USA) according to the manufacturer's instructions.

2.6. Western immunoblot analysis

Equal amounts of protein were separated on SDS polyacrylamide

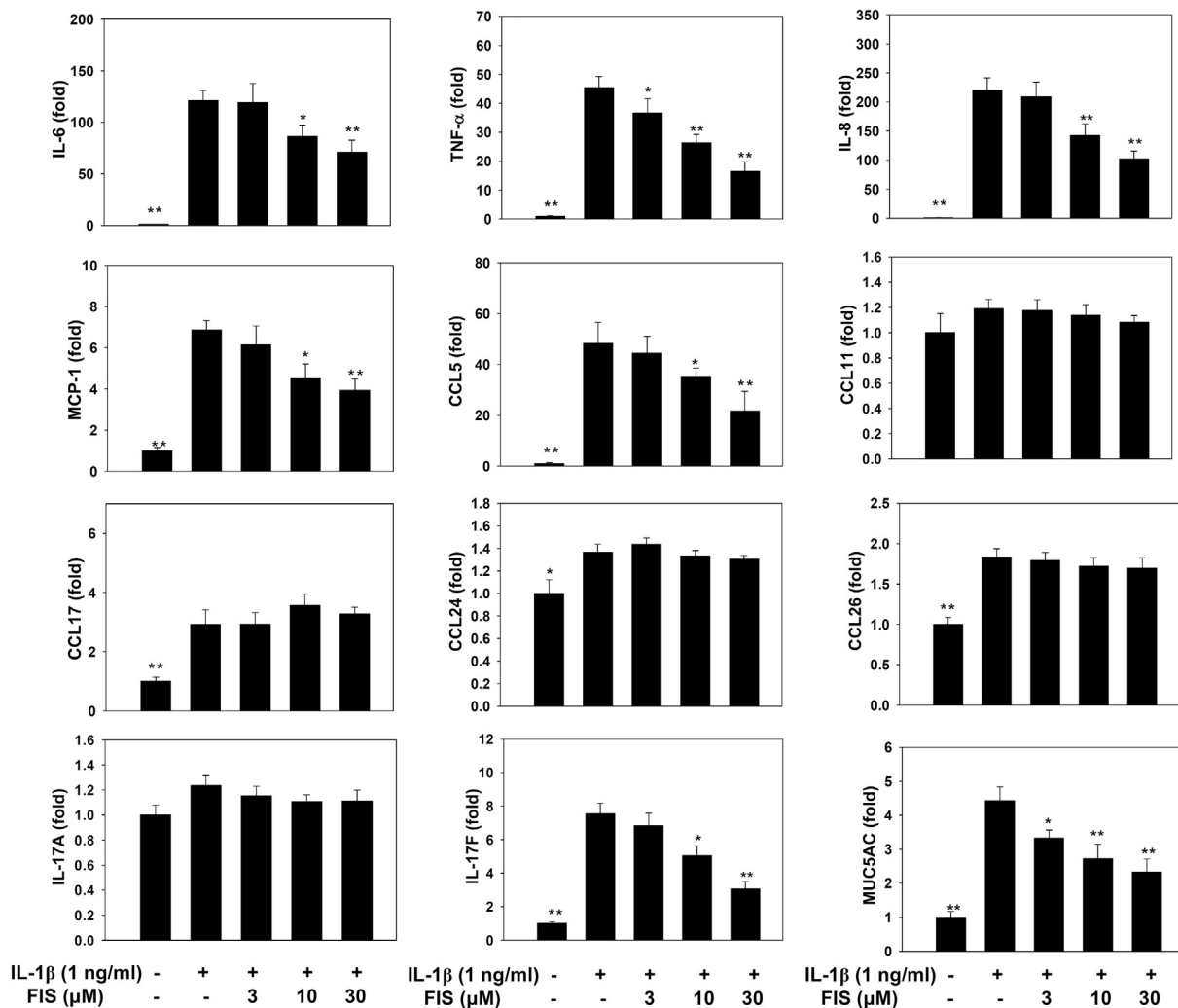


Fig. 3. The effects of fisetin (FIS) on IL-1 β -induced gene expression. A549 cells were treated with fisetin for 1 h and then stimulated with IL-1 β for 4 h. The fold-changes in gene expression levels were determined using real-time RT-PCR with β -actin as an internal control. The data are presented as means $\pm$ SDs of three independent experiments (n = 3); *p < 0.05, **p < 0.01 compared with the IL-1 β -treated group.

gels and transferred to polyvinylidene fluoride (PVDF) membranes. The PVDF membranes were incubated overnight at 4 °C with specific primary antibodies and then washed and incubated with secondary antibodies at room temperature for 1 h. The secondary antibodies were detected with ECL reagent, and the bands were quantified using the BioSpectrum 600 system (UVP, Upland, CA, USA). The specific primary antibodies included antibodies against the following proteins: ICAM-1 (SAB4501486) and β -actin (A1978) from Sigma; ERK1/2 (137F5, 4695), phosphorylated-ERK 1/2 (Thr202/Tyr204, 9101), p38 (9212), phosphorylated-p38 (Thr180/Tyr182, 9216), JNK (9252), and phosphorylated-JNK (Thr183/Tyr185, 4668) from Cell Signaling Technology, Inc. (Danvers, MA, USA); and COX-2 (sc-19999), heme oxygenase (HO-1) (sc-136256), I κ B- α (sc-203), phosphorylated-I κ B- α (sc-8404), lamin B1 (sc-6216), nuclear factor erythroid 2-related factor-2 (Nrf2) (sc-81342), and p65 (sc-372) from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The ERK inhibitor PD98059 (Enzo Life Sciences, Inc., Farmingdale, NY, USA) was added to cultured cells that were pretreated with fisetin to detect ICAM-1 protein expression.

2.7. Transient transfection and reporter gene assays

NF- κ B and activator protein 1 (AP-1) activity was evaluated using cells transfected with the pNF κ B-Luc or the pAP1-Luc plasmid (Stratagene, CA, USA), respectively, as described previously [17]. A549

cells were transfected with pNF κ B-Luc or pAP1-Luc plasmid using Lipofectamine 2000 (Life Technologies) for 24 h. After transfection, the cells were treated with fisetin for 1 h and stimulated with IL-1 β for 4 h. Luciferase reporter gene expression was detected using the luciferase reporter assay kit (GeneDireX, Brussels, Belgium) and measured using a Multi-Mode Microplate Reader (BioTek Synergy HT, Bedfordshire, United Kingdom).

2.8. Cell-cell adhesion assay

A549 cells (5×10^5 cells/ml) were seeded in 6-well plates and treated with fisetin for 1 h. The cells were then stimulated with IL-1 β (1 ng/ml) for 24 h. Next, human monocytic THP-1 cells were treated with calcein AM (Sigma) and co-cultured with the A549 cells for 1 h, as described previously [19]. Adhered THP-1 cells were observed using fluorescence microscopy (Olympus, Tokyo, Japan).

2.9. Statistical analysis

All experimental data were expressed as the mean $\pm$ SD of at least three independent experiments. Significant differences between groups were analyzed by one-way analysis of variance, and post-hoc analysis was performed with Dunnett's test using the SPSS statistical software package version 19.0 (SPSS Inc., Chicago, USA). Differences were

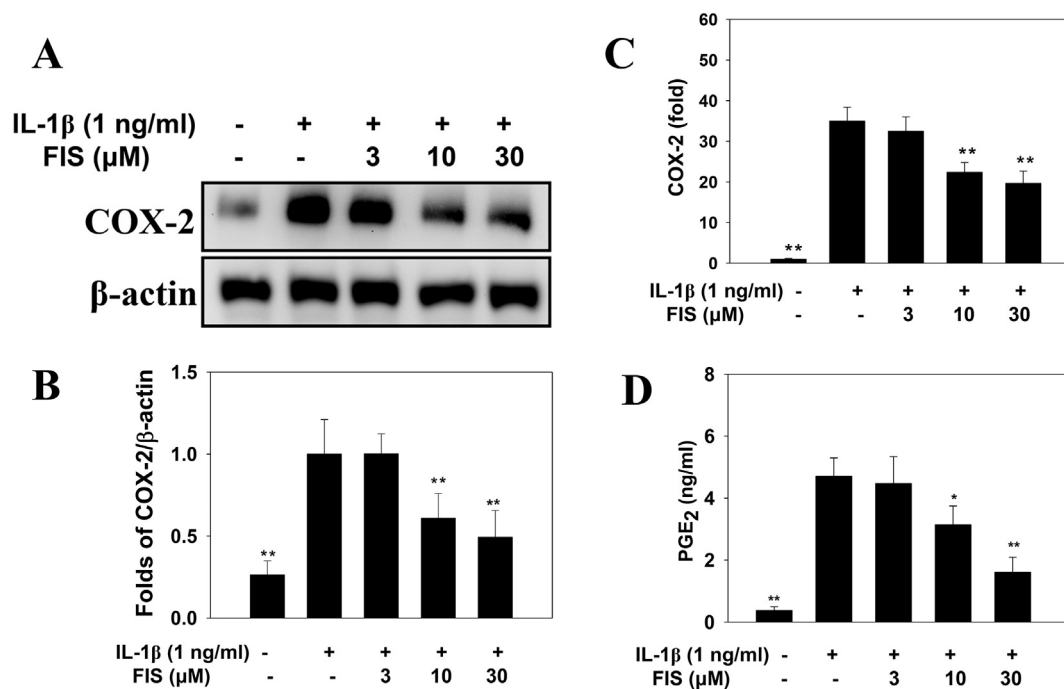


Fig. 4. (A) The effects of fisetin on IL-1β-induced expression of the COX-2 protein, and (B) the fold-change in the expression levels of the COX-2 protein were measured relative to the expression of β-actin. (C) The fold-changes in the expression of the COX-2 gene were measured using real-time PCR, with β-actin as an internal control. (D) PGE₂ levels were detected by ELISA. The data are presented as means ± SDs of three independent experiments (n = 3); *p < 0.05, **p < 0.01 compared with the IL-1β-treated group.

considered statistically significant at $P < 0.05$.

3. Results

3.1. Fisetin inhibits proinflammatory cytokine and chemokine production

The cytotoxicity of fisetin in A549 cells was determined using the MTT assay. Fisetin did not show significant cytotoxic effects at doses ≤ 50 μM, and subsequent experiments used fisetin at 3–30 μM concentrations. A549 cells were pretreated with fisetin or dexamethasone for 1 h and then stimulated with 1–10 ng/ml of IL-1β for 24 h. Of the tested concentrations, 30 μM fisetin or 5 μM dexamethasone, plus 1 ng/ml IL-1β, reduced IL-6 and IL-8 production the most (Fig. 1B, C). Hence, we subsequently used 1 ng/ml IL-1β to stimulate an inflammatory response in A549 cells. We also treated A549 cells with fisetin plus IL-1β at the same time and then cultured the cells for 24 h. Only 30 μM fisetin significantly reduced the levels of IL-6 and IL-8 in IL-1β-stimulated A549 cells in these conditions (Fig. 1D, E). Next, A549 cells were treated with various concentrations of fisetin and stimulated with IL-1β for 24 h. Fisetin significantly decreased the levels of IL-6 and tumor necrosis factor alpha (TNF-α) compared with IL-1β-stimulated cells (Fig. 2A, B). Moreover, fisetin significantly decreased the levels of CCL5, monocyte chemoattractant protein 1 (MCP-1), and IL-8 in a concentration-dependent manner (Fig. 2C–E). We investigated the expression of chemokine and inflammatory cytokine genes using real-time PCR and found that fisetin reduced the expression of IL-6, TNF-α, IL-8, CCL5, MCP-1, IL-17F, and MUC5AC (Fig. 3). Notably, fisetin did not significantly regulate CCL11, CCL17, CCL24, CCL26, or IL-17A gene expression in A549 cells.

3.2. Fisetin reduces COX-2 expression in IL-1β-stimulated A549 cells

A549 cells that were treated with fisetin and then stimulated with IL-1β showed significantly decreased COX-2 protein and gene expression compared with non-fisetin-treated IL-1β-stimulated cells (Fig. 4A–C). Fisetin also significantly suppressed PGE₂ production in a

concentration-dependent manner (Fig. 4D).

3.3. Fisetin reduces ICAM-1 expression in A549 cells

ELISAs and Western blot protein assays showed that fisetin significantly inhibited ICAM-1 production (Fig. 5A, B) and decreased the release of soluble ICAM-1 into the culture medium (Fig. 5C). Fisetin also reduced ICAM-1 gene expression compared with non-fisetin-treated IL-1β-stimulated A549 cells (Fig. 5D). Furthermore, fisetin reduced the adhesion of monocytic THP-1 cells to IL-1β-stimulated A549 cells (Fig. 5E, F).

3.4. Fisetin suppresses NF-κB and phosphorylation in ERK1/2 pathway proteins in A549 cells

Fisetin suppressed IκB-α degradation and attenuated IκB-α phosphorylation compared with non-fisetin-treated IL-1β-stimulated A549 cells. In unstimulated A549 cells, the NF-κB p65 subunit was found mostly in the cytoplasm. However, IL-1β-stimulation of A549 cells increased p65 localization to the nucleus. Interestingly, pretreatment with fisetin maintained the localization of the p65 subunit in the cytoplasm and blocked p65 translocation into the nucleus compared with non-fisetin-treated IL-1β-stimulated A549 cells (Fig. 6A–C).

The phosphorylation of ERK1/2, but not p38 or JNK, was reduced in fisetin-treated IL-1β-activated A549 cells compared to control cells that were not treated with fisetin (Fig. 6A, D, E). Hence, we used ERK1/2 inhibitors to investigate how fisetin inhibits ICAM-1 expression. Interestingly, fisetin enhanced the ability of ERK1/2 inhibitors to suppress ICAM-1 expression (Fig. 7A, B). Moreover, the results of the NF-κB and AP-1 promoter assays demonstrated that fisetin significantly decreased luciferase activity compared with non-fisetin-treated IL-1β-stimulated A549 cells (Fig. 6F, G).

3.5. Fisetin enhances HO-1 and Nrf2 expression

Our results showed that 30 μM fisetin did not increase HO-1 and

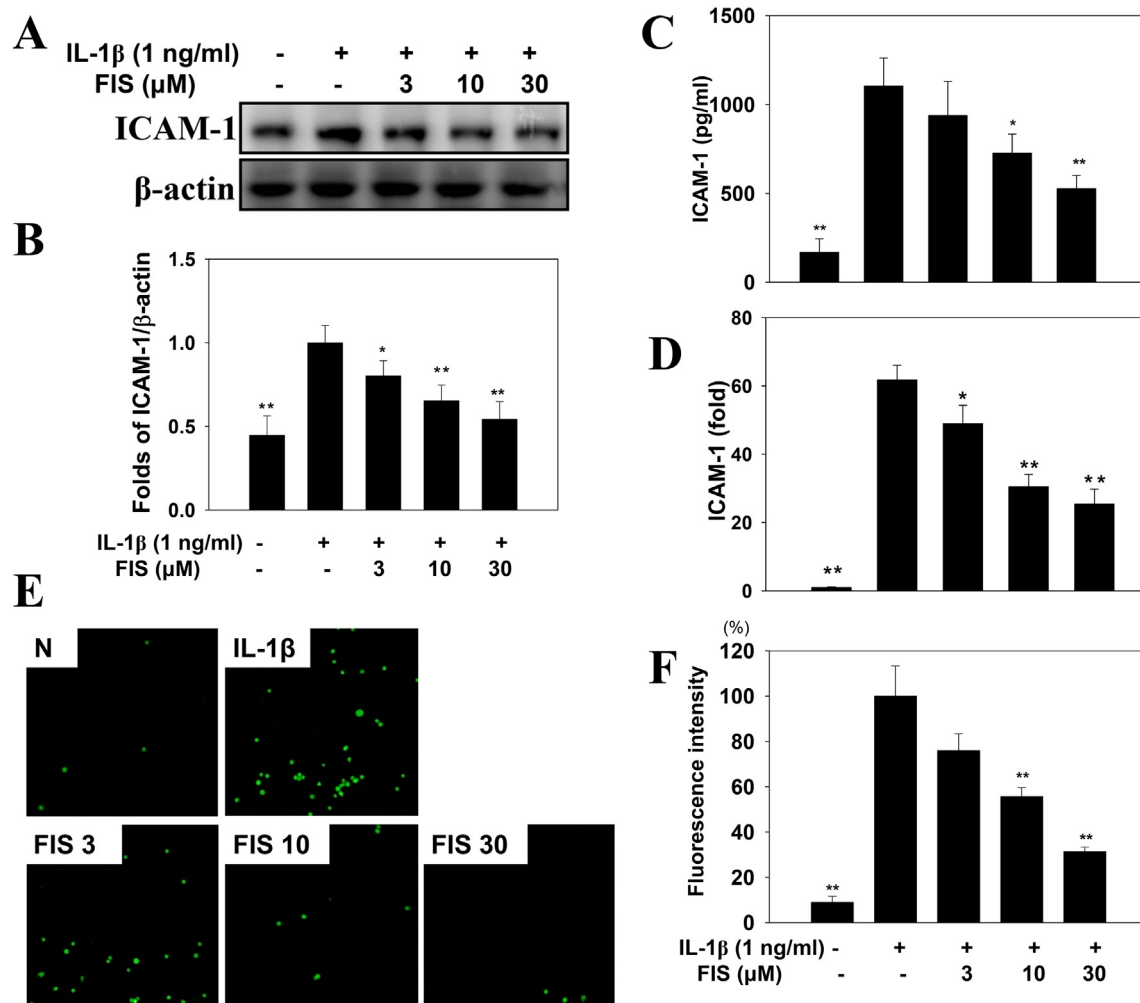


Fig. 5. The effects of fisetin (FIS) on IL-1 β -induced production of ICAM-1. (A) ICAM-1 protein expression determined by Western blot analysis. (B) The fold-changes in ICAM-1 protein expression levels were measured relative to β -actin expression. (C) ICAM-1 levels in the culture medium were detected by ELISA. (D) The fold-changes in ICAM-1 gene expression levels were determined using real-time RT-PCR with β -actin as an internal control. (E) THP-1 cells were labeled with calcein AM and co-cultured with A549 cells then observed using fluorescence microscopy. (F) The fluorescence intensity reflects THP-1 cell adhesion to A549 cells. The data are presented as means $\pm$ SDs of three independent experiments ($n = 3$); * $p < 0.05$, ** $p < 0.01$ compared with the IL-1 β -treated group.

Nrf2 production in unstimulated A549 cells. IL-1 β -stimulated A549 cells showed increased HO-1 and Nrf2 expression, and pretreatment of these cells with fisetin significantly increased the effects of IL-1 β on HO-1 and Nrf2 production in A549 cells (Fig. 7C–F).

4. Discussion

IL-1 β is an inducible pro-inflammatory cytokine that is not secreted by many healthy tissues or cells [20]. Studies have found that pulmonary bacterial infections in the lungs cause respiratory inflammation or bacteria-induced asthma attacks, and higher levels of IL-1 β are detected in the bronchoalveolar lavage fluid of patients with asthma [21]. Notably, IL-1 β gene expression is significantly higher in the lungs of asthmatic children than in the lungs of healthy children [22]. IL-1 β is secreted mainly by activated macrophages and neutrophils. This chemokine stimulates lung epithelial cells to release additional inflammatory cytokines and chemokines, thereby triggering inflammatory reactions in lung epithelial cells and attracting more inflammatory cells that infiltrate into the lung and cause serious inflammation and cell damage [17,23]. Accordingly, inhibiting IL-1 β -induced inflammation in lung epithelial cells should improve the lung function of patients with asthma.

Fisetin is found in some vegetables and fruits [13]. It has important

anti-inflammatory effects in LPS-induced murine macrophages and suppresses proinflammatory cytokines in IL-1 β -induced human chondrocytes [14,24]. Fisetin inhibits the proliferation of laryngeal carcinoma by regulating the AKT, NF- κ B, and ERK1/2 pathways [25]. In this study, fisetin decreased the cellular levels of IL-6, IL-8, TNF- α , CCL5, and MCP-1 and downregulated COX-2 expression in IL-1 β -stimulated A549 cells. Fisetin also reduced the expression of ICAM-1, which is involved in monocyte adhesion, by suppressing NF- κ B and the phosphorylation of proteins in the ERK1/2 pathway. We also found that fisetin regulated the antioxidant pathway by increasing the expression of Nrf2 and HO-1. These data suggest that fisetin has an anti-inflammatory effect on IL-1 β -stimulated A549 cells.

Nonsteroidal anti-inflammatory drugs and COX-2 inhibitors are often used to treat inflammatory diseases [26]. Etoricoxib, a selective COX-2 inhibitor, effectively improves lung function and airway responsiveness and reduces sputum eosinophils in patients with asthma by decreasing the levels of PGE₂, which damages lung tissue [27]. However, long-term oral administration of COX-2 inhibitors can lead to drug tolerance or can even induce an allergic response [28]. Thus, researchers continue to seek drugs or natural products to treat pulmonary inflammatory disease. This study found that fisetin significantly reduced COX-2 and PGE₂ expression in IL-1 β -stimulated human lung epithelial cells. Fisetin may thus have an important protective effect as

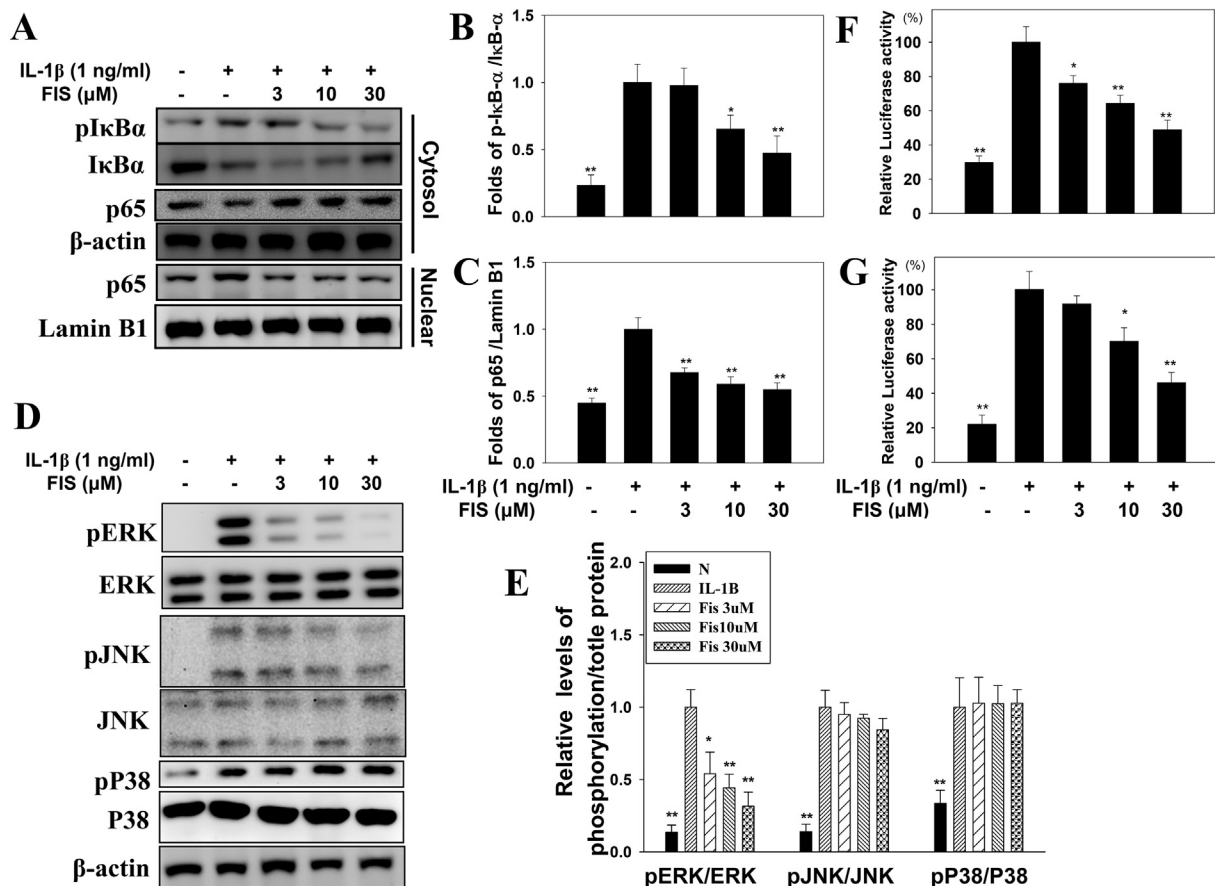


Fig. 6. The inhibitory effects of fisetin (FIS) on the nuclear translocation of NF- κ B and MAPK in A549 cells. (A) The effects of fisetin on IL-1 β -induced phosphorylation of I κ B- α and on the nuclear translocation of NF- κ B. β -actin and lamin B1 were used as internal controls for the cytosol and nucleus, respectively. (B, C) The fold-changes in the densitometry values were analyzed and compared with results in the IL-1 β -treated group. (D) The effects of fisetin on IL-1 β -induced phosphorylation of MAPK. (E) The fold-changes in the densitometry values were analyzed and compared with results in the IL-1 β -treated group. A promoter assay was used to evaluate (F) NF- κ B and (G) AP-1 promoter activity. The data are presented as means $\pm$ SDs of three independent experiments ($n = 3$); * $p < 0.05$, ** $p < 0.01$ compared with the IL-1 β -treated group.

a COX-2 inhibitor that could improve the inflammatory response in lungs.

Neutrophils are important in the development of steroid-resistant asthma [29]. Lung epithelial cells from patients with steroid-resistant asthma release IL-6 and IL-8, thereby attracting neutrophils into the lungs and causing more serious inflammatory reactions [30]. IL-6 is a major inflammatory cytokine, and high levels IL-6 are found in lung tissue and in the bronchoalveolar lavage fluid of asthma patients [31]. Indeed, IL-6 increases C-reactive protein expression and complement C3 production and promotes fibrinogen expression, thereby attenuating lung function in the acute phase response of patients with asthma [32].

MCP-1 is a chemotactic monocyte protein that acts as a potent chemoattractant for macrophage infiltration of the lungs of asthma patients via CCR2 [33]. Macrophages and neutrophils release more IL-1 β , which stimulates lung epithelial cells to secrete more TNF- α , IL-6, MCP-1, and CCL5, which activates inflammatory signaling [34,35]. Our study found that the levels of CCL5, MCP-1 IL-6, and IL-8 were reduced after A549 cells were treated with fisetin, indicating that fisetin might attenuate the inflammatory response in lung epithelial cells and improve lung disease symptoms in asthma patients. A previous study found that IL-4 stimulated A549 cells could induce eotaxin (CCL26) production [36]. Interestingly, fisetin did not regulate eotaxin expression (CCL11, CCL24, or CCL26) in IL-1 β -stimulated A549 cells. We thought that IL-1 β -stimulated lung epithelial cells attracted neutrophils and macrophages, but not eosinophils, into the lungs. Therefore, it seems likely that fisetin would not regulate eosinophil infiltration into

the lungs via IL-1 β -stimulated lung epithelial cells. Fisetin did not regulate CCL17 gene expression compared to control cells. Notably, CCL17 increases Th2 cell infiltration and activation in asthmatic mice [37]. Hence, it seemed likely that fisetin would not reduce CCL17 expression or Th2 cell infiltration into the lungs of patients with asthma.

Lung epithelial cells are part of innate immunity and can induce an inflammatory response. This response produces more inflammatory mediators, which activate the immune system and damage lung tissue [38,39]. Lung epithelial cells also express the ICAM-1 cell adhesion molecule, which plays a role in neutrophil and monocyte adhesion and infiltration into lung tissue in steroid-resistant asthma [40]. In the current work, fisetin decreased ICAM-1 protein and gene expression and attenuated monocyte THP-1 cell adhesion to inflammatory A549 cells compared with IL-1 β -stimulated A549 cells. Excess ICAM-1 is cleaved to form soluble ICAM and released into the supernatant [41]. Some studies found that IL-1 β , TNF- α , IL-6, and angiotensin II stimulate epithelial cells and endothelial cells to secrete soluble ICAM-1 [42]. However, the function of soluble ICAM-1 remains unclear. Interestingly, synovial tissue in rheumatoid arthritis and lung tissue in asthma both express more ICAM-1 than the same tissues in healthy subjects [41,43]. Furthermore, we found that fisetin decreased Muc5AC gene expression; this implies that fisetin could reduce excessive mucus production, which causes breathing difficulty.

HO-1, an antioxidant enzyme, is a potential treatment for reducing oxidative stress and inflammation in the lungs of patients with asthma [44], and Nrf2 is a transcription factor that regulates redox reactions

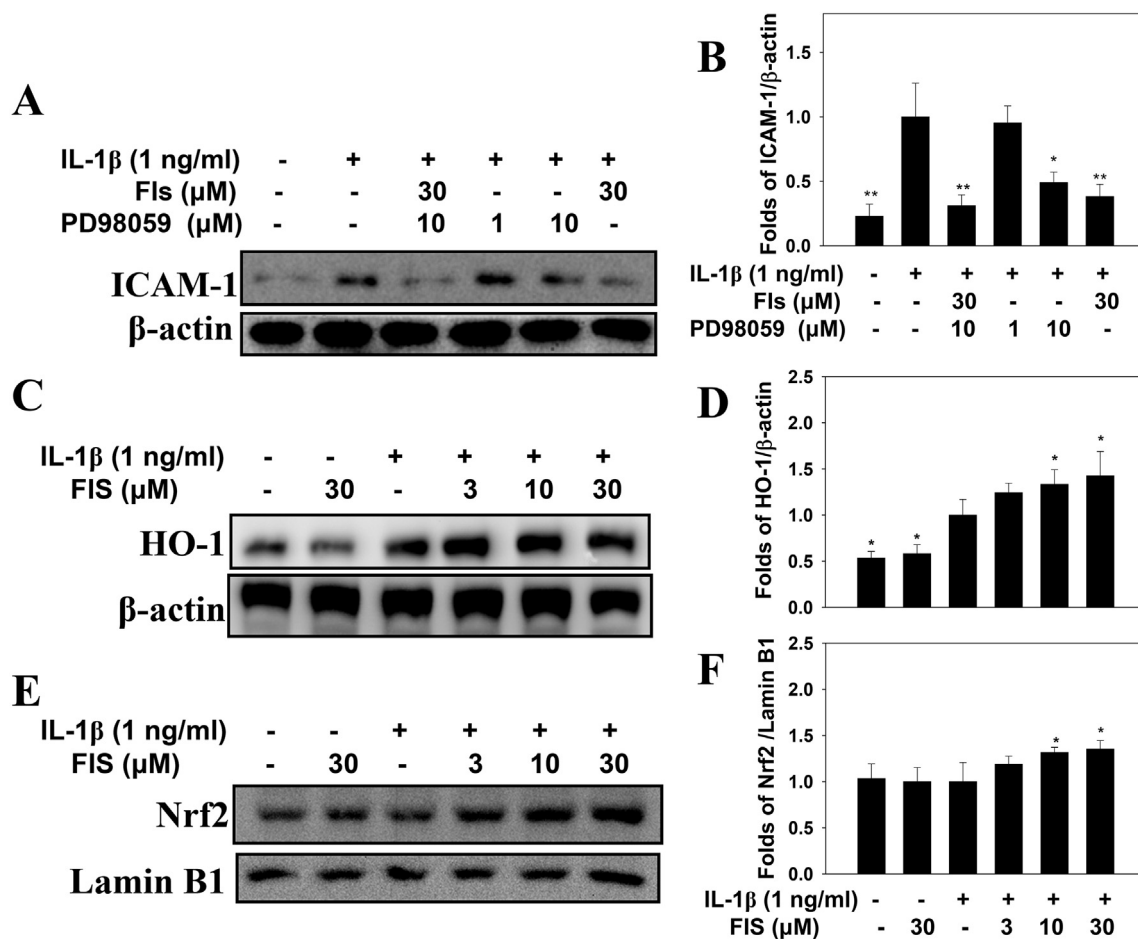


Fig. 7. (A) The inhibitory effects of ERK1/2 inhibitors or fisetin on IL-1β-induced ICAM-1 protein expression in A549 cells. A549 cells were treated with ERK1/2 inhibitors (1 or 10 μM PD98059) with or without 30 μM fisetin (FIS) for 1 h, followed by IL-1β stimulation for 24 h. ICAM-1 protein levels were detected with β-actin as an internal control. (B) The fold-changes in the densitometry values were analyzed and compared with the IL-1β-treated group. (C) The effects of fisetin on IL-1β-induced HO-1 expression and (D) the fold-changes in the densitometry values of HO-1 were analyzed and compared with the IL-1β-treated group. (E) The effects of fisetin on IL-1β-induced Nrf2 expression and (F) the fold-changes in the densitometry values of Nrf2 were analyzed and compared with the IL-1β-treated group. The data are presented as means ± SDs of three independent experiments (n = 3); *p < 0.05, **p < 0.01 compared with the IL-1β-treated group.

[45]. In the nucleus, activated Nrf2 binds to the antioxidant response element to activate HO-1 expression and thereby reduce lung tissue oxidative damage in mice with asthma [44]. This suggests that fisetin promoted HO-1 expression which in turn modulated oxidative stress in IL-1β-stimulated A549 cells.

In unstimulated A549 cells, NF-κB was inhibited by IκB and stayed in the cytoplasm. When A549 cells were stimulated with IL-1β, the inflammatory signal caused IκB phosphorylation and degradation, and NF-κB translocated from the cytoplasm into the nucleus and activated inflammatory gene expression [46,47]. Fisetin significantly reduced NF-κB translocation due to attenuated expression of inflammatory mediators. We found that fisetin significantly decreased the phosphorylation of ERK1/2, but not p38 and JNK, in IL-1β-stimulated A549 cells. ERK1/2 signaling can directly affect the AP-1 transcription factor and increase activation of inflammatory genes [48]. To confirm that fisetin reduced ERK1/2 activity, IL-1β-stimulated A549 cells were treated with ERK1/2 inhibitors plus fisetin; fisetin treatment alone significantly reduced ICAM-1 expression more than cells treated with both fisetin and ERK1/2 inhibitors.

In conclusion, based on these data, we suggest that fisetin reduces the levels of inflammatory chemokines and cytokines and decreases ICAM-1 expression by suppressing the activation of the NF-κB and ERK1/2 pathways in IL-1β-stimulated human lung epithelial cells. Fisetin thus represents a potential therapeutic treatment for the allergic inflammatory response in asthma and merits further investigation.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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