

Dioscin exhibits protective effects on in vivo and in vitro asthma models via suppressing TGF- β 1/Smad2/3 and AKT pathways

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Abstract

Dioscin is a natural product that possesses protective effects on multiple chronic injuries, but its effects on asthma are not fully understood. Herein, we evaluated its effects on asthmatic mice established by ovalbumin (OVA) sensitization and challenges and further explored the mechanism. Inflammatory cells in bronchoalveolar lavage fluids (BALFs) were analyzed using Diff-Quik staining. OVA-specific immunoglobulin E (IgE)/IgG1 in serum and inflammatory cytokines (interleukin 4[IL-4], IL-5, IL-13, and tumor necrosis factor- α) in BALFs and lung tissues were measured using Enzyme-Linked Immunosorbent Assay Kits. Hematoxylin and eosin, periodic acid-Schiff, and immunohistochemistry staining showed histopathological changes in lung tissues. Epithelial-mesenchymal transition (EMT) in human bronchial epithelial (16HBE) cells was assessed by immunofluorescence staining. Hydroxyproline content was used to evaluate collagen deposition. Polymerase chain reaction and Western blot were performed to measure messenger RNA and protein expression. We found that dioscin treatment (particularly at the dose of 80 mg/kg) significantly inhibited pulmonary inflammation in asthmatic mice, as evidenced by the decreased serum OVA-specific IgE/IgG1 and the reduced inflammatory cells and cytokines in BALFs and lung tissues. Moreover, dioscin effectively ameliorated the goblet cell hyperplasia, mucus hypersecretion, collagen deposition, and smooth muscle hyperplasia in the airways of asthmatic mice. Mechanistically, dioscin restrained the activated TGF- β 1/Smad2/3 and protein kinase B (AKT) signal pathways in lung tissues and potently reversed the TGF- β 1-induced EMT and phosphorylation of Smad2/3 and AKT in 16HBE cells. Collectively, dioscin displayed protective effects on OVA-induced asthmatic mice via adjusting TGF- β 1/Smad2/3 and AKT signal pathways, supporting the fact that dioscin could be a candidate for chronic asthma prevention in the future.

KEYWORDS

airway inflammation, airway remodeling, asthma, dioscin, TGF- β 1/Smad2/3/AKT pathway

1 | INTRODUCTION

Asthma is a chronic inflammatory disease of the airways and has become a global health concern due to its increasing morbidity and mortality.^[1,2] At present, treatments with inhaled medications are the common therapeutic option for asthma.^[3] There are two main types of medications: bronchodilators (such as salbutamol) that open the air passages and relieve symptoms, and steroids (such as dexamethasone [DEX]) that reduce inflammation in the air passages. However, long-term usage of inhaled corticosteroids brings the risk of a series of adverse effects, involving thrush, cataract development, and a decrease in bone mineral density.^[4,5] Hence, exploring a novel strategy for asthma with minimized side effects is important for enabling people with this chronic disease to enjoy a normal and active life.

Over the centuries, phytochemicals have attracted lots of attention because they have less toxic side effects. As one such phytochemical, dioscin (a steroidal saponin) has shown potent protective effects on necrosis, lipid peroxidation, and mitochondrial dysfunction.^[6–9] For instance, dioscin has significant effects on cisplatin-induced renal injury^[8] and doxorubicin-induced cardiotoxicity.^[10] Previous studies also revealed that dioscin could alleviate crystalline silica-induced pulmonary inflammation and fibrosis^[7] and protect against lipopolysaccharide (LPS)-induced acute lung injury through adjusting oxidative stress and inflammation.^[9] Interestingly, a study based on ovalbumin (OVA)-sensitized and challenged mice model of asthma has reported that diosgenin, a hydrolyzed material of dioscin, functions by decreasing the secretion of inflammatory factors, including tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6, thereby preventing chronic inflammation.^[11] However, the mechanism of the effects of dioscin on asthma remains unknown. In addition, dioscin potently inhibits the transforming growth factor (TGF)- β 1-induced epithelial-mesenchymal transition (EMT) in HepG2 cells,^[12] reduces hepatic stellate cell activation and collagen accumulation, and downregulates the expression of TGF- β 1, α -smooth muscle actin (α -SMA), collagen, and vimentin.^[13,14] Importantly, EMT induced by TGF- β 1 may participate in chronic airway changes and remodeling in asthma.^[15] Besides this, TGF- β 1 could elicit proliferation and hypertrophy of smooth muscle cells, thus leading to dysfunctions of the smooth muscle of the airways in asthma.^[16,17] Hence, we speculated that dioscin has a protective effect on asthma and established an *in vitro* asthma model using TGF- β 1.

Asthma results from chronic inflammation of the airway conduction area (often in the bronchi and bronchioles), which is characterized by wheezing episodes, coughing, chest tightness, and breathlessness leading by airway hyperresponsiveness (AHR).^[18,19] These episodes are usually associated with elevated allergen-specific type 2 T helper (Th2) cytokines, including IL-4, IL-5, and IL-13.^[20] Th2 cytokines are involved in typical changes in the airways, such as increased eosinophils, thickened lamina reticularis, and production of immunoglobulin E (IgE) and mucus.^[21,22] Chronically, as the mucous gland number increases, the airway smooth muscle may gradually

increase in size, and finally, airway remodeling,^[23] in which the components of the immune system are also involved, including immune cells, cytokines, and chemokines.^[24,25] This evidence highlights the importance of suppressing the inflammatory response to control asthma.

Protein kinase B (AKT) is a serine/threonine kinase that phosphorylates and regulates vital downstream effectors, which control numerous cellular processes including EMT.^[26] Pharmacological blockade of AKT signaling significantly inhibits the EMT process through downregulating EMT transcription factors like Snail and Twist.^[27,28] And its cross-talk with TGF- β signaling also participates in inflammation and airway remodeling in asthma.^[29,30] Additionally, the activation of phosphoinositide 3-kinase (PI3K)/AKT signaling is observed in asthmatic mice, while treatment with AKT signaling inhibitor significantly suppresses inflammation and EMT in the lungs of asthmatic mice as well as suppresses the TGF- β induced EMT and migration in human bronchial epithelial (16HBE) cells.^[31,32] Furthermore, dioscin has been reported to inhibit the inflammation and AKT signaling activation to alleviate LPS-induced acute lung injury.^[9] However, whether dioscin regulates AKT signaling in asthma remains unknown.

Hence, the present study aimed to explore the effects of dioscin in an OVA-challenged asthmatic mice model by analyzing inflammatory cells, cytokine levels, and airway remodeling, and DEX served as the positive control. Additionally, we further explored the mechanisms associated with the effects of dioscin *in vivo* and *in vitro*, specifically the levels of TGF- β 1, Smad2/3, and AKT.

2 | MATERIALS AND METHODS

2.1 | Animals

Female BALB/c mice (6- to 8-week-old, Changsheng Biotechnology) were housed in the standard environment (22 $\pm$ 1°C, relative humidity of 45%–55%, and dark/light cycle of 12/12 h), with food and water freely available. All experimental animal protocols were approved by the Henan Institute for Food and Drug Control.

2.2 | Mouse model of OVA-induced asthma

BALB/c mice were randomly divided into control, asthma, asthma + dioscin (40 mg/kg), asthma + dioscin (80 mg/kg), and asthma + DEX (3 mg/kg; Aladdin) groups. As previously described,^[23] OVA (Aladdin) sensitization and airway challenges are schematically shown in Figure 1. Specifically, mice were sensitized intraperitoneally with 20 μ g OVA emulsified in 2 mg Al(OH)₃ in 200 μ l normal saline on Day 0 and Day 7 and were exposed to 2% OVA dissolved in normal saline (M/V) using an ultrasonic nebulizer for 30 min from Day 14.^[23] OVA challenges were conducted 3 consecutive days a week for 30 min each time. Mice in the control group received the same procedures with normal saline. From Day 14, mice in asthma + dioscin groups

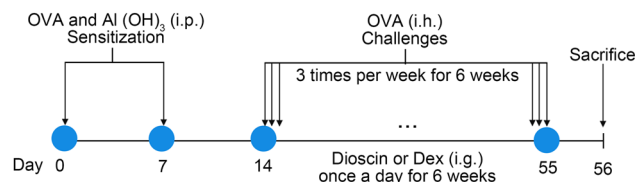


FIGURE 1 Schematic diagram of the establishment of a murine model of asthma. DEX, dexamethasone; OVA, ovalbumin.

were administrated (i.g., QD, 30 min before OVA challenge) with dioscin at the doses of 40 or 80 mg/kg for 6 weeks. In parallel, DEX was dissolved in normal saline and administered (i.g., QD, 30 min before OVA challenge) to mice in the asthma + DEX (3 mg/kg) group. Twenty-four hours after the OVA challenge on Day 55, mice were killed, and the bronchoalveolar lavage fluids (BALFs), blood, and lung tissues were obtained.

2.3 | Differential cell counts in BALFs

As described previously with minor modifications,^[20] the BALFs were collected using lavage with phosphate-buffered saline (PBS) via a tracheal catheter. For differential cell counting, BALFs were centrifuged (1500 rpm) for 10 min and the supernatant is discarded, and then the cells were stained with Diff-Quik. The number of total cells, eosinophils, neutrophils, lymphocytes, and macrophages in BALFs were counted in random fields of the slide under the microscope.

2.4 | Lung histopathology

The lung tissues were immersed in 4% paraformaldehyde before being embedded in paraffin and sectioned into 5- μ m slices. Hematoxylin and eosin staining was performed to evaluate inflammatory cell infiltration in lung tissues. As previously described, the inflammation was scored as follows: Grade 0, no inflammation; Grade 1, occasional observation of cuffing with inflammatory cells; Grades 2–4, most bronchi or vessels surrounded by a thin (1–2 cells), moderate (3–5 cells), or thick (>5 cells) layer of inflammatory cells, respectively.^[33,34] Periodic acid–Schiff (PAS; Leagene Biotech) staining was conducted to examine the goblet cell hyperplasia. The PAS-positive area and the total epithelial area were measured of seven bronchioles on each slide. The percentage of PAS-stained area in the total epithelium area was calculated.^[22] To further determine the total content of collagen in lung tissues (100 mg/per mouse), hydroxyproline (HYP) assay was performed using the HYP Kit (Jiancheng Bioengineering Institute). The lung sections were also immunohistochemically stained for α -SMA. The scoring method for quantitating α -SMA expression was modified from a previously reported study.^[35]

2.5 | Determination of *Muc5ac* level in lung tissues

The RNA was obtained using TRIpure (BioTeke) following the manufacturer's instructions. Complementary DNA was synthesized using BeyoRT II M-MLV reverse transcription reagent (Beyotime Biotechnology). Polymerase chain reaction (PCR) was performed using the 2X Taq PCR MasterMix Kit and SYBR Green (Solarbio). The messenger RNA expression was normalized against the level of β -actin. The sequences of primer are as follows: *Muc5ac*, forward 5'-CGTGGTCTGGAAGGATGCTAT-3'; *Muc5ac*, reverse 5'-GAAGTGTGCGGCTGTAGGT-3'; *Actb*, forward 5'-CTGTGCCCATCTACGAGGGCTAT-3'; *Actb*, reverse 5'-TTTGATGTACGCACGATTTC-3'.

2.6 | Enzyme-linked immunosorbent assay

OVA-specific IgE and IgG1 levels in serum were detected by means of the Mouse OVA sIgE/sIgG1 Kits (Fine Biotech; Jianglai Bio). BALFs were centrifuged at 300g for 10 min and the supernatants were collected. The lung tissue homogenates (tissue weight [g]: normal saline volume [ml] ratio = 1:9) were centrifuged at 430g for 10 min and the supernatants were collected. To evaluate the effect of dioscin on airway inflammation, the concentrations of Th2 cytokines (IL-4, IL-5, and IL-13) that play pivotal roles in allergic asthma^[20] and the proinflammatory cytokine TNF- α were measured in the supernatants using the commercially available Enzyme-Linked Immunosorbent Assay Kits purchased from the Lianke Biotech Co., Ltd., following the manufacturer's instructions.

2.7 | Cell culture and dioscin toxicity assay

16HBE cells (Procell Life Science) were cultured at 37°C and 5% CO₂ in Roswell Park Memorial Institute-1640 medium (Solarbio) with 10% fetal bovine serum. The cytotoxicity of dioscin on 16HBE cells was examined using the MTT method. 16HBE cells were treated with dioscin at 50, 100, 200, 400, and 800 ng/ml for 24 h. Optical density at 570 nm was measured with a microplate reader (BioTek).

2.8 | Immunofluorescence

Immunofluorescence was used to label α -SMA or E-cadherin. 16HBE cells were initially pretreated with dioscin (400 ng/ml) for 2 h and were co-treated with TGF- β 1 (10 ng/ml) for a further 24 h or stimulated with 10 ng/ml TGF- β 1 alone. Afterward, primary antibodies against α -SMA (A17910; ABclonal) or E-cadherin (AF0131; Affinity Biosciences) were applied separately, followed by fluorescein-labeled secondary antibody Cy3-conjugated goat anti-rabbit IgG (A0516; Beyotime) for 16HBE cells. All primary antibodies were diluted at 1:200 with PBS and applied overnight at 4°C. The secondary antibody (1:100) was applied for 1 h. Finally, samples were stained with 4',6-diamidino-2-phenylindole and photographed at 400-fold magnification.

2.9 | Western blot assay

The protein samples were obtained using lysis buffer (Beyotime) on ice, and the protein content was detected using a BCA Protein Assay Kit (Beyotime). Proteins were separated by sodium

dodecyl sulphate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membranes (Millipore). After blocking, the membranes were individually incubated overnight at 4°C with antibodies against TGF- β 1 (#3711, 1:1000; CST), AKT (AF6261, 1:1000; Affinity), phospho-AKT

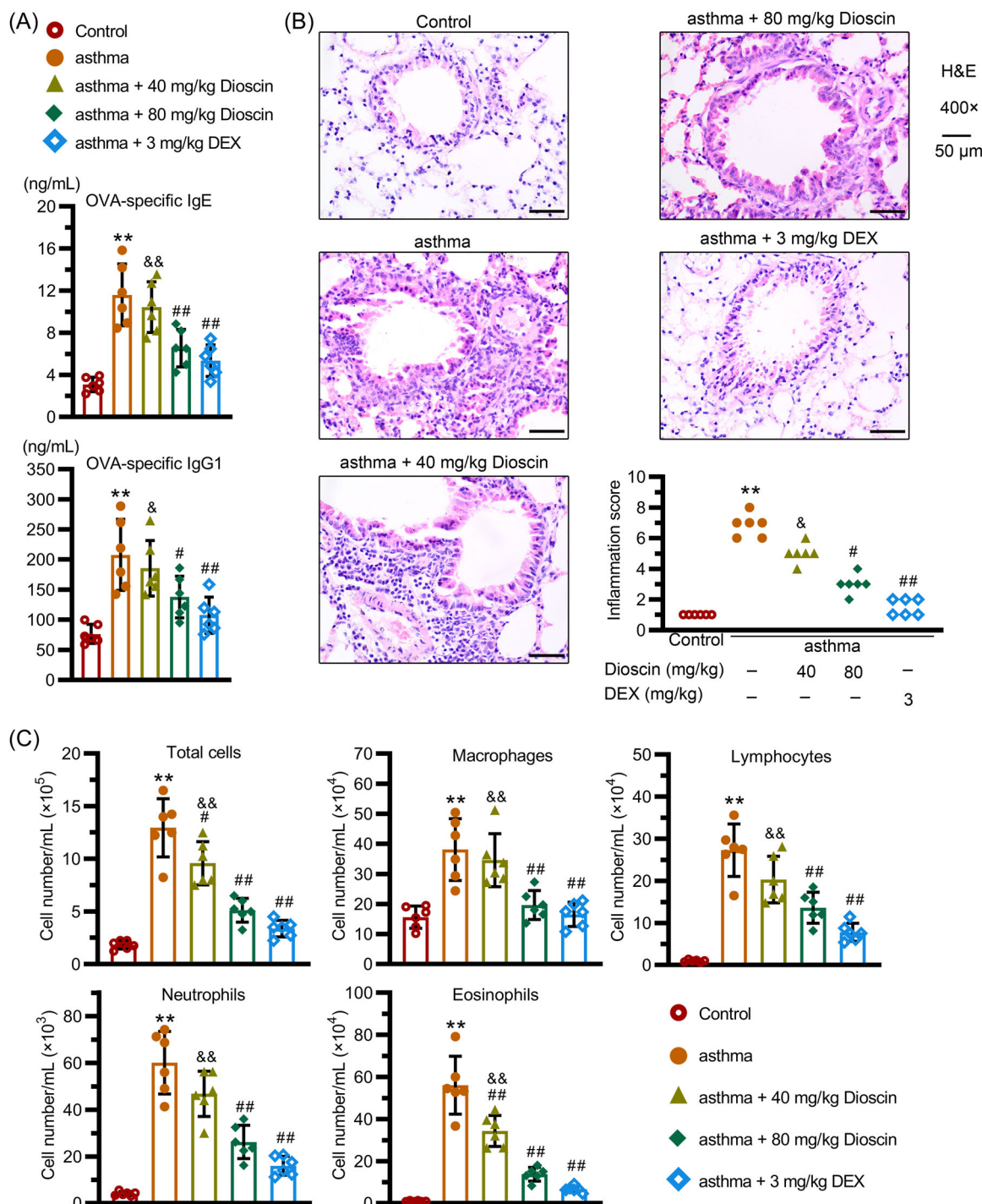


FIGURE 2 Effects of dioscin on inflammatory cells. (A) The levels of serum OVA-specific IgE and IgG1. (B) Lung sections stained with H&E and quantitative analysis of the total inflammation score. (C) Total white blood cell and differential white blood cell (eosinophils, neutrophils, lymphocytes, and macrophages) counts ($n = 6$ per group). ** $p < 0.01$ versus control; # $p < 0.05$ and ## $p < 0.01$ versus asthma; & $p < 0.05$ and && $p < 0.01$ versus asthma + 3 mg/kg DEX. DEX, dexamethasone; H&E, hematoxylin and eosin; IgE, immunoglobulin E; OVA, ovalbumin.

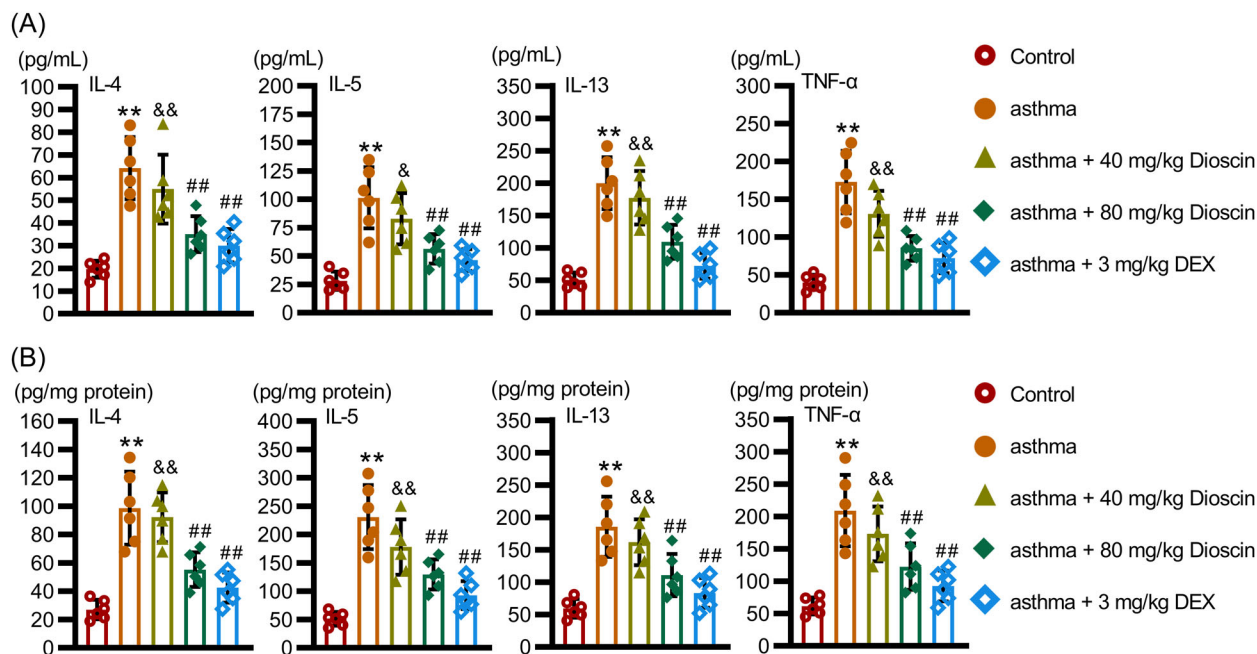


FIGURE 3 Effects of dioscin on inflammatory cytokines. Concentrations of inflammatory cytokines in the BALFs (A) and lung tissues (B) ($n = 6$ in each group). ** $p < 0.01$ versus control; ## $p < 0.01$ versus asthma; & $p < 0.05$ and && $p < 0.01$ versus asthma + 3 mg/kg DEX. BALF, bronchoalveolar lavage fluid; DEX, dexamethasone; IL-4, interleukin 4.

(p-AKT) (AF0016, 1:1000; Affinity), Smad2/3 (A18674, 1:1000; ABclonal), and p-Smad2/3 (AP0548, 1:1000; ABclonal). Subsequently, goat anti-rabbit IgG-horseradish peroxidase (A0208, Beyotime) at a 1:5000 dilution was used to detect antibody binding. The protein bands were visualized using enhanced chemiluminescence by the Gel-Pro-Analyzer software and normalized with β -actin (sc-47778, 1:1000; Santa Cruz).

2.10 | Statistical analysis

The data are shown as the mean $\pm$ standard deviation. All tests were performed using Prism 8.0. Groups were compared using a one-way analysis of variance followed by a Tukey test. Kruskal-Wallis and Dunn's tests were used to determine statistically significant differences between groups for the inflammatory scores and α -SMA staining scores. $p < 0.05$ indicated a statistical significance.

3 | RESULTS

3.1 | Effect of dioscin on inflammatory cells in OVA-sensitized mice

We determined the antiallergic activity of dioscin by assessing the concentration of OVA-specific IgE and IgG1 in the serum. As illustrated in Figure 2A, the concentration of OVA-specific IgE and IgG1 in OVA-induced asthmatic mice was significantly elevated compared with those seen in control mice. Administration of dioscin

(40 or 80 mg/kg) decreased the upregulated IgE and IgG1 levels in asthmatic mice in a dose-dependent manner. Compared to the control group, mice in the OVA group showed severe airway inflammation, including a markable increase in white blood cell counts present in BALFs (Figure 2C) and massive infiltration of inflammatory cells into lung tissues (Figure 2B). Treatment with dioscin (80 mg/kg) induced an obvious decrease in cell counts of total cells, macrophages, lymphocytes, neutrophils, and eosinophils in asthmatic mice, whereas 40 mg/kg dioscin did not display such obvious effects (Figure 2C). In addition, inflammation scores also showed that inflammatory cells of mice treated with dioscin (40 and 80 mg/kg) and DEX were fewer (Figure 2B). We further compared the results among the dioscin treatment groups and DEX treatment group and found that the anti-inflammatory effect of DEX was better than that of 40 mg/kg dioscin but displayed no statistically significant difference compared to that of 80 mg/kg dioscin (Figure 2A-C). All results suggested that the pretreatment of dioscin ameliorated the inflammation in asthmatic mice's airways.

3.2 | Effect of dioscin on inflammatory cytokines in OVA-sensitized mice

The concentrations of IL-4, IL-5, IL-13, and TNF- α in the BALFs and lung tissues were obviously elevated after OVA aerosol challenge. Dioscin and Dex decreased the secretions of cytokines in BALFs of the asthmatic mice (Figure 3A). Consistent alterations were found in lung tissues (Figure 3B). Although the inhibitory effect of 40 mg/kg dioscin on the secretion of inflammatory

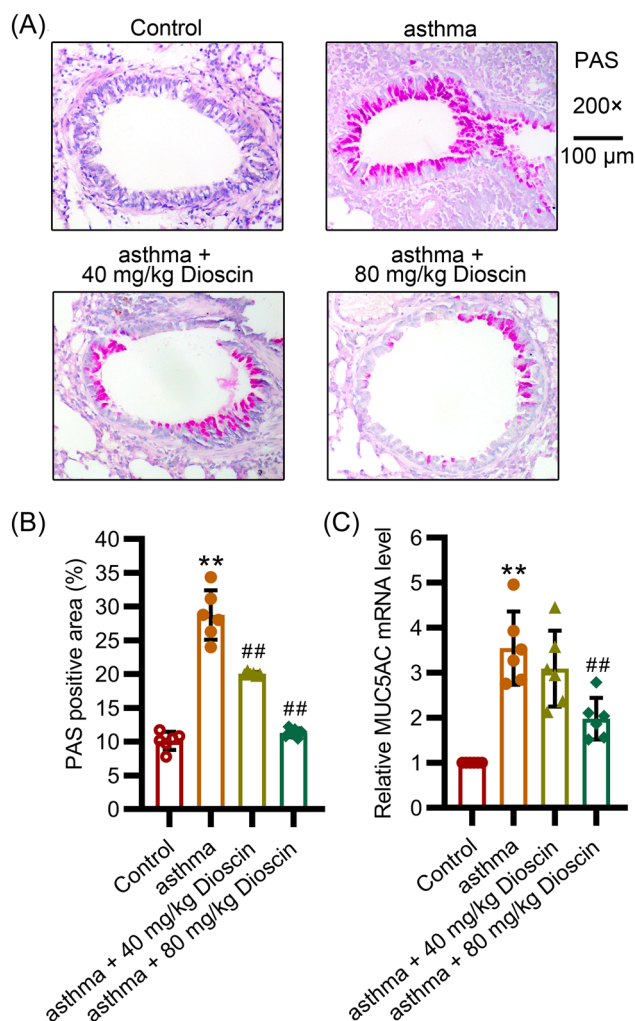


FIGURE 4 Effects of dioscin on mucus production in the airway. (A) Lung sections stained with PAS. (B) Determination of PAS-positive area. (C) Relative MUC5AC mRNA levels in lung tissues ($n = 6$ per group). ** $p < 0.01$ versus control; ## $p < 0.01$ versus asthma. mRNA, messenger RNA; PAS, periodic acid–Schiff.

factors was not as obvious as that of DEX, the effect of 80 mg/kg dioscin had no significant differences from that of DEX (Figure 3A,B). These results implied that dioscin suppressed airway inflammations by regulating inflammatory cytokines in the OVA-challenged asthmatic mice.

3.3 | Effect of dioscin on the production of tracheal mucus in OVA-sensitized mice

The goblet cell hyperplasia was assessed by PAS staining. As shown in Figure 4A, OVA-induced mice had elevated mucus secretion and goblet cell hyperplasia in bronchioles. The dioscin-treated mice displayed fewer PAS-positive cells in lung tissues than those in the asthmatic mice (Figure 4B). Moreover, the level

of mucin MUC5AC in lung tissues was evaluated, and we found that dioscin markedly decreased its expression (Figure 4C), which suggested that dioscin significantly prevented mucus hypersecretion.

3.4 | Effect of dioscin on fibrosis and collagen deposition

The HYP content was detected to evaluate the total collagen level. As shown in Figure 5A, the HYP level was significantly upregulated by OVA, and reduced by treatment of dioscin in a dose-dependent manner. Moreover, immunohistochemistry staining (Figure 5B) showed that the staining density of α -SMA around the bronchioles in OVA-sensitized mice was higher compared to control mice (Figure 5C), which was reversed by dioscin, especially at the dosage of 80 mg/kg (Figure 5C). These observations suggested that dioscin might have a potential effect on preventing airway remodeling.

3.5 | Effect of dioscin on TGF- β 1, Smad2/3, and AKT

The expression of TGF- β 1 (Figure 6A), and the phosphorylation levels of AKT (Figure 6B) and Smad2/3 (Figure 6C) were profoundly increased in the OVA-induced asthmatic mice. Administration of dioscin significantly reduced the TGF- β 1 expression (Figure 6A). Furthermore, it was observed that the treatment of dioscin can inhibit the phosphorylation of AKT and Smad2/3 proteins (Figure 6B,C).

3.6 | Effect of dioscin on the TGF- β 1-treated 16HBE cells

As presented in Figure 7A, 50, 100, 200, and 400 ng/ml dioscin did not influence the cell viability of 16HBE cells. To validate whether dioscin regulates TGF- β 1/Smad2/3 and AKT signaling pathways, 16HBE cells were pretreated with 2-h of 400 ng/ml dioscin and cocultured with 24-h of 10 ng/ml TGF- β 1. The phosphorylation of AKT and Smad2/3 was significantly induced by TGF- β 1, whereas the elevated phosphorylation levels of those proteins were notably reduced by the treatment of dioscin (Figure 7B,C). In addition, immunofluorescence assay showed that TGF- β 1 treatment markedly increased the α -SMA expression but decreased the E-cadherin level in 16HBE cells, whereas those changes were restrained after dioscin treatment (Figure 7D,E). In brief, we further verified that dioscin could suppress the TGF- β 1-induced upregulation of p-Smad2/3 and p-AKT expressions in 16HBE cells and display a protective effect against fibrotic remodeling of the bronchial epithelial cell.

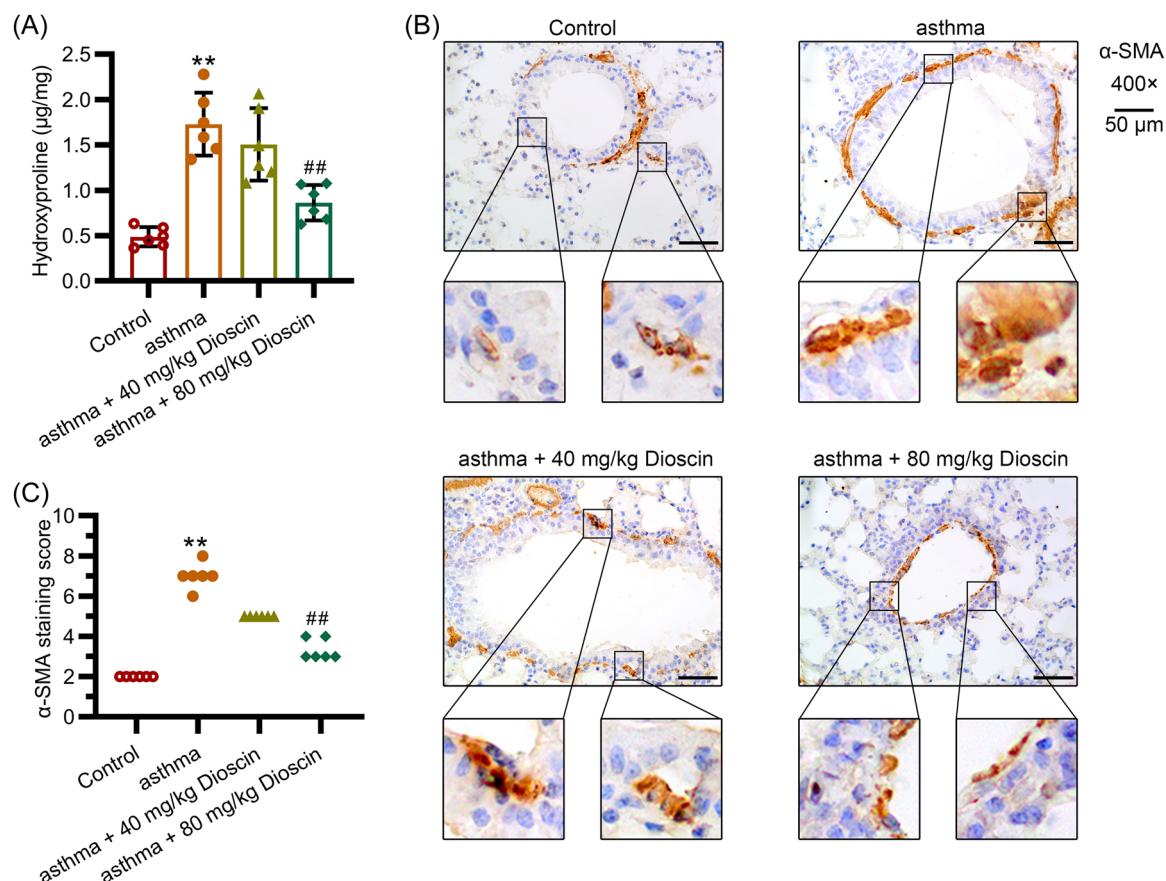


FIGURE 5 Effects of dioscin on airway remodeling. (A) Contents of hydroxyproline in lung tissues. (B) IHC staining for α -SMA in lung tissues. (C) Analysis of α -SMA staining score ($n = 6$ per group). ** $p < 0.01$ versus control; ## $p < 0.01$ versus asthma. IHC, immunohistochemistry; α -SMA, α -smooth muscle actin.

4 | DISCUSSION

Asthma is a long-term pulmonary inflammatory disorder, and it is associated with AHR to nonspecific stimuli, mucus production, and alterations in structure.^[36,37] Airway remodeling includes a variety of airway structural changes, such as mucus cell hyperplasia and subepithelial fibrosis.^[38] In this study, we illustrated that dioscin ameliorated airway inflammation and protected against airway remodeling by inhibiting mucus hypersecretion, airway smooth muscle hyperplasia, and collagen deposition in asthmatic mice. In addition, we also uncovered the mechanisms of the protective impacts of dioscin.

The present study found that dioscin attenuated the lung injuries of the OVA-sensitized mice, along with effective reduction of white blood cells in BALFs and the OVA-specific IgE and IgG1 levels in serum. IgE contributes to the production of Th2 cytokines, TNF- α , thymic stromal lymphopoietin, and other chemokines, which induce smooth muscle contraction and proliferation.^[21] A previous study has confirmed that down-regulation of IgE/IgG1 (OVA-specific) participates in the improvement of allergic airway inflammation.^[23] Leukocytosis, such as eosinophil infiltration, is an important sign of airway

inflammation. Accumulation of eosinophils in inflammation areas leads to asthma progress.^[39] More recent studies have suggested that recruitments of neutrophils, macrophages, and eosinophils into the airways exhibit complicated functions during different phases of asthma.^[40,41] Moreover, dioscin suppressed the elevated expression of inflammation cytokines in both BALFs and lung tissues of the OVA-induced mice. IL-4 stimulation upregulated collagen levels in bronchial fibroblasts from asthma patients, implying that IL-4 might link inflammation with airway fibrosis.^[42] IL-5 is associated with eosinophil maturation and secretion.^[43] IL-13 promotes B cell (IgE-producing) proliferation and might play a role in asthma.^[44] Additionally, inhibition of TNF- α decreased the secretions of inflammatory cytokines (IL-4 and IL-13), thereby inhibiting the airway inflammation in asthma.^[33,45] Therefore, the effects of dioscin in suppressing the elevated inflammation cytokines supported the anti-inflammatory impacts of dioscin on asthma. Besides this, we demonstrated that the anti-inflammatory effect of a high dose of dioscin (80 mg/kg) did not exhibit significant differences from that of DEX, a common and widely used drug to treat allergic asthma, further supporting the clinical application of dioscin in asthma.

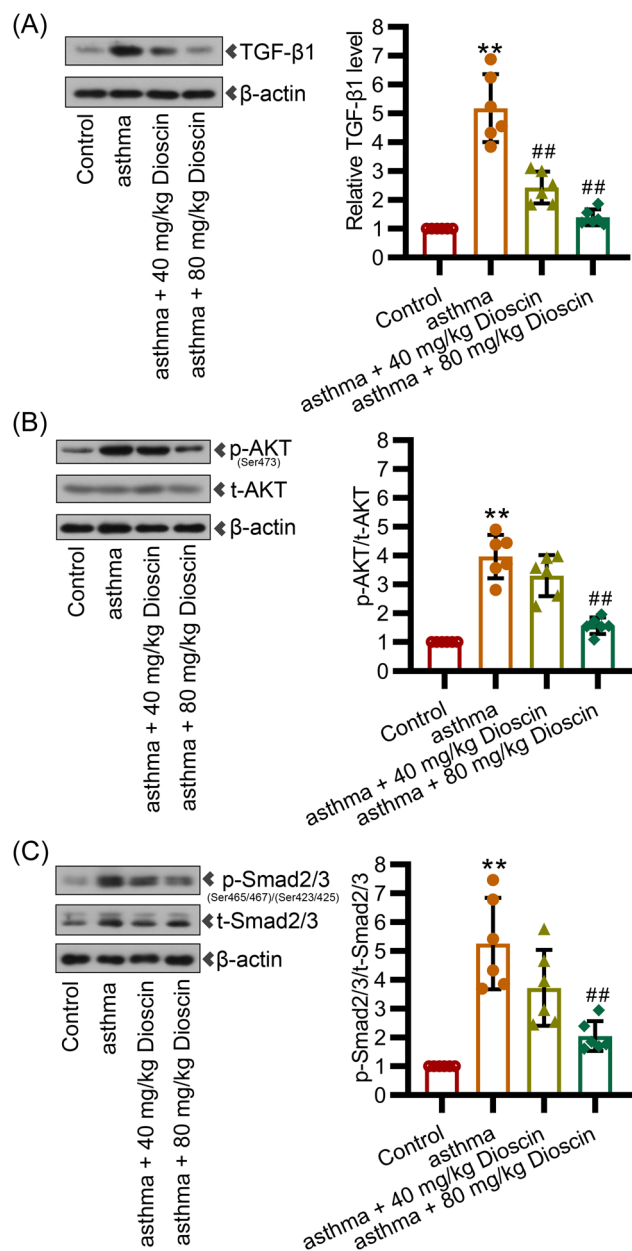


FIGURE 6 Effects of dioscin on TGF-β1, Smad2/3, and AKT in a murine model of chronic asthma. Western blot analysis for TGF-β1 (A), p-AKT/t-AKT (B), and p-Smad2/3/t-Smad2/3 (C) in lung tissues ($n = 6$ per group). ** $p < 0.01$ versus control; ## $p < 0.01$ versus asthma. AKT, protein kinase B; p-AKT, phospho-AKT; t-AKT, total AKT; TGF-β1, transforming growth factor-β1.

Airway remodeling is closely linked to chronic inflammation. Hence, we further investigated the inhibitory impacts of dioscin on airway remodeling by evaluating mucus contents and fibrosis. Irregular goblet cells and mucus are important pathological features of asthma that can affect both large and small airways.^[46,47] MUC5AC, the marker of goblet cell metaplasia, is major airway mucin.^[48] Inflammation cytokines can upregulate the expression of MUC5AC, thereby leading to mucus hypersecretion.^[49,50] Shimizu et al.^[22] also discovered that AHR was

ameliorated by suppressing mucus production. Our data indicated that dioscin could regulate the goblet cell hyperplasia in asthmatic mice's airways and significantly reduced the MUC5AC expression. Besides this, dioscin could alleviate pulmonary inflammation and fibrosis by enhancing autophagy of alveolar macrophages,^[7] but the effects of dioscin on airway fibrosis in asthma are unknown. Here, dioscin significantly decreased HYP content, and accumulation of HYP is evident in the airway remodeling of many chronic asthmatics.^[51] Moreover, we found that dioscin treatment suppressed α-SMA expression in asthmatic mice, which is important for airway smooth muscle contraction and proliferation.^[52] Additionally, patients with severe asthma exhibited significantly higher α-SMA expression.^[35] Thus, our findings verified that dioscin might affect airway remodeling by reducing mucus production and suppressing airway fibrosis.

TGF-β1 contributes to the production of extracellular matrix and is considered to be an important modulator of fibrosis in asthma.^[52] Several studies have reported that TGF-β1 exhibits an important role in the remodeling of the airway and is involved in multiple cellular processes, such as smooth muscle remodeling, fibrosis, and microvascular alternations.^[53–55] Activation of TGF-β leads to phosphorylation of Smads.^[56] The elevated TGF-β1 and p-Smad2/3 took part in fibrosis and inflammation in mice with OVA-induced asthma.^[57,58] Additionally, TGF-β1 could enhance chronic airway inflammation in bronchial asthma by recruiting inflammatory cells to the airway.^[53] Administration of a TGF-β1 receptor inhibitor significantly elevated IL-6, TNF-α, and IL-17a but decreased IL-10, thus reversing the protective effect of epigallocatechin gallate against airway inflammation in asthmatic mice.^[54] In addition to the classical TGF-β1/Smad signaling, PI3K/AKT signaling and its cross-talk with TGF-β also plays a role in inflammation and airway remodeling in asthma.^[29,30] In the lung tissues of OVA mice, the PI3K/AKT signaling pathway was activated, and inhibition of PI3K/AKT signaling attenuated allergic airway inflammation in asthmatic mice.^[29,32,55] Mechanistically, TGF-β signaling can activate the PI3K/AKT pathway in 16HBE cells,^[56] and downregulation of TGF-β signaling was found to slow down the activation of PI3K/AKT signaling, thereby reducing EMT and inflammation, in bleomycin-induced fibrosis of mice.^[57] PI3K/AKT inhibitor, in turn, reduced the increased TGF-β1 levels in lung tissues of OVA-sensitized and -challenged mice and reversed TGF-β1-induced EMT and migration of 16HBE cells.^[32,58]

Consistent with the previously reported inhibitory effects of dioscin on TGF-β and AKT signaling pathways.^[9,12,59] Herein, the inhibition of dioscin on the expression of TGF-β1, as well as the phosphorylated Smad2/3 and AKT in asthmatic mice induced by OVA were observed. Furthermore, TGF-β1-treated 16HBE cells were utilized to determine the impacts of dioscin on subepithelial fibrosis. We have identified that the p-Smad2/3 and p-AKT are upregulated in TGF-β1-treated 16HBE cells, while the phosphorylation levels of those proteins are restored by dioscin, suggesting that dioscin can inhibit the activation of TGF-β1/Smad2/3 and AKT pathways. TGF-β1 promotes the transition from epithelial cells to mesenchymal phenotypes,^[56] accompanied by the reduced epithelial E-cadherin

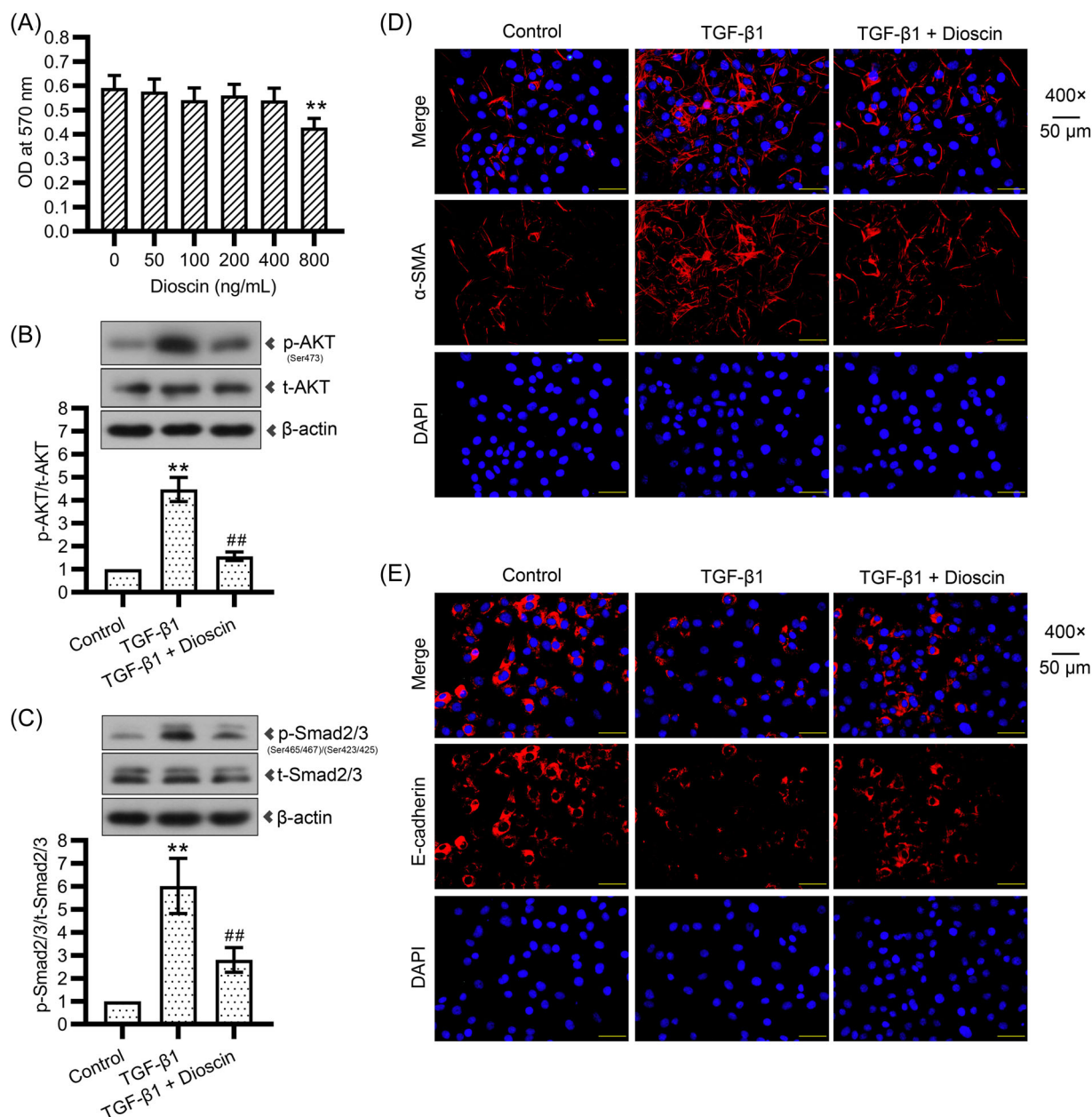


FIGURE 7 Effects of dioscin on TGF-β1-treated 16HBE cells. (A) Effects of 24-h incubation of dioscin (0, 50, 100, 200, 400, and 800 ng/ml) on cell viability. ** $p < 0.01$ versus the dioscin (0 ng/ml) treatment. Western blot for p-AKT/t-AKT (B) and p-Smad2/3/t-Smad2/3 (C) in 16HBE cell lysates. IF staining for α-SMA (D) and E-cadherin (E) in 16HBE cells. ** $p < 0.01$ versus control; ## $p < 0.01$ versus TGF-β1. 16HBE, human bronchial epithelial; AKT, protein kinase B; DAPI, 4',6-diamidino-2-phenylindole; IF, immunofluorescence; OD, optical density; p-AKT, phospho-AKT; t-AKT, total AKT; TGF-β1, transforming growth factor-β1; α-SMA, α-smooth muscle actin.

and elevated mesenchymal α-SMA.^[32,60,61] Herein, we surprisingly found that dioscin increased E-cadherin and decreased α-SMA. Based on the results of the in vivo and in vitro studies, dioscin might display beneficial effects by mediating the TGF-β1/Smad2/3 and AKT pathways. However, whether TGF-β and AKT signaling pathways are also involved in the effect of dioscin on inflammation requires further evidence.

In conclusion, we demonstrated that dioscin has the potential to improve airway inflammatory responses and remodeling in

OVA-challenged asthmatic mice and to inhibit the activation of the TGF-β1/Smad2/3 and AKT signal pathway in vivo and in vitro. This study supports the protective effect of dioscin on asthma and proposes that dioscin has the potential to be applied in asthma treatment.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

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