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The protective effect of solidagenone from *Solidago chilensis* Meyen in a mouse model of airway inflammation

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Abstract

Solidagenone is the main active constituent present in *Solidago chilensis* Meyen which is used in folk medicine to treat pain and inflammatory diseases. This study aimed to evaluate the anti-inflammatory activity of solidagenone in vitro and in a model of allergic airway inflammation. In vitro studies were performed in activated macrophages and lymphocytes. BALB/c mice were sensitized and challenged with ovalbumin and treated with solidagenone orally (30 or 90 mg/kg body weight) or dexamethasone, as a positive control in our in vivo analysis. Supernatant concentrations of nitrite, TNF and IL-1β, as well as gene expression of pro-inflammatory mediators in macrophages cultures, were reduced after solidagenone treatment, without affecting macrophages viability. Besides, solidagenone significantly decreased T cell proliferation and secretion of IFNγ and IL-2. Th2 cytokine concentrations and inflammatory cell counts, especially eosinophils, in bronchoalveolar lavage fluid were reduced in mice treated with solidagenone. Histopathological evaluation of lung tissue was performed, and morphometrical analyses demonstrated reduction of cellular infiltration and mucus hypersecretion. Altogether, solidagenone presented anti-inflammatory activity in vitro and in vivo in the OVA-induced airway inflammation model, suggesting its promising pharmacological use as an anti-inflammatory agent for allergic hypersensitivity.

KEYWORDS
allergic airway inflammation, cytokines, immunomodulation, mouse model, solidagenone

1 | INTRODUCTION

Natural products have been vital in the pharmaceutical and biotechnology industries, as shown by the vast range of modern medicines based upon either naturally occurring molecules or their derivatives.[1] *Solidago chilensis* Meyen (Asteraceae), which is an original species from Chile with distribution in South America, popularly

known in Brazil as 'arnica', 'arnica-brasileira', 'arnica-do-campo' and 'erva-lanceta', has a variety of uses in folk medicine, used as a diuretic, anti-inflammatory, antispasmodic and anthelminthic.[2]

S. chilensis is mainly used for the treatment of inflammatory conditions.[3] Despite the broadly popular therapeutic use, only recent experimental studies investigated the mechanisms of action of extracts obtained from rhizomes, leaves and inflorescences of *S. chilensis*, on inflammatory processes.[4,5] The main constituent described for *S. chilensis* is solidagenone, a diterpene isolated from the extract obtained from its inflorescences,[6,7] which showed a gastroprotective effect in a model of gastric lesion and inhibits skin inflammation in croton oil-induced ear oedema, arachidonic acid-induced ear oedema and phenol-induced ear oedema.[7–9]

Allergic diseases represent a group of conditions caused by hypersensitivity of the immune system to allergens present in the environment. In allergic disorders, such as anaphylaxis, allergic rhinitis, some food allergies and allergic asthma, these responses are characterized by the involvement of allergen-specific IgE and T helper 2 (Th2) cells that recognize allergen-derived antigens.[10,11] Allergic asthma is characterized by airway oedema, remodelling and hyperresponsiveness, causing the airways to narrow and reducing the flow of air into and out of the lungs.[12] It is a complex disease associated with the recruitment and activation of inflammatory cells and the production of cytokines that are involved with the maintenance of inflammation.[13]

Many new pharmacological or biological agents that target the various steps in the cell and mediator pathways implicated in allergic inflammation are being investigated, but due to their strong immunosuppressive, anti-inflammatory and anti-allergic effects on immune cells, tissues and organs, glucocorticoids are the most used drug to treat diseases caused by a dysregulated immune system.[14] Unfortunately, rising dosages and duration of administration simultaneously increase the risk of adverse events like drug-induced hyperglycemia, glaucoma, osteoporosis, obesity, gastritis and cardiovascular disease.[15] Thus, the present work aimed to investigate the possible anti-allergic activity of solidagenone in a mouse model of allergic airway inflammation.

2 | MATERIALS AND METHODS

2.1 | Solidagenone obtention

The bioactive diterpene solidagenone was obtained from *Solidago chilensis* inflorescences using the methodology previously described by our group.[6,7] The plant material was cultivated in PAF-FIOCRUZ (Agroecological Platform of Phytomedicines of Pharmaceutical Technology Institute; RJ, Brazil) (22.9407°S, 43.4046°W), confirmed and authenticated by Marcelo Neto Galvão, and one voucher specimen was deposited at HFV (Herbal Green Pharmacies), under number HFV 646.

The SIS (solidagenone isolated standard) was accurately weighed, diluted in the ratio of 10 mg/ml and 10 µl and filtered through a 0.5 membrane filter before the chromatographic analysis. All solvents and chemicals were of analytical grade. The structure and identification of SIS were evaluated by TLC, FTIR and UV spectroscopy and confirmed by MS and NMR analyses as described before.[6,7]

2.2 | Animals

Four- to six-week-old male BALB/c mice weighing approximately 25 g were raised and maintained at the animal facilities of the Gonçalo Moniz Institute, FIOCRUZ, BA. Animals were handled according to the NIH guidelines for animal experimentation, and this study was conducted in accordance with the Basic and Clinical Pharmacology and Toxicology policy for experimental and clinical studies.[16] All procedures had prior approval from the local animal ethics committee (29/2009).

2.3 | Cytotoxicity assay

Peritoneal exudate macrophages were obtained by washing, with cold phosphate-buffered saline (PBS), the peritoneal cavity of BALB/c mice 4 days after injection of 3% thioglycolate (Sigma-Aldrich, St. Louis, MO, USA) in saline (1.5 ml per mice). Then, cells were seeded into 96-well plates at a cell density of 2×10^5 cells/well in Dulbecco's modified Eagle medium (DMEM; Life Technologies, GIBCO-BRL, Gaithersburg, MD, USA) medium supplemented with 10% foetal bovine serum (FBS; GIBCO) and 50 µg/ml of gentamicin (Life, Carlsbad, CA, USA) and incubated for 24 h at 37°C and 5% CO_2. After that time, cells were incubated with LPS (500 ng/ml, Sigma-Aldrich) and IFNγ (5 ng/ml; Sigma-Aldrich) plus solidagenone (50, 25 or 12.5 µM), in triplicate and incubated for 72 h. Then, 20 µl per well of AlamarBlue (Invitrogen, Carlsbad, CA, USA) was added to the plates for 10 h. Colorimetric readings were performed at 570 and 600 nm.

2.4 | Macrophage cultures

Peritoneal exudate macrophages were seeded in 96-well plates at 2×10^5 cells/well in DMEM medium

supplemented with 10% of FBS and 50 μg/ml of gentamicin for 24 h at 37°C and 5% CO_2. Cells were then stimulated or not with LPS (500 ng/ml) and IFNγ (5 ng/ml) in the absence or presence of solidagenone at different concentrations (50, 25 and 12.5 μM) and incubated at 37°C. Cell-free supernatants were collected 4 h (for TNFα measurement) and 24 h (for IL-1β and nitrite quantifications) and kept at −80°C until use. Dexamethasone was used as a positive control, at 12.5 μM. Three independent experiments were performed.

2.5 | Real-time reverse transcription polymerase chain reaction (qRT-PCR)

Peritoneal exudate macrophages were seeded in 24-well plates at 1×10^6 cells/well in DMEM medium supplemented with 10% of FBS and 50 μg/ml of gentamicin for 24 h at 37°C and 5% CO_2. Cells were then pretreated or not with solidagenone at different concentrations (50, 25 and 12.5 μM) or dexamethasone for 1 h and then stimulated or not with LPS (500 ng/ml) and IFNγ (5 ng/ml) and incubated at 37°C for 3 h. Then, RNA samples were extracted from macrophages using TRIzol (Invitrogen, Molecular Probes, Eugene, OR, USA) and fluorometrically quantified using Qubit RNA BR Assay Kit (Thermo Fisher Scientific). RNA samples (1 μg) were converted to cDNA using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). The qRT-PCR reaction was prepared using SYBR™ Green PCR Master Mix (Applied Biosystems™). Primer sequences are detailed in Table S1. Gene expression was normalized using the endogenous *Gapdh* gene, and the samples were amplified in triplicate. qRT-PCR amplification was performed in an ABI7500 Real-Time PCR System (Thermo Fisher Scientific) under standard thermal cycling conditions. The threshold cycle method of comparative PCR was used to analyse the results.

2.6 | Lymphoproliferation assay

BALB/c splenocyte suspensions were prepared in DMEM medium supplemented with 10% of FBS and 50 μg/ml of gentamicin. Splenocytes (5×10^6 cells/well) were plated in 24-well plates, in triplicate, and stimulated or not with concanavalin A (Con A; 5 μg/ml, Sigma-Aldrich). To evaluate the lymphoproliferation, splenocytes were activated in the absence or presence of various concentrations of solidagenone (50, 25 and 12.5 μM) or dexamethasone (12.5 μM) for 72 h. Quantitative evaluation of the exponential cell expansion was estimated by the carboxyfluorescein succinimidyl ester–CFSE assay (Invitrogen/Molecular Probes). CFSE staining was performed according to the methodology previously described.[17] Acquisition was performed using a BD LSRFortessa SORP cytometer, and data were analysed using FlowJo software (Tree Star, Ashland, OR, USA). A total of 10 000 events were acquired.

Splenocytes from BALB/c mice were also plated into 24-well plates at a cell density of 5×10^6 cells/in DMEM medium supplemented with FBS containing or not 5 μg/ml of Con A in the absence or presence of different concentrations of solidagenone (50, 25 and 12.5 μM) and dexamethasone (12.5 μM). After 24 h, cell-free supernatants were collected and kept at −80°C until use for cytokine quantification.

2.7 | OVA-induced model of allergy and treatment

Allergic airway inflammation was induced by ovalbumin (OVA) immunization as described before.[18] Groups of mice received systemic immunization by subcutaneous injection of 10 μg of chicken egg ovalbumin (Grade V, N98% pure; Sigma, St Louis, MO, USA) diluted in 2 mg/ml alum (AlumImject; Pierce, Rockford, IL, USA) followed by a booster injection at day 14. A nasal OVA challenge was performed for 15 min/day, on five consecutive days, in an acrylic box. A solution of 1% ovalbumin in saline was aerosolized by delivery of compressed air to a sidestream jet nebulizer (RespiraMax, NS, Brazil). Twenty-six hours before each OVA challenge, mice were treated orally with solidagenone (OVA + SOL, 30 or 90 mg/kg), dexamethasone (OVA + Dexa, 2 mg/kg) or vehicle (OVA) ($n = 8$ animals/per group), performing 6 days of treatment. Mice not immunized and nor challenged with chicken egg ovalbumin were used as naive mice ($n = 6$ animals).

2.8 | Collection of bronchoalveolar lavage

Twenty-four hours after the last inhalation exposure, mice were anaesthetized with a lethal dose of ketamine (Vetanarcol™; Konig, Avellaneda, Argentina) and 2% xylazine (Sedomin™; Konig) by intraperitoneal injection. Bronchoalveolar lavage (BAL) was performed by intratracheal instillation of 1 ml of PBS. The lavage fluid was centrifuged, and the pellet was resuspended in a PBS final volume of 1 ml for leukocytes quantification in a Neubauer chamber. Cytospin slides were prepared from BAL fluid and stained with Giemsa stain to perform

differential counts. Slides were analysed under light microscopy at 60X magnification by an observer blinded to the specimen identities. The supernatant of BAL fluid was collected for cytokine quantification and kept at −80°C until use.

2.9 | Assessment of cytokine and nitric oxide production

Cytokine concentrations in supernatants from peritoneal macrophages or spleen cells cultures collected at 4 h (TNFα) and 24 h (IFNγ, IL-1β and IL-2) and from the supernatant of BAL fluid (IL-4, IL-5 and IL-13) were determined by enzyme-linked immunosorbent assay (ELISA), using DuoSet kits from R&D Systems (Minneapolis, MN, USA), according to the manufacturer's instructions. Nitric oxide production was estimated in macrophage culture supernatants harvested at 24 h using the Griess method for nitrite quantification.[19]

2.10 | Histopathological and morphometric analyses

After euthanasia, the lungs were removed for histological analysis, fixed in 4% buffered formalin for 24 h and then processed for histological preparations. HE stained sections were used for the quantification of inflammatory cells by optical microscopy. For each lung, 10 fields (40×) per section were analysed to calculate the average number of cells per mm^2. The presence of mucus was

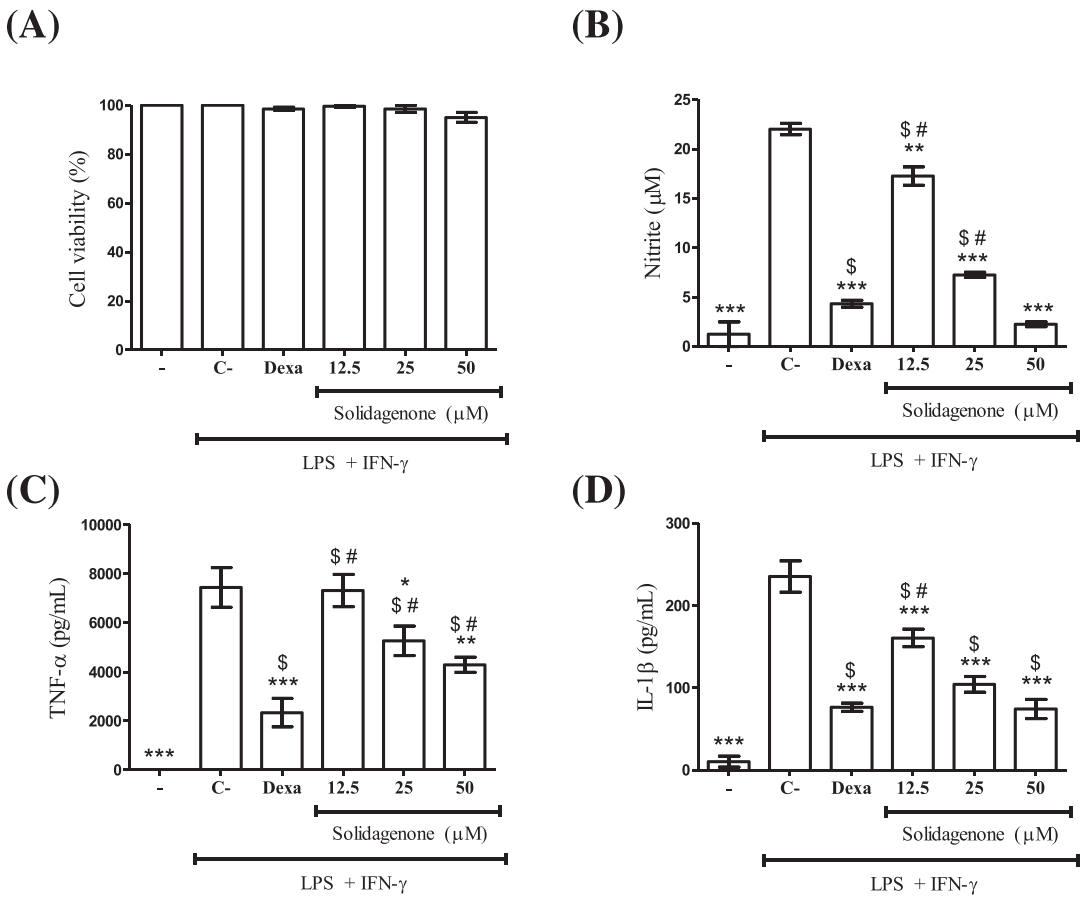

FIGURE 1 Effects of solidagenone on macrophages in vitro. Mouse peritoneal exudate macrophages stimulated or not with LPS + IFNγ were cultured in the absence or presence of solidagenone (50, 25 or 12.5 μM) or dexamethasone (Dexa; 12.5 μM). Cell viability (A) was determined by the Alamar Blue method. Cell-free supernatants were collected for nitrite (B), TNFα (C) and IL-1β (D) quantification. "-" refers to the group of untreated and unstimulated cells. C- refers to the group of untreated cells stimulated with LPS + IFNγ. Values represent the means ± S.E.M. $^{***}P < 0.001$ compared to stimulated and untreated cells; $^{**}P < 0.01$ compared to stimulated and untreated cells; $^{*}P < 0.05$ compared to stimulated and untreated cells; $^{\#}P < 0.05$ compared to unstimulated and untreated cells; $^{\$}P < 0.05$ compared to dexamethasone-treated cells (ANOVA followed by Newman-Keuls multiple comparison tests, $n = 9$ determinations obtained in three independent experiments performed in triplicate)

analysed in periodic alcian blue-stained sections. Ten fields (20×) per section were analysed to calculate the percentual of mucus production. All images were digitalized using a colour digital video camera (CoolSnap cf) adapted to a BX41 microscope (Olympus, Tokyo, Japan) calibrated with a reference measurement slide and were analysed using Image-Pro image program (version 6.1; Media Cybernetics, San Diego, CA, USA).

2.11 | Statistical analysis

Results were expressed as means ± SEM of six to eight mice per group for in vivo study or nine replicates per group for in vitro analysis. Statistical comparisons between groups were performed by one-way analysis of variance followed by Newman-Keuls multiple comparison tests using Graph Pad Prism version 5.01 (Graph Pad Software, San Diego, CA, USA). Differences were considered significant when the values of P were <0.05.

3 | RESULTS

3.1 | Chromatographic analysis and Characterization

Solidagenone was obtained by traditional chromatographic techniques with a good yield (~6.5 to 8.0%), 98.10% pure as described before.[6,7] The SIS (solidagenone isolated standard) was accurately weighed, diluted in the ratio of 10 mg/ml and 10 µl and filtered through a 0.5 membrane filter before the chromatographic analysis. SIS was submitted to chromatographic and spectroscopic

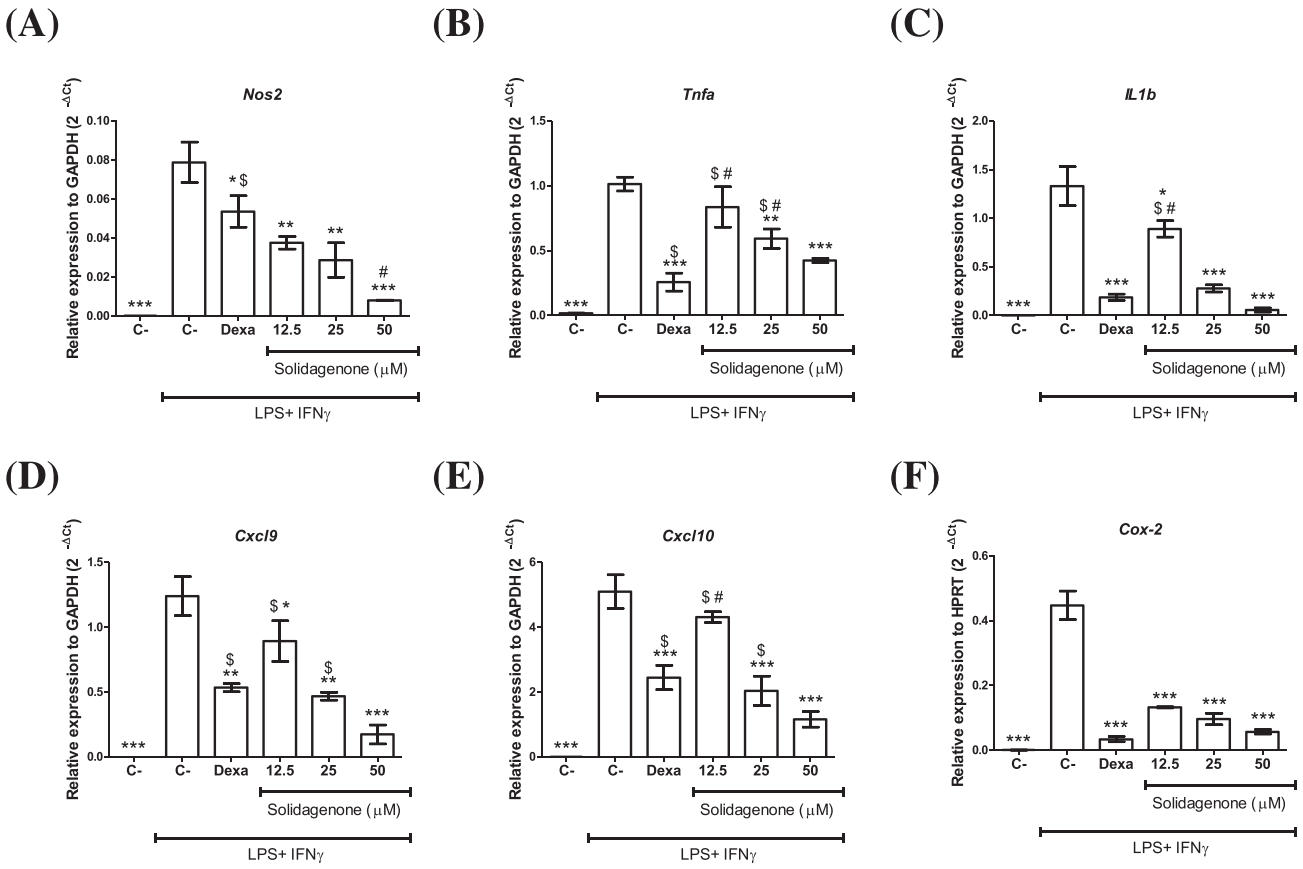

FIGURE 2 Gene expression in macrophages treated or not with solidagenone. Analysis of gene expression was performed by real-time qRT-PCR using cDNA samples prepared from mRNA extracted from cultures of macrophages stimulated or not with LPS + IFN-γ and treated or not with solidagenone (12.5, 25 and 50 µM) or dexamethasone (Dexa; 12.5 µM). (A) *Nos2*, (B) *Tnfa*, (C) *IL1b*, (D) *Cox2*, (E) *Cxcl9* and (F) *Cxcl10* gene expression. Values represent the means ± S.E.M. $^{***}P < 0.001$ compared to stimulated and untreated cells; $^{**}P < 0.01$ compared to stimulated and untreated cells; $^{*}P < 0.05$ compared to stimulated and untreated cells; $^{\#}P < 0.05$ compared to unstimulated and untreated cells; $^{\$}P < 0.05$ compared to dexamethasone-treated cells (ANOVA followed by Newman-Keuls multiple comparison tests, $n = 9$ determinations obtained in three independent experiments performed in triplicate)

analysis by UFLC, IV, NMR ^{1}H, ^{13}C and spectrometric analysis by ESI in positive mode through which its structure could be confirmed (Figures S1–S5 and Table S2).

3.2 | Solidagenone reduces the release of inflammatory mediators by activated macrophages and also reduces lymphoproliferation in vitro

Initially, the cytotoxicity of solidagenone on peritoneal macrophages was evaluated. All tested concentrations had a non-significant effect on macrophage viability, and so they were adopted and used in the following in vitro assays (Figure 1A). Next, the anti-inflammatory activity of solidagenone was investigated in cultures of activated macrophages. As revealed in Figure 1B–D, solidagenone significantly inhibited, in a concentration-dependent manner, the production of nitric oxide, TNFα and IL-1β. In addition, we performed RT-qPCR analysis in culture of macrophages from the different experimental groups. As expected, untreated macrophages stimulated with LPS and IFNγ upregulated the expression of several inflammation-related genes, including *Nos2*, *Tnfa*, *Il*β,

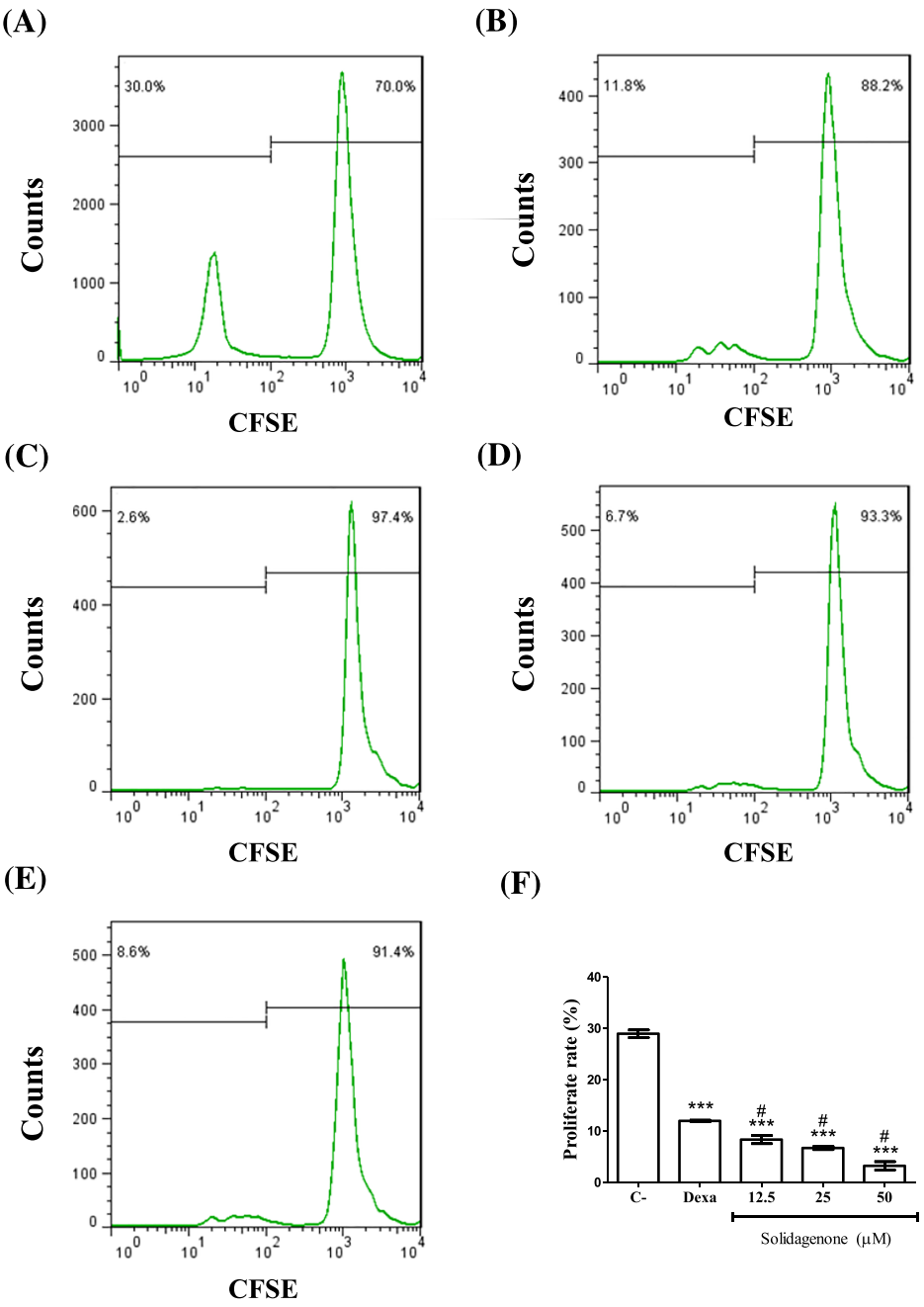

FIGURE 3 Inhibition of lymphocyte proliferation by solidagenone. Spleen cells activated with Con A were treated or not with different concentrations of solidagenone or dexamethasone for 72 h. Proliferation rates were assessed through CFSE staining. (A) untreated splenocytes. (B) Splenocytes treated with 12.5 μM of dexamethasone. (C) Splenocytes treated with 50 μM of solidagenone. (D) Splenocytes treated with 25 μM of solidagenone. (E) Splenocytes treated with 12.5 μM of solidagenone. (F) Proliferate rate based on CFSE staining. Values represent means ± S.E.M. $^{***}P < 0.001$ compared to stimulated and untreated cells; $^{\$}P < 0.05$ compared to dexamethasone-treated cells (ANOVA followed by Newman-Keuls multiple comparison tests, $n = 9$ determinations obtained in three independent experiments performed in triplicate)

Cxcl9, *Cxcl10* and *Cox2*, compared to untreated and unstimulated macrophages (Figure 2A–F). Treatment with solidagenone or dexamethasone promoted a significant reduction in the gene expression of all the genes investigated.

To evaluate the immunosuppressive effects of solidagenone on lymphocytes, we first performed a Con A induced lymphoproliferation assay. As shown in Figure 3A–F, solidagenone significantly decreased T cell proliferation assessed through CFSE staining, as well as dexamethasone. Then, in comparison with untreated and Con A-stimulated cultures, treatment with solidagenone decreased in a concentration-dependent manner the secretion of IFNγ and IL-2 (Figure 4A,B).

3.3 | Suppression of OVA-induced inflammation and Th2 response by solidagenone in the lungs

After the in vitro results, we tested solidagenone in a murine model of allergic airway inflammation, and the treatment caused a marked reduction of leukocytes, especially of eosinophils, in BAL fluid (Figures 5A,B). At the higher administered dose, solidagenone had a similar effect compared to dexamethasone, a glucocorticoid. We also observed a reduced production of Th2 cytokines (IL-4, IL-5 and IL-13) in the BAL when compared to OVA group (Figures 5C–E). In this model, we used the lungs of naive mice as a control (Figure 6A) and compared them to the lungs in which immunized mice had been treated with vehicle only, OVA group (Figure 6B). We observed an intense peribronchiolar inflammation in mice of the group OVA compared to a feature that was reversed by treatment with dexamethasone (Figures 6C) or solidagenone (Figures 6D,E). Solidagenone caused a reduction of tissue inflammation, as shown by the lower cellularity in the lungs demonstrated in Figure 6F. In the lung sections stained with alcian blue, we observed a representative section from a naïve mouse without presence of mucus (Figure 7A), a great amount of mucus in the lumen of mice from the OVA group (Figure 7B), and less mucus deposit in bronchioles after dexamethasone or solidagenone treatment (Figures 7C–E). The percentual of the area occupied by mucus was quantified in all animals; solidagenone treatment reduced the presence of mucus in both doses used when compared with OVA group (Figure 7F).

4 | DISCUSSION

Allergic airway inflammation is a chronic airway disease characterized by peribronchial inflammation, reversible airway obstruction and airway hyperresponsiveness that involves pro-inflammatory functions of diverse leukocytes of the innate and adaptive immune response, typically driven by type 2 immunity.[20,21] The mechanisms

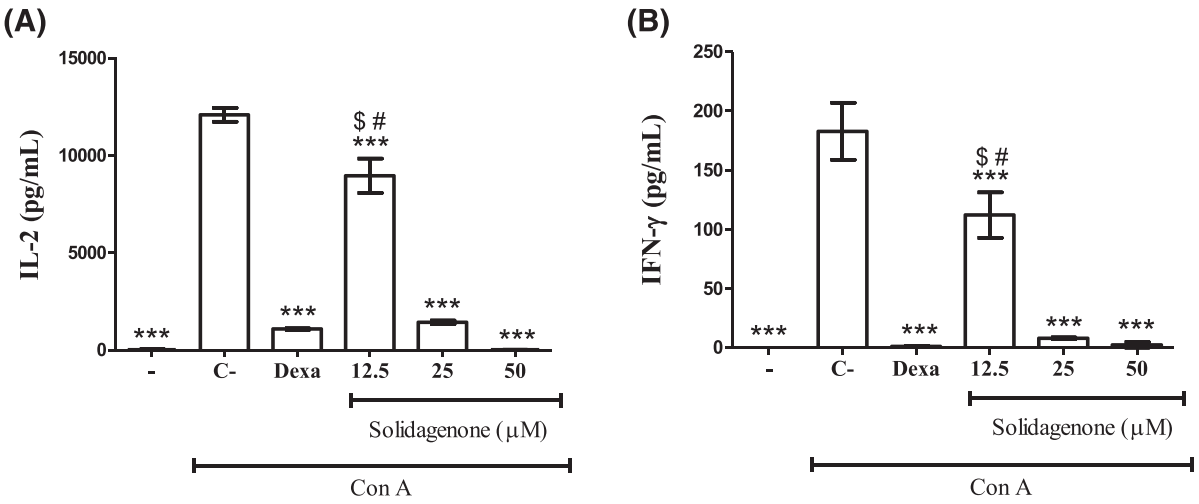

FIGURE 4 Assessment of cytokine production by splenocytes treated with solidagenone. Concentrations of IL-2 (A) and IFNγ (B) were determined in cell-free supernatants from splenocyte cultures treated or not with solidagenone (50, 25 or 12.5 μM) or dexamethasone (Dexa; 12.5 μM) in the presence of concanavalin A (Con A; 5 μg/ml) for 48 h. Cell-free supernatants were then collected for cytokine measurement by ELISA. "-" refers to the group of untreated and unstimulated cells. C- refers to the group of untreated cells but stimulated with Con A. Values represent the means ± S.E.M. $^{***}P < 0.001$ compared to stimulated and untreated cells; $^{\#}P < 0.05$ compared to unstimulated and untreated cells; $^{\$}P < 0.05$ compared to dexamethasone-treated cells (ANOVA followed by Newman-Keuls multiple comparison tests, $n = 9$ determinations obtained in three independent experiments performed in triplicate)

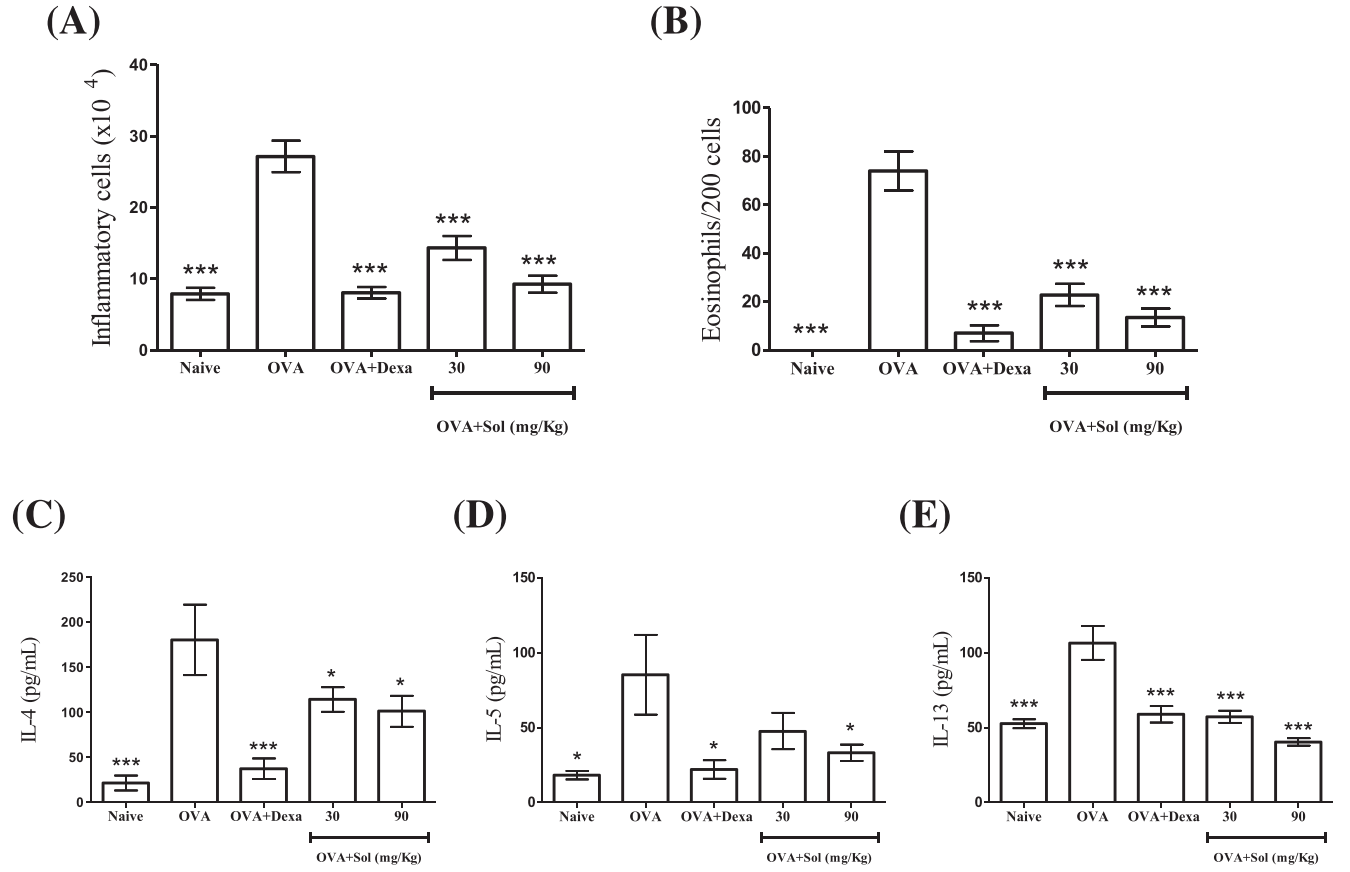

FIGURE 5 Leukocyte and Th2 cytokine quantification in BAL samples obtained from mice treated with solidagenone compared to the controls. The cellularity in BAL fluid from naïve ($n = 6$) or OVA-challenged mice ($n = 8$/each group) treated with saline (OVA), dexamethasone (OVA+Dexa) or solidagenone (OVA+SOL; 30 or 90 mg/Kg) was evaluated. Differential counts were completed on 200 cells per slide using standard morphological criteria. (A) Total cell counts. (B) Number of eosinophils in 200 cells. Concentration of plasma IL-4 (C), IL-5 (D) and IL-13 (E) were measured by ELISA. Values are expressed as means ± S.E.M. $^{***}P < 0.001$; $^{*}P < 0.05$ compared to OVA group (ANOVA followed by Newman-Keuls multiple comparison test, $n = 6$–8)

underlying inflammation may vary between clinical conditions, but they share many common mediators, like specific patterns of eicosanoid and cytokine production.

Numerous studies have demonstrated that the extract of *S. chilensis* has anti-inflammatory properties[4,5] including on activated macrophages, but here, we demonstrated for the first time the capacity of solidagenone, the main constituent of *S. chilensis*, to inhibit the inflammatory responses in an OVA-induced allergic airway inflammation model. In this model, animals present several features of allergic hypersensitivity, like histopathological changes in the lower airways, mainly triggered by the activity of leukocyte infiltrates, as well as by mucus hypersecretion, and by Th2 cytokines production. These characteristics are similar to those observed in acute human allergic asthma.[22,23]

We observed that solidagenone inhibits the production of nitric oxide, TNFα and IL-1β on activated macrophages, initial innate cells that display diverse function including the development and sustainability of innate and adaptive immune responses.[24] A previous study has shown the capacity of solidagenone to modulate activated peritoneal macrophages in vitro, by reducing NO production without presenting cytotoxicity.[25] They also demonstrated that some solidagenone derivatives presented anti-inflammatory effects in vitro. Here, we demonstrated for the first time the ability of solidagenone to modulate the production of inflammatory mediators by inhibition of NOS-2 and Cox-2 enzymes, corroborating data published with its derivatives. Cox-2 expression in vitro is reduced by solidagenone in agreement with data using docking analyses, which demonstrated that solidagenone had molecular interactions both with Cox-1 and Cox-2.[7] In addition, solidagenone downregulated the gene expression of CXCL9 and CXCL10; both chemokines were involved in respiratory allergic response and asthma exacerbations.[26,27]

In agreement with these data, solidagenone also induced a potent anti-inflammatory effect in mouse models of skin inflammation, where it significantly

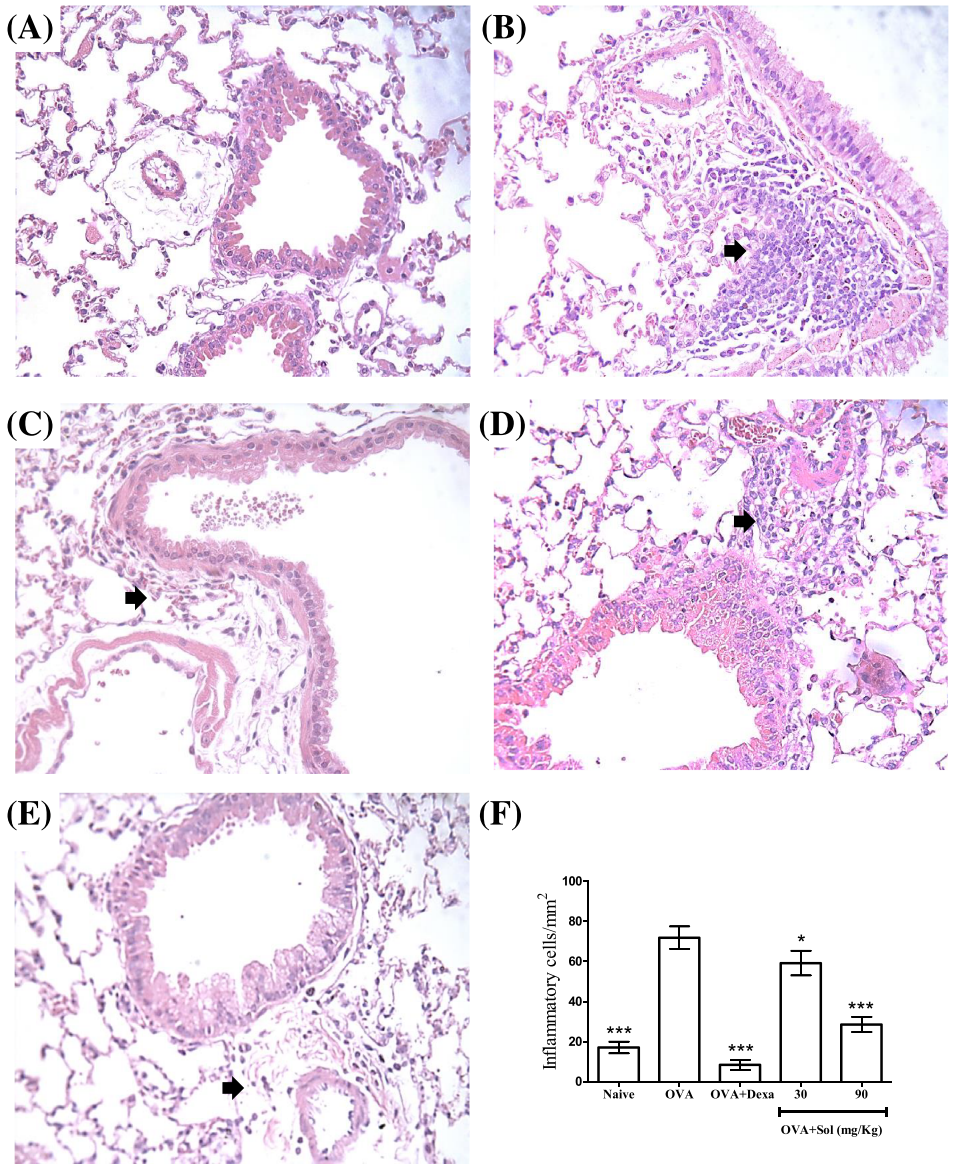

FIGURE 6 Representative sections of mice lungs induced airway inflammation by OVA and quantification of inflammation. (A) Naive. (B) OVA. (C) Dexamethasone (OVA + Dexa). Solidagenone (OVA + Sol) 30 mg/kg (D) and (E) 90 mg/kg. The number of inflammatory cells was evaluated on H&E-stained sections (magnification 400X). Large arrows indicate presence of inflammatory cells. (F) Quantification of inflammatory cells in the lungs of mice from the groups Naive, OVA + Dexa, or OVA + Sol (30 or 90 mg/kg) compared to OVA group. Values are expressed as means ± S.E.M. $^{***}P < 0.001$; $^{*}P < 0.05$ compared to OVA group (ANOVA followed by Newman-Keuls multiple comparison test, $n = 6–8$)

reduced IL-6, nitric oxide, TNFα levels and inflammatory parameters in the histopathological analysis as oedema thickness, leucocyte infiltration and vasodilation.[7] Adding to the effect of solidagenone on macrophages, we also showed a reduction of IFNγ and IL-2 production by lymphocytes, key cytokines involved in their proliferation and activation.[28,29] The effects observed after in vitro solidagenone treatment is similar to what is described for natural anti-inflammatory drugs; they typically reduce IL-1β, IL-6, leukotriene, prostaglandin and TNF-α levels, as well as inhibiting Cox-2, iNOS and NFκB.[30]

After in vitro experiments, we tested solidagenone in a murine model of allergic airway inflammation induced by OVA. In doses up to 600 mg/kg, solidagenone did not show signs of toxicity or mortality in mice.[8] Here, we tested 30 and 60 mg/kg of solidagenone and observed a marked reduction of leukocytes, especially of eosinophils, in BAL fluid and lungs. In addition to the cellular infiltrate during bronchial remodelling, hypertrophy of the submucosal glands and hyperplasia of goblet cells was also observed, which contributes to the narrowing of the airways.[31] Mucus hypersecretion after goblet cell metaplasia is recognized as an important contributor to morbidity and mortality in patients with lung diseases such as asthma and chronic obstructive pulmonary disease.[32] Besides the reduction of cellularity in the lungs, treatment with solidagenone also reduced mucus production and modulated the production of Th2 cytokines in vivo.

Inflammation is amplified by local responses of the epithelium, smooth muscle and fibroblasts through the production of chemokines, cytokines and proteases. Th2 cytokines are involved in mucus production by activating mast cells and eosinophils, cell types that produce autacoids, such as histamine and leukotrienes.[33] Thus, the

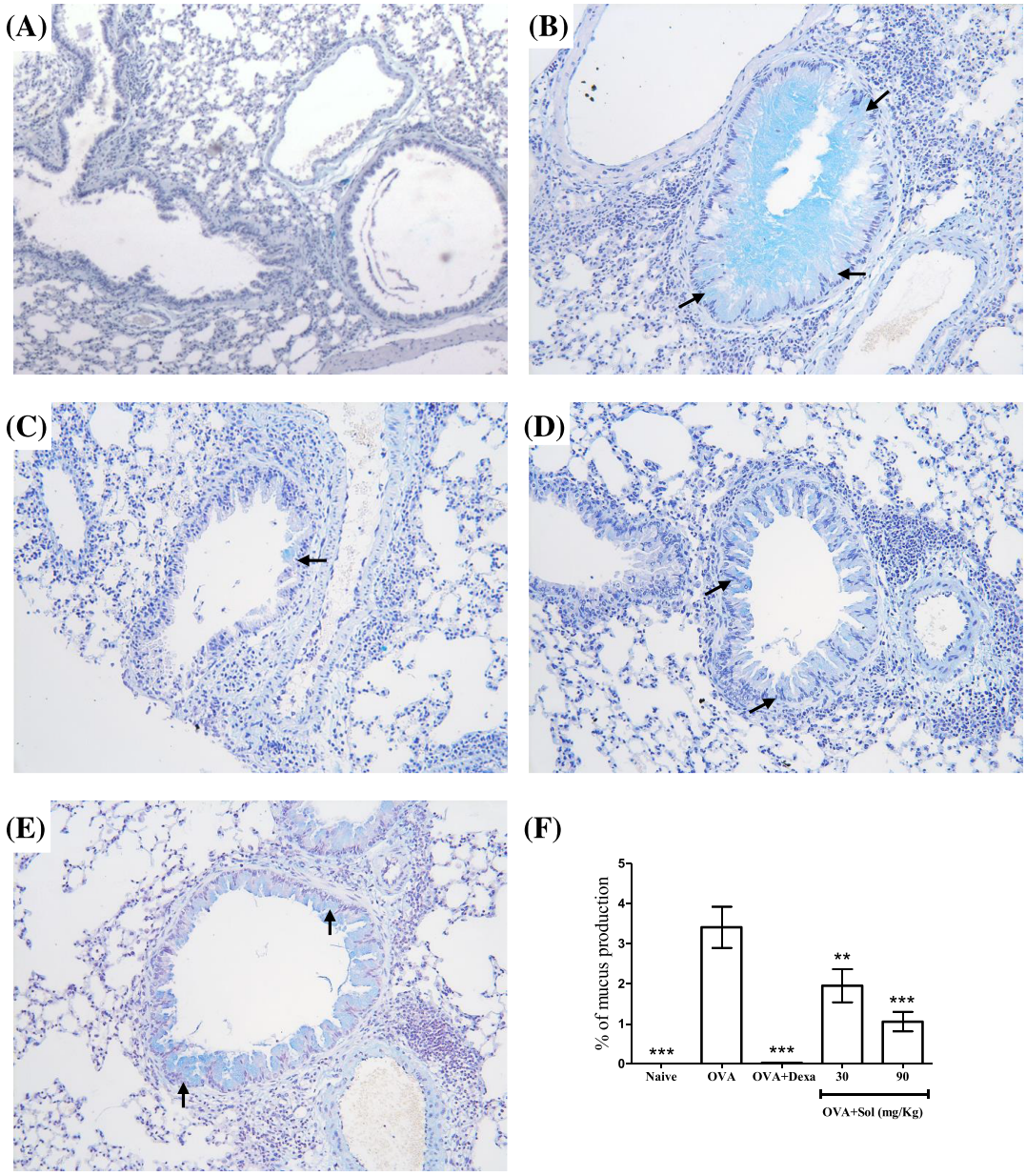

FIGURE 7 Mucus production in the lungs of mice induced to airway inflammation. (A) Naive. (B) OVA. (C) OVA + Dexa. OVA + Sol 30 mg/kg (D) and (E) 90 mg/kg. (F) Analysis of mucus production on alcian blue-stained lung sections (magnification 200X). The area of alcian blue staining was estimated by morphometric analysis. Narrow arrows indicate areas of alcian blue+ cells. Values are expressed as means ± S.E.M. $^{***}P < 0.001$; $^{**}P < 0.01$ compared to OVA group (ANOVA followed by Newman-Keuls multiple comparison tests, $n = 6–8$)

reduced mucus production found in solidagenone-treated mice may be due to the reduced inflammatory stimulus acting on goblet cells. A reduction in the exudate containing both mucus and cells implies a functional gain since it minimizes airflow obstruction to the airways,[34] being relevant for the potential anti-inflammatory effect of solidagenone described here. Although we did not evaluate airway hyperresponsiveness, the modulation of inflammatory response and the reduction of leukocytes in BAL and in the lungs after solidagenone treatment would protect mice from harmful stimulation in the lung.

Those effects described for solidagenone derivatives were accompanied by the downregulation of NF-κB activation and mitogen-activated protein kinase (MAPK) phosphorylation.[25] Since activation of NF-κB signalling is regarded as an important event in inducing allergic responses, such as airway inflammation and mucus production,[35] the effects of solidagenone seen in the present study may be due to inhibitory effects on NFκB

expression or on its phosphorylation. Further studies are needed to investigate the mechanisms by which solidagenone reduces the inflammatory response in this murine airway allergy model.

The adverse effects caused by chronic use of corticoids limit their systemic administration and therefore stimulate the search for new anti-inflammatory agents.[36,37] Solidagenone in the dose of 90 mg/kg presented similar effects to that of dexamethasone, as far as it showed efficacy in reducing Th2 cytokines, inflammatory cell counts and mucus production in the lungs in the allergic airway inflammation model. Further safety studies must be carried out to evaluate its safety profile; however, previous experimental findings suggest that solidagenone did not present toxicity or mortality in doses of up to 600 mg/kg.[8] Overall, these results suggest that solidagenone could be used as a potential substitute for corticosteroid therapy or even as a combination drug to associate with low doses of corticosteroids to decrease the risk of adverse effects, providing a strong rationale for further developing solidagenone as a candidate treatment for allergic hypersensitivity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

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