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Original Research Article

Rutin has anti-asthmatic effects in an ovalbumin-induced asthmatic mouse model

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Abstract

Purpose: *To investigate the anti-asthmatic effects of the flavonoid rutin in an ovalbumin (OVA)-induced asthmatic mouse model.*
Methods: *Asthma was induced by OVA induction. Effects of rutin (25–100 mg/kg BW) on interleukins (IL)-4, IL-5, and IL-13 and interferon (IFN)-γ in bronchoalveolar lavage fluid (BALF) and serum OVA-specific IgE levels were assessed by enzyme linked immunosorbent assay (ELISA). Airway hyperresponsiveness (AHR) was measured by determining lung compliance and airway resistance. Expression levels of p-I-κBα, p-NF-κB p65, TNF-α, and Foxp3 were also assessed. The $CD4^+$ $CD25^+$ $Foxp3^+$ T regulatory cell population was determined by flow cytometry.*
Results: *Rutin significantly ($p < 0.05$) decreased Th2 cytokines (IL-4, IL-5, IL-13), whereas it enhanced IFN-γ levels in BALF. Decreased OVA-specific serum IgE was also observed with improved lung histology. Rutin was effective in reducing AHR and regulating the expression levels of p-I-κBα, p-NF-κB p65, and TNF α, and also increased the $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cell population and Foxp3 mRNA and protein levels.*
Conclusion: *Rutin effectively suppresses OVA-induced asthma and improves airway function by suppressing inflammatory mediators and inflammatory cell infiltration.*

Keywords: *Asthma, $CD4^+$ $CD25^+$, Cytokines, Inflammation, Ovalbumin, Rutin, Protein levels*

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INTRODUCTION

Allergic asthma is a chronic airway disorder characterised by airway inflammation, airway hyperresponsiveness (AHR), and mucus hypersecretion [1]. The prevalence of asthma is increasing globally, especially in children [2]. An imbalance in the equilibrium between T helper cell type 1 (Th1) and T helper type 2 (Th2) immune responses appears to be the underlying mechanism that leads to the asthmatic inflammatory response, with an increased Th2 response [3]. Th2 cell-derived cytokines (IL-4, IL-5, IL-13) play key roles in the pathogenesis of asthma and have been implicated in AHR development, eosinophil accumulation, mucus hypersecretion, and lung remodelling [4].

It has also been demonstrated that NF-κB plays an important role in the pathogenesis of asthma [5]. NF-κB has been shown to be involved in the production of Th2 cytokines and in the recruitment of inflammatory cells [6], making NF-κB a potential molecular target in therapeutic strategies for asthma. Corticosteroids have long been used as the main therapeutic drug in asthma [7]. However, such treatments do not 'cure' the disease or even alter disease

progression; indeed, the symptoms return soon after treatment termination and the prolonged use of corticosteroids causes systemic and local side effects [8].

The use of natural plant products as alternative medicines in the treatment of various disease conditions has increased [9]. The flavonoids are a family of common phenolic plant pigments that possess anti-carcinogenic and anti-oxidant properties. Studies have also shown profound immunoregulatory activities [10]. Rutin, a flavonoid found especially in citrus fruits, such as lemon, grapefruit, lime, and orange, has anti-oxidant and anti-inflammatory activities [11]. Here, we investigated whether rutin could offer protection in asthmatic mice, via downregulation of inflammatory mediators, and improve airway function.

EXPERIMENTAL

Chemicals and antibodies

Antibodies against p-NF-κB p65, p-Iκ-Bα, TNF-α, Foxp3, and β-actin were from Cell Signaling Technology (Beverly, MA, USA). Rutin and ovalbumin (OVA) were from Sigma-Aldrich (St. Louis MO, USA). FITC-labelled anti-rat CD4, APC-labelled anti-rat CD25, and PE-labelled anti-rat Foxp3 were from eBioscience Co. (San Diego, CA, USA). All other chemicals and reagents used were from Sigma-Aldrich unless stated otherwise.

Experimental animals

Female BALB/c mice (18–22 g) were obtained from the Experimental Animal Centre of China Pharmaceutical University (Nanjing, China). All animals were housed in a sterile room (24 ± 1 °C, 40–60 % humidity) and were supplied with water and food *ad libitum*. The mice were acclimatised to the conditions for 1 week prior to experimentation. All experiments were performed in compliance with the Guidelines For the Care and Use of Laboratory Animals [12] and the study was approved by the Ethics Committee of Jining Medical University (Approval reference number:YXSA12443).

Ovalbumin sensitisation and challenge

Mice were sensitised and challenged with OVA as described previously [13] with minor modifications. OVA at 500 µg/mL in PBS was mixed with equal volumes of 10 % (w/v) aqueous aluminium potassium sulphate (alum; Sigma) and incubated at room temperature for about 1 h at pH 6.5 and centrifuged (750*g*, 5 min). The OVA/alum pellet was suspended to its original volume in distilled water. The animals were given 100 µg of OVA (0.2 mL of 500 µg/mL solution in normal saline) intraperitoneally (i.p.) on the first day. Mice were exposed to 250 µg OVA (100 µL of a 2.5 mg/mL solution) on day 8, and on days 15, 18, and 21, mice were challenged with 125 µg OVA (50 µL of 2.5 mg/mL solution) intranasally (i.n.), as described previously [13]. Mice were exposed to ketamine anaesthesia (i.p. injection of 0.2 mL of a mixture of ketamine, 0.44 mg/mL, and xylazine, 6.3 mg/mL, in normal saline) prior to OVA challenge. In the treatment group, rutin was administered at 25, 50, 100 mg/kg bodyweight (BW) orally every day from day 1 to day 21. On the days of OVA challenge, the mice received rutin 60 min before injections. A separate group of mice received dexamethasone (Dex; 2 mg/kg) i.p. 1 h before OVA injections. Dex served as a positive control. The normal control mice received no injections or rutin.

Collection and analysis of bronchoalveolar lavage fluid (BALF)

The mice were sacrificed after 24 h following the last OVA challenge, by pentobarbital overdose, and a tracheotomy was performed. Ice-cold PBS (0.5 mL) was infused into a lung and BALF was obtained by three successive aspirations (total volume 1.5 mL) via tracheal cannulation [14]. The collected BALF was then centrifuged (250 *g*, 5 min, 4 °C) and the supernatant was collected and stored at -70 °C. The supernatant was used for cytokine assays. The cell pellets were resuspended in PBS and differential cell counts were determined by staining with the Wright-Giemsa staining method.

BALF levels of IL-4, IL-5, IL-13 and IFN-γ were determined using ELISA. Kits were purchased from R&D Systems (Minneapolis, MN, USA).

Determination of OVA-specific IgE

Whole blood was collected and centrifuged (3, 000 rpm, 10 min, 4 °C) and the serum was separated and stored at -80 °C. OVA-specific serum IgE levels were determined using the Legend Max mouse OVA-specific IgE ELISA kit (BioLegend, San Diego, CA, USA) according to the manufacturer's protocol.

Measurement of airway hyperresponsiveness

Change in airway function was assessed as AHR, following challenge with aerosolised methacholine via the airway. AHR was determined using Buxco's modular and invasive

system (Buxco Electronics Inc., Wilmington, NC, USA). Changes in lung compliance (Cdyn) and airway resistance (RI) in response to increasing concentrations of methacholine were recorded directly, as described previously [15]. Briefly, the rats were tracheotomised and cannulated and placed in a whole body plethysmograph chamber connected to the ventilator. The rats were given various concentrations of methacholine (3.125, 6.25, 12.5, 25 mg/mL) via a jet nebuliser into the head chamber. The values are expressed as percentage of the respective basal values in response to phosphate-buffered saline (PBS).

Histological analysis

Lung tissues were collected and fixed in 10 % formalin, and embedded in paraffin wax; 5-µm sections were cut and stained with haematoxylin and eosin (H&E). For examination of mucus production, periodic acid-fluorescence Schiff stain (PAFS) was used. The mucin granules emit red fluorescence at excitation wavelengths of 380–580 nm and were observed at 600–650 nm using a Leica TCS SP5 confocal microscope (Leica Microsystems, Richmond, IL, USA) as described previously [16]. The inflammatory scores and expression levels of mucus-positive cells were calculated as described previously [17].

Flow cytometric analysis for $CD4^+$ $CD25^+$ $Foxp3^+$ expression

Cells from the BALF were analysed for $CD4^+$ $CD25^+$ $Foxp3^+$ expression. Briefly, prepared cells (1×10^6) were washed by centrifugation in flow cytometry staining buffer. For Treg analysis, cells were incubated with FITC-labelled anti-CD4 and APC-labelled anti-CD25 antibodies in staining buffer and incubated for 30 min at 4 °C. Following surface staining, the cells were fixed, permeabilised, and subsequently stained with anti-rat Foxp3 and finally resuspended in staining buffer and subjected to analysis by flow cytometry (FACSCalibur instrument with CellQuest software; BD Biosciences, Mountain View, CA, USA).

Quantitative real-time (RT)-PCR

The lung tissues of each group were homogenised in 1 mL of TRIzol (Invitrogen) using a glass homogeniser. Total RNA was isolated according to the manufacturer's protocol. RNA specific for Foxp3 was amplified using specific primers: Foxp3, 5'-CCC TCA AAG TTA CAA TCCTG-3' (forward) and 5'-GGG TTA GTG GCA AGT GAT A-3' (reverse), and GAPDH, 5'-GGG GAG CCA AAA GGG TCA TC-3' (forward) and 5'-GAC GCC TGC TTC ACC ACC TTC TTG-3' (reverse). Amplification was performed under the following conditions: initial denaturation at 94°C for 10 min followed by 40 cycles of 94 °C for 20 s and annealing at 53 °C for 25 s, and a final extension at 72 °C for 30 s in a Line-Gene FQD-33A instrument (Bioer Inc., Hangzhou, China).

Western blot analysis

Following homogenisation of the lung tissue, the cells were subjected to lysis on incubation with 0.5 mL ice-cold whole-cell lysate buffer (0.5 M EDTA, 5 M NaCl, 10 % Nonidet P-40, 0.1 M phenylmethylsulphonyl fluoride, 0.1 M EGTA, 1 M sodium fluoride, 1 M HEPES, 0.2 M sodium orthovanadate, 2 µg/mL aprotinin and 2 µg/mL leupeptin) on ice for 30 min. Protein concentrations were determined by a Bradford assay using a kit from Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of protein (60 µg) from each group were subjected to SDS-PAGE. Separated proteins were blotted and transferred to a nitrocellulose membrane and incubated with primary antibodies against p-NF-κB p65 (Ser 536), p-Iκ-Bα, TNF-α, and Foxp3 and further incubated with peroxidase-labelled secondary antibody. The immunoreactive bands were visualised with a chemiluminescence system (Amersham Bioscience, Little Chalfont, UK). The band densities of the proteins analysed were normalised to those of β-actin.

Statistical analysis

Results are presented as mean ± standard deviation (SD), from three or six independent experiments. Data were analysed for statistical significance at $p < 0.05$ by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) as a *post hoc* analysis.

RESULTS

Rutin reduced inflammatory cells in BALF

Changes in total cell levels in the BALF were examined at 24 h after the last OVA challenge. In the OVA-sensitised mice, infiltration of inflammatory cells was observed. OVA challenge significantly ($p < 0.05$) induced influx of eosinophils and neurophils into BALF (Figure 1). Suppression of cellular infiltration was observed in OVA-challenged and rutin-administered mice. OVA-sensitised mice that were treated with rutin at 100 mg showed reduced eosinophil and neutrophil counts versus mice treated with lower doses of rutin. The drug control Dex also exerted

similar suppressive effects on cellular influx into BALF after OVA challenge.

Rutin decreased the level of IgE in serum

Th2-type cytokines exert important roles in the pathogenesis of allergic asthma by regulating IgE production. Expression and cross-linking of allergen-specific IgE on surfaces of mast cells upon specific allergen challenge initiates early allergic asthmatic responses. Serum levels of IgE specific to OVA were measured at 24 h after the last OVA challenge. OVA challenge resulted in a several-fold elevation in serum OVA-specific IgE levels versus the control mice (Figure 2). Rutin administration caused a significant decrease ($p < 0.05$) in the levels of OVA-specific IgE in a dose-dependent manner, with the 100 mg dose of rutin causing more marked reductions.

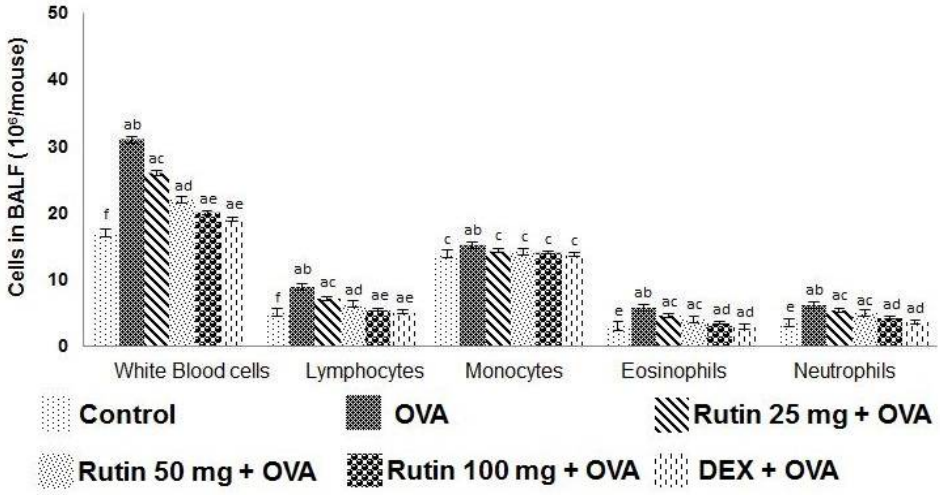

Figure 1: Effect of rutin on cell accumulation in bronchoalveolar lavage fluid (BALF). Rutin significantly reduced cellular infiltration in the BALF. Values are presented as means ± SD, $n = 6$. a indicates statistical significance at $p < 0.05$ vs. the respective controls; b–f indicate significant differences ($p < 0.05$) between mean values as determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT)

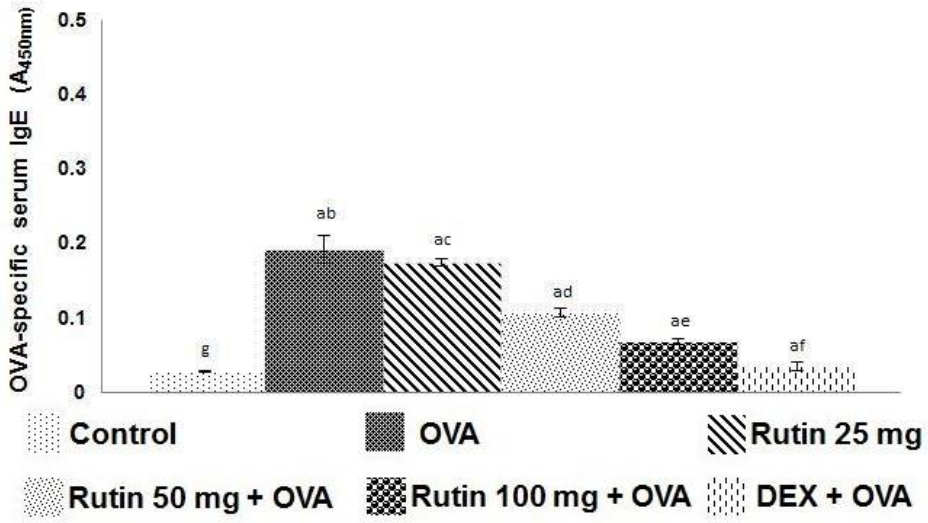

Figure 2: Rutin reduced ovalbumin (OVA)-specific IgE levels in serum. Values are presented as means ± SD, $n = 3$. a indicates statistical significance at $p < 0.05$ versus the respective controls. b–f indicate significant differences ($p < 0.05$) between mean values as determined by one-way ANOVA followed by DMRT analysis

Influence of rutin on Th2 cytokine levels and IFN-γ in BALF

The influence of rutin on the expression of cytokines, (Th2 cytokines: IL-4, IL-5, and IL-13, and the Th1 cytokine, IFN-γ) in BALF was determined by ELISA 24 h after final OVA challenge. OVA challenge caused significantly increased ($p < 0.05$) BALF levels of IL-4, IL-5, and IL-13 versus the control group (Figure 3). However, the level of IFN-γ was slightly lower than the control group level. Rutin administration

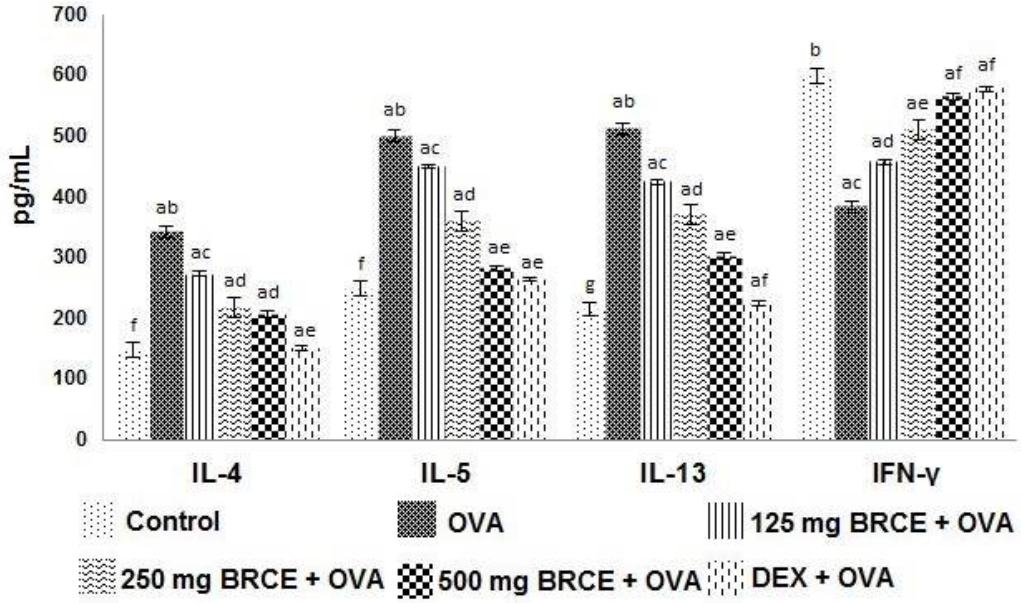

Figure 3. Effects of rutin on the levels of Th2 cytokines and IFN-γ in BALF. Values are presented as means ± SD, $n = 3$; a indicates statistical significance at $p < 0.05$ versus the respective controls. b–f represent significant differences ($p < 0.05$) between mean values within the groups of the same cell line, as determined by one-way ANOVA followed by DMRT analysis

caused a marked decrease in the level of Th2 cytokines, in a dose-dependent manner, with a gradual increase in the level of IFN-γ in BALF. Rutin at 25 mg caused a reduction in levels of IL-4, IL-5, and IL-13; the higher doses of 50 and 100 mg caused more pronounced decreases with a significant increase in BALF IFN-γ levels.

Rutin improved airway function and reduced airway hyperresponsiveness

The effect of rutin on AHR in response to increasing concentrations of methacholine was determined. AHR is the excessive narrowing of the airways in response to various allergic stimuli as contractile agonists of OVA. Asthma is characterised by AHR. RI and Cdyn were measured following OVA challenge. Cdyn refers to lung distensibility and is defined as the change in volume of the lung produced by pressure change across the lung. RI can be defined as the pressure driving respiration divided by the air flow. In our study, the OVA-challenged mice developed high AHR, indicated by high RI and low Cdyn (Figure 4a, b). Treatment with rutin (25, 50, or 100 mg) markedly ($p < 0.05$) restored Cdyn and reduced RI in OVA-challenged mice in response to methacholine in a dose-dependent manner.

Rutin restored lung architecture

Histological analysis was conducted to assess changes in the architecture of the lung tissue. H&E analysis showed that OVA challenge induced marked infiltration of inflammatory cells into the peribronchiolar and perivascular connective tissues versus normal lung tissue (Figure 5). Rutin (25, 50 or 100 mg/kg) strikingly suppressed infiltration of eosinophils and neutrophils in the lung tissues and also restored the normal histology. Dexamethasone treatment also resulted in tissue sections with almost negligible alterations and infiltration versus the control. Rutin at 100 mg showed almost no change in the extent of inflammatory cell infiltration, similar to normal histology. Furthermore, goblet cell hyperplasia with hyper-secretion of mucus was observed in OVA-challenged mice, and was almost normalised upon rutin treatment (Figure 6).

Rutin modulated expression levels of proteins in the inflammatory pathway

The pathogenesis of asthma development is associated with NF-κB activation. OVA challenge resulted in a robust increase in the expression levels of p-NF-κB p65 (Ser 536), p-I-κBα, and TNF-α, whereas Foxp3 expression was downregulated (Figure 7). However rutin administration caused significant decreases in the levels of NF-κB p65, p-I-κBα, and TNF-α, with s substantially elevated level of Foxp3. Furthermore, to assess the effects of rutin on Foxp3 expression at the mRNA level, RT-PCR was performed. Rutin at 25, 50, and 100 mg upregulated Foxp3 mRNA in a dose-dependent manner (Figure 7). The anti-inflammatory effects of rutin could also be due in part to the increased

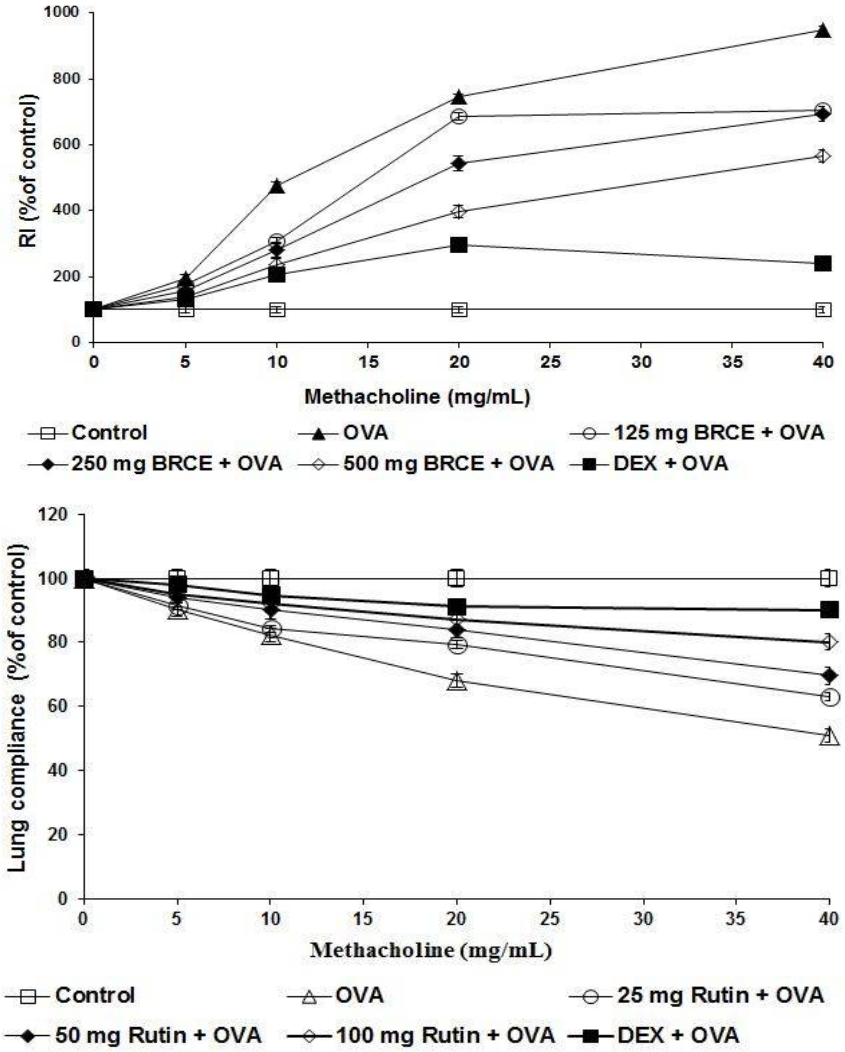

Figure 4: Effect of rutin on airway hyperresponsiveness. Rutin markedly reduced airway resistance and improved lung compliance in mice exposed to OVA. Values are presented as means ± SD; $n = 6$

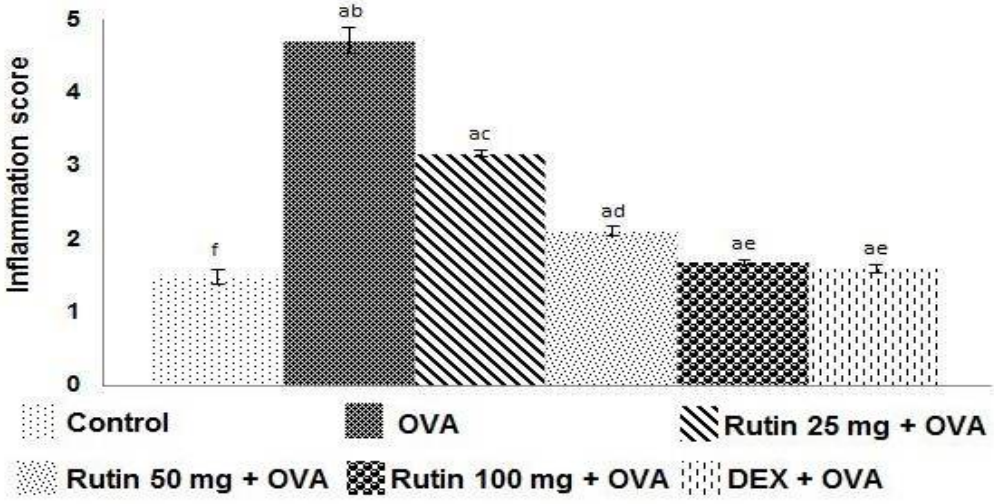

Figure 5: Rutin reduced inflammatory cell infiltration. Rutin markedly inhibited inflammatory cell recruitment into lung tissue. Values are presented as means ± SD, $n = 3$; a indicates statistical significance at $p < 0.05$ versus the respective controls; b–f represent significant differences ($p < 0.05$) between mean values, as determined by one-way ANOVA followed by DMRT analysis

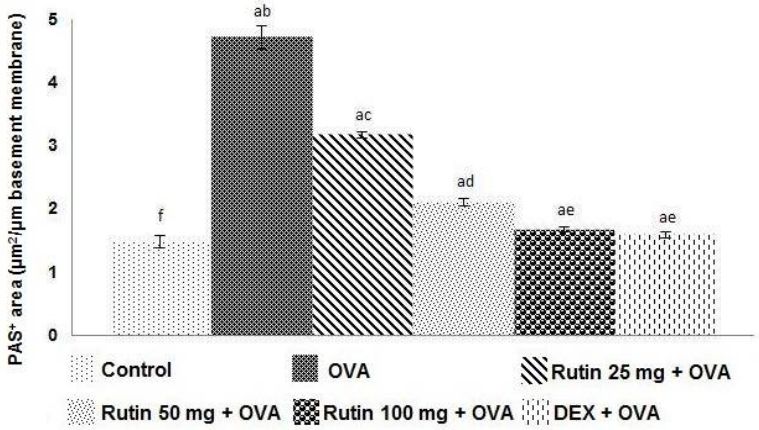

Figure 6: Rutin reduced mucus hypersecretion. Mucus hypersecretion is a characteristic of allergic asthma. Rutin markedly reduced mucus accumulation and hypersecretion in the bronchioles. Values are presented as means ± SD, n = 3. a indicates statistical significance at $p < 0.05$ versus the respective controls; b–f indicate significant differences ($p < 0.05$) between mean values, as determined by one-way ANOVA followed by DMRT analysis

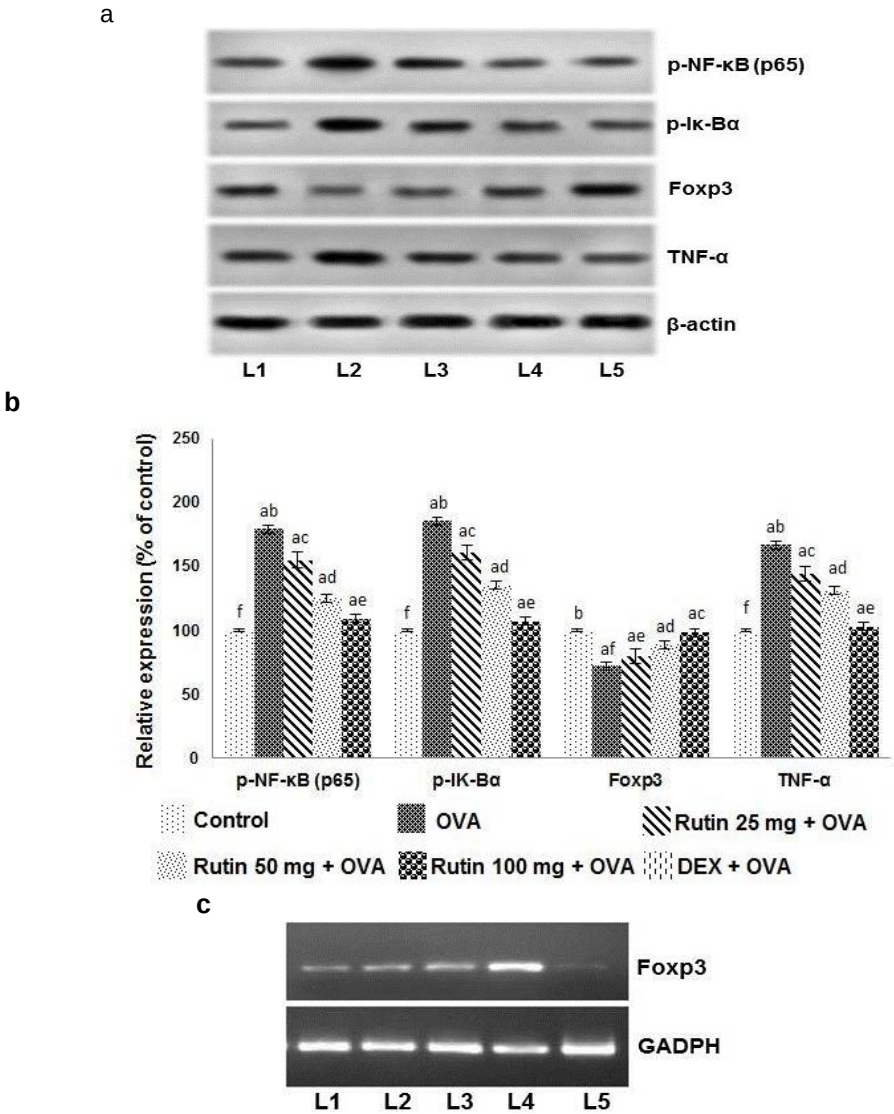

Figure 7: Effects of rutin on the levels of inflammatory mediators and Foxp3. OVA-induced enhanced expression of NF-κB signalling pathway proteins and decreased Foxp3 expression were modulated effectively by rutin. Values are presented as means ± SD, n = 3; a indicates statistical significance at $p < 0.05$ versus the respective controls; b–f represent significant differences ($p < 0.05$) between mean values. as determined by one-way ANOVA followed by DMRT analysis; (L1-Control; L2-OVA; L3-25 mg Rutin + OVA; L4-50 mg Rutin + OVA; L5-100 mg Rutin + OVA)

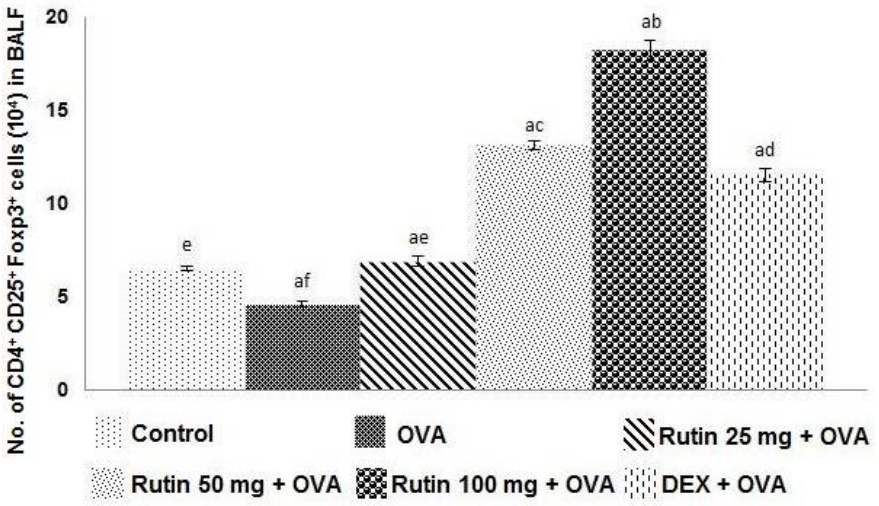

Figure 8. Rutin increased the $CD4^+$ $CD25^+$ $Foxp3^+$ Treg population in BALF. $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs are key in maintaining immune homeostasis. Rutin markedly increased the $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cell population. Values are presented as means ± SD, $n = 3$. a indicates statistical significance at $p < 0.05$ versus the respective controls. b–f represent significant differences ($p < 0.05$) between mean values within groups of the same cell line, as determined by one-way ANOVA followed by DMRT analysis

expression of Foxp3. These results suggest anti-inflammatory effects of rutin.

Influence of rutin on the $CD4^+$ $CD25^+$ $Foxp3^+$ Treg population in BALF

$CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells play an important role in the regulation of asthma pathogenesis. Furthermore, allergen-specific Tregs are key in asthma therapy. In our study, we examined the population of $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs in BALF of OVA-challenged mice. OVA challenge significantly reduced the $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cell population (Figure 8). However, rutin administration caused a striking increase in the level of $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs that was consistent with Foxp3 mRNA expression. The 100 mg dose of rutin resulted in an increased population of these cells, suggesting an effect of rutin against OVA-induced allergic asthma.

DISCUSSION

Asthma is a chronic inflammatory disease of the lungs, characterised by infiltration of inflammatory cells, especially eosinophils, into the lungs and airways, leading to AHR, bronchoconstriction, and mucus production [14]. The prevalence of asthma is increasing rapidly worldwide; the disease may cause severe morbidity and even mortality in aggravated episodes. Currently, inhaled corticosteroids (ICSs) and bronchodilators are employed for asthma management for relieving bronchoconstriction [18]. Nevertheless, prolonged use of ICS at higher doses, in particular, has been reported to be associated with side effects [18] and oesophageal candidiasis [19]. Thus, there is a continuing need for the identification of new and targeted approaches. In our study, we investigated the role of rutin in exerting protective effects in an OVA-induced asthma model.

In asthma, bronchoconstriction occurs due to contraction or hypertrophy of airway smooth muscles and inflammation, leading to decreased lung function [20]. AHR is a measure of such bronchial constriction. OVA-induced mice showed severely impaired Cdyn and increased AR. However, rutin reduced AR and improved lung function in response to inhaled methacholine.

Migration of inflammatory cells, specifically eosinophils and lymphocytes, into the lungs is a key event in allergic asthma and is a major cause of allergic airway inflammation [21]. An increase in the number of eosinophils and neutrophils in BALF is a characteristic of asthma. In the present study, rutin significantly reduced the infiltration of inflammatory cells in BALF and lung tissues.

The marked increase in levels of cytokines (IL-4, IL-5, IL-13) in BALF observed in our study following OVA sensitisation and challenge, along with the increased recruitment of inflammatory cells, is suggestive of AHR. In allergic asthma, the Th2 cytokines have been found to play vital roles in the pathogenesis of inflammation and also induce many features of asthma [22]. Effective decreases in the levels of Th2 cytokines with a substantial increase in IFN-γ levels showed the efficacy of rutin in inhibiting OVA-induced airway inflammation and enhancing Th2

responses. Additionally, the balance of Th1/Th2 cytokines is essential in regulating inflammatory responses. It is known that asthma and inflammatory responses are associated with the increased production of Th2 cytokines (IL-4, IL-5, IL-13) and reduced levels of the Th1 cytokine IFN-γ. Here, rutin reduced Th2 cytokine levels while improving IFN-γ levels, indicating a positive Th1/Th2 cytokine balance and anti-inflammatory effects. These observed decreases in Th2 cytokines could also have caused decreased inflammatory cell recruitment in BALF. Because IL-4, IL-5, and IL-13 are associated with activation of eosinophils and production of IgE by B cells, the decrease in cytokine levels by rutin could have also contributed to the decreased IgE levels in serum, which could also have been due to the suppression of IgE production by rutin itself.

Consistent with the BALF levels of cytokines and cellular infiltration, the histopathological analysis with H&E and PAS staining revealed increased infiltration of inflammatory cells, and the characteristic morphology of cells, with hyperproduction of mucus by goblet cells upon OVA challenge. Activation of allergen-induced Th2 cells has been reported to be associated with goblet cell hyperplasia and increased mucus secretion [23]. Rutin treatment caused the suppression of PAS-positive cell counts and area in bronchial tissues of OVA-challenged mice. Hypersecretion of mucus in the airway, along with allergic inflammation, plays a major role in the pathogenesis of asthma. Mucus accumulation in the bronchus narrows the airway and increases AHR. Rutin-induced inhibition of inflammatory cell infiltration, in BALF and in lung tissues, along with the decrease in cytokine secretion could also have contributed to decreased mucus production.

Moreover, continued activation of NF-κB has been observed in allergic airway inflammation and is believed to be involved in airway remodelling in human and animal models of asthma [24]. NF-κB, as a vital transcription factor in Th2 cell differentiation [25], regulates innate and adaptive immune responses by causing the expression of various genes that are involved in inflammation, as well as leukocyte migration and activation. Nevertheless, in cells not stimulated by allergens, NF-κB remains sequestered in the cytoplasm by an inhibitor of κB (IκB) [26], whereas upon activation, NF-κB unit p65 translocates from the cytoplasm to the nucleus, eventually leading to the synthesis and subsequent release of proinflammatory cytokines [27]. The increased expression of NF-κB p65, p-IκB, and TNF-α suggests the activation of NF-κB in OVA-challenged mice, thus leading to expression of cytokines and inflammatory mediators. Rutin was found to also suppress the activation of NF-κB p65, IκB, and TNF-α, thus suppressing the NF-κB signalling pathway leading to inflammatory responses.

Tregs, a specialised sub-population of T cells, exert important roles in autoimmunity and immune tolerance. A considerable amount of research has focused on naturally occurring $CD4^+$ $CD25^+$ Tregs that have been shown to be critically involved in preventing autoimmunity [28]. $CD4^+$ $CD25^+$ Tregs that co-express forkhead/winged helix transcription factor (Foxp3) exhibit strong anti-inflammatory roles and function to maintain tolerance to self-components, either through direct contact with cells or by releasing anti-inflammatory cytokines such as IL-10 [29]. Rutin at the doses tested was found to markedly enhance the percentage of $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs in BALF versus the OVA control, with the 100 mg dose showing the maximum effect. Moreover, rutin treatment also upregulated the expression of Foxp3.

The observed reductions in airway inflammation upon rutin treatment could be due, in part, to the inhibitory effects on IL-4, IL-5, and IL-13, and also to enhanced numbers of $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs. Foxp3 functions as a transcriptional repressor for various transcription factors, such as nuclear factor of activated T cells (NFAT), NF-κB, and Runx1 [30], thus suggesting possible multi-target molecular effects of rutin. Furthermore, $Foxp3^+$ Tregs are known to inhibit eosinophil recruitment, mucus hyper-production, and Th2 cytokine release [29]. Thus, the enhanced Foxp3 expression and increased $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs on rutin treatment indicate potent anti-inflammatory effects of rutin in OVA-challenged mice.

CONCLUSION

Rutin effectively suppressed the expression of inflammatory mediators and regulated NF-κB signalling, indicating the effectiveness of rutin as a potential candidate for the treatment of allergic asthma. More clinical research is needed to further explore the molecular events involved.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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