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ORIGINAL ARTICLE



Combination of Sarsasapogenin and Fluticasone attenuates ovalbumin-induced airway inflammation in a mouse asthma model

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ABSTRACT

Objective: Asthma is a very common airway inflammatory disease for which the existing drug therapy options are insufficient. In this study, we explored the mechanisms underlying the anti-inflammatory potential of Sarsapogenin (SG) and its combination with Fluticasone (FC) in ovalbumin (OVA)-induced allergic asthma in mice.

Methods: In a standard experimental model, asthma in mice was sensitized and challenged by OVA. The mice were treated with SG and SG + FC during OVA challenge. At the completion, lung weight, inflammatory cell count in bronchoalveolar lavage fluid (BALF), serum cytokines levels, immunoglobulin E (IgE) levels, lung nitrate/nitrite (NO) levels, and lung tissue oxidative stress biomarkers were determined. Histopathological evaluation of the lung tissue was also performed.

Key findings: Treatment of mice with SG and SG + FC combination intensely diminished the trafficking of total and differential inflammatory cells count into BALF. SG and SG + FC administration significantly reduced the production of inflammatory cytokines, serum IgE levels and restoration of antioxidant stress markers. Histopathological analysis of lung samples effectually weakened bronchial inflammation and mucus production in the lung with a significant reduction in inflammation and mucus score.

Conclusion: Our study results suggested that SG and SG + FC effectively reduced allergic airway inflammation via inhibiting pro-inflammatory cytokines, NO expressions and oxidative stress parameters. So, it could be used as a therapeutic potential agent for the treatment of asthma by decreasing its dose in combination with FC to avoid the chronic adverse effects of FC.

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KEYWORDS

Allergic asthma; ovalbumin; Sarsapogenin; Fluticasone; pro-inflammatory cytokines; histopathology

Introduction

Asthma is a long-lasting airways disorder affecting about eighteen percent of the world populace. According to 'The Global Initiative for Asthma,' around 300 million of asthmatic patients were reported and triggered 346,000 demises each year in the globe [1]. It is a familiar allergic disease characterized by bronchial airways inflammation [2]. The symptoms of asthma are bronchoconstriction, cough, wheezing, dyspnea, obstruction of airflow, and tightness in the chest [3]. On exercise or at night time, the symptoms of asthma become more provoked [4]. The pathophysiological features of asthma include overproduction of mucus, infiltration of lymphocytes, eosinophils, and mast cells at the site of inflammation [5]. Ovalbumin (OVA) is a key allergen, which is used for the experimental induction of asthma in animal models [6]. The key mechanism behind the OVA-encouraged asthma comprises of overexpression of nitric oxide, Type-II T helper (Th2) cytokines such as interleukin (IL)-4, IL-5, and IL-13. Cytokine IL-4 is responsible for activation of B cells for immunoglobulin production [7]. IL-5 is an eosinophil regulatory cytokine participated in the activation, differentiation, and eosinophils survival [8]. However, IL-13 is associated with the induction of allergic asthma involving airway-hyper-

responsiveness, mucus over production and airway remodeling [9]. Together with other Th-1 (pro-inflammatory) cytokines such as tumor necrosis factor (TNF)- α , IL-6, IL-12, and IL-1 β contribute to the mechanism of OVA induced of allergic asthma [10]. Abundant research literature suggests that nitric oxide content and reactive oxygen radical play a vital part in the bronchial swelling that denotes the severity of asthma [11].

Sarsasapogenin (SG) (Figure 1) is a steroidal sapogenin obtained from many smilax species such as *Smilax ornata*, *S. aspera*, *S. febrifuga*, *S. china*, *S. aristolochiifolia*, and *S. officinalis* belonging to the family *Smilacaceae* [12]. It is also found in the rhizome of *Anemarrhena asphodeloides*, which is used in Chinese traditional medicine. It is used as a preliminary material for the synthesis of steroids. It shows numerous pharmacological activities, such as antitumor and anti-depressant activities. Hu et al. [13] have studied the memory-enhancing activity of SG by increasing the concentration of muscarinic acetylcholinergic receptor in the brains of memory-deficit rat models. SG obtained from *S. officinalis* has anti-inflammatory activity comparable to conventional anti-inflammatory drugs like dexamethasone [14]. It is also used for the treatment of psoriasis, arthritis and rheumatism

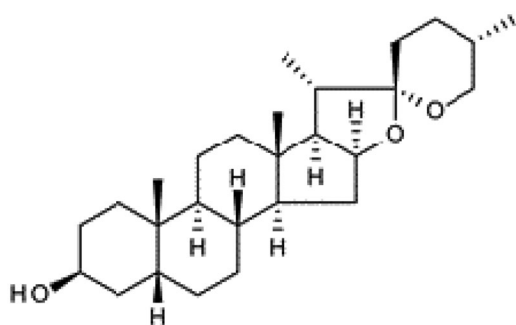


Figure 1. Structure of Sarsapogenin.

[15–17]. Recently, Liu et al. [18] have studied the effects of SG in rats against the early stage of diabetic nephropathy by inhibition of IL-18, NLRP3 gene, and caspase 1 in renal cortex of diabetic rats. To the other side, different preclinical research studies have shown significant anti-inflammatory and antioxidant effects of SG [19]. However, none of the study has been reported on the probable protective effect of SG in the treatment of OVA-induced allergic asthma inflammation. Glucocorticosteroids (GCs) are the choice of drug for the regular treatment of asthma. However, these drugs are normally associated with major adverse effects. The chronic use of minimum dose of GCs with phytosteroid like SG can be a possible therapeutic substitute for the treatment of asthma patients with fewer side effects.

Glucocorticoids are the gold standard therapy used for the treatment of asthma. Fluticasone (FC) is a locally acting glucocorticoid used in clinical practice for asthma therapy [20]. FC at increased dose concentration interrelates with deoxyribonucleic acid to stimulate transcription process *via* improved histone acetylation of anti-inflammatory genes associated with adverse effects of glucocorticoid known as trans-activation. However, the prolonged clinical use of FC at high doses is associated with numerous side effects such as diabetes, metabolic syndromes, peptic ulcers, high blood pressure, glaucoma, gastrointestinal tract irritation, and bone marrow suppression [21]. FC also decreases the stability of pro-inflammatory mRNAs due to its post-transcriptional effects [22]. Still, the combination of glucocorticoids with bronchodilators is incapable to regulate the asthmatic signs and caused several difficulties [23]. Hence, the formulation of novel drugs which can directly diminish or relieve the indications of asthma is instantly desired.

Therefore, the basis behind such type of study was to reduce the dosage of conventional glucocorticoid by administering SG with FC. Hence, the work was planned to explore the anti-inflammatory potential of SG and SG + FC combination in allergic asthma induced by OVA in mice by studying its effect on total and differential cell count in BAL fluid, oxidative markers, and serum cytokines. Immunoglobulin E and histopathological analysis of lung sections were also performed.

Materials and methods

Drugs and reagents

SG, OVA, trichloroacetic acid (TCA), hexadecyltrimethylammonium bromide (HTAB), O-dianisidine hydrochloride, Griess

reagent, thiobarbituric acid (TBA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and pyrogallol were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). A gift sample of FC was acquired from Sun Pharma (Gujarat, India). Mouse TNF- α , IL-6, IL-12, TXB₂ (thromboxane B₂) and IgE enzyme-linked immunosorbent assay (ELISA) kits were purchased from Krishgen Biosystems (Mumbai, Maharashtra, India). Other chemicals and reagents used in the research were analytical grades.

Animals

Female Swiss albino mice and male BALB/c mice (18–20 g) were purchased from the National Institute of Biosciences (Pune, Maharashtra, India). Mice were maintained in the specific pathogen-free condition with a 12-h light/dark cycle and free access to standard pellet food and water. All experiments procedures were sanctioned by the Institutional Animal Ethics Committee (IAEC) of Sinhgad College of Pharmacy, Pune (SCOP/IAEC/2014-15/202).

Acute toxicity study

Acute oral toxicity study was executed in female Swiss albino mice (20–25 g) as per the Organization for Economic Cooperation and Development (OECD) guidelines-425. The mice were kept on fasting condition 3 h before starting the study. The mice were treated with 2000 mg/kg dose of SG and were monitored for clinical signs of toxicity or death for the next forty-8 h [24].

Selection of SG and FC dose

The research work was carried out to minimize the side effects of FC by combining FC with Phytosteroid SG. The dose of SG (50 μ g/mice) and SG + FC (25 μ g/mice, each) were chosen on the basis of pilot study. At the starting, the study was performed by selecting various doses of SG such as 50, 75, and 100 μ g/mice and all of the above doses show significant anti-inflammatory activity in mice. Hence, the lowest dose of SG (50 μ g/mice) was selected for further anti-asthmatic activity.

Induction of OVA-induced allergic asthma and treatments

Thirty BALB/c mice were arbitrarily divided into five groups ($n = 6$) as follows: normal control (NC) group, OVA group, SG (50 μ g/mice) group, FC (50 μ g/mice) group, and SG + FC (25 μ g/mice, each) group. Briefly, BALB/c mice were sensitized by intraperitoneal injection of 20 μ g of OVA and 2 mg of aluminum hydroxide emulsified in 200 μ L of phosphate-buffered saline (PBS) (pH 7.4) on 1 and 14 days. From 21, 22, and 23 days, mice were challenged by intranasal inhalations of 100 μ g of OVA in 50 μ L of PBS once/day. The drug treatments such as 20 μ L of SG (50 μ g/mice), FC (50 μ g/mice) and SG + FC (25 μ g/mice, each) were administered intranasally before 1 h of OVA challenge. The mice of the NC group were sensitized

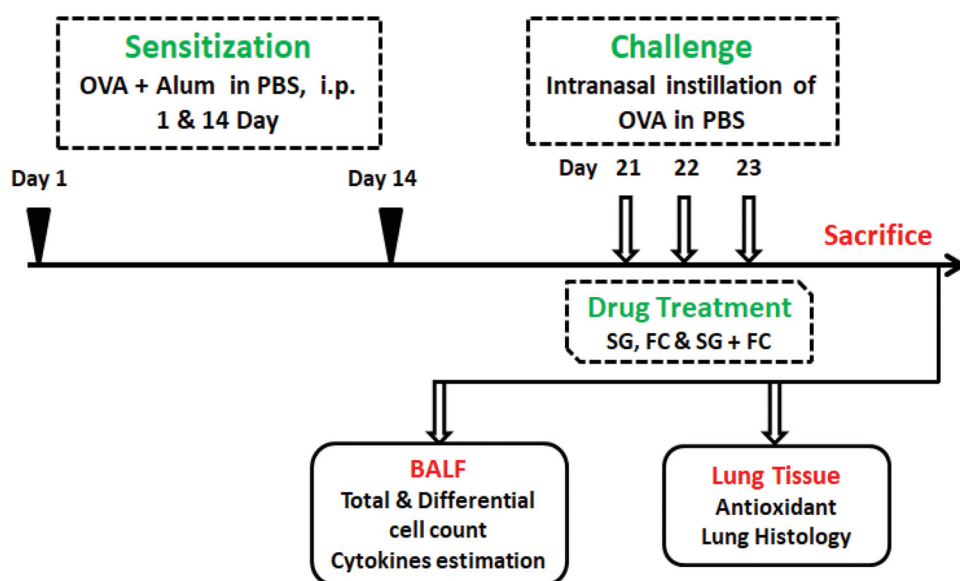


Figure 2. Experimental protocol for induction of allergic asthma and treatment in mice.

and challenged with an equal volume of PBS by using the same method. 24 h after the last OVA challenge (24 days), the mice were sacrificed to explore the anti-inflammatory potential of SG and SG + FC on bronchial airway inflammation [25]. The experimental induction of asthma is depicted in Figure 2.

Collection of blood and bronchoalveolar lavage fluid

On day 24 after the last OVA challenge, mice were euthanized with thiopental (40 mg/kg, i. p.). The blood samples were collected by puncturing the vena cava. The blood samples were subjected to centrifugation at 3000 rpm (4°C for 10 min) and the serum samples were stored (–80°C) for the estimation of cytokines and immunoglobulin. The mice were tracheostomized and cannulated to collect BALF. The lungs were lavaged with cold PBS (0.8 mL) thrice to collect BALF. The collected BALF samples were centrifuged instantly at a speed of 1000 × g (4°C for 10 min) and kept at –80 °C.

Total and differential cell count in BALF

The cell pellets from BALF were resuspended in PBS (0.5 mL) and used for total and differential inflammatory cell counting. Total cell counting was done with a hemocytometer. Wright's and Giemsa stain were used for counting of differential cell counts [26].

Determination of lung weight/body weight ratio and preparation of lung homogenate

After collection of BALF, lung samples were removed and washed with ice-cold potassium chloride (1.15%; pH 7.45) and then weighed. Then, lung weight/body weight ratio was determined [27]. Chilled potassium chloride was used for the homogenization of the left lung to obtain lung homogenate (10% w/v) [28]. Then, a variety of oxidative stress biomarkers were measured from the prepared lung homogenate.

Estimation of oxidative stress biomarkers

Estimation of malondialdehyde (MDA)

MDA levels were evaluated in the lung homogenate according to the procedure of Ohkawa et al. [29].

Estimation of reduced glutathione (GSH)

Estimation of GSH was carried out by the method of Ellman [30].

Estimation of superoxide dismutase (SOD)

The SOD activity was measured by the Marklund and Marklund's [31] method.

Estimation of myeloperoxidase (MPO)

The MPO activity was determined by the method of Barone et al. [32].

Estimation of nitrite/nitrate content in lung

The total nitrite/nitrate ($\text{NO}_2^-/\text{NO}_3^-$) content of lung homogenate was estimated by the procedure of Green et al. [33].

Measurement of serum cytokine levels

The levels of IL-6, IL-12, TNF- α , and TXB₂ cytokines were measured by ELISA kits, purchased from Krishgen Biosystems according to the manufacturer's instructions.

Measurement of serum IgE levels

The level of IgE was evaluated by ELISA kits, purchased from Krishgen Biosystems according to the manufacturer's instructions.

Histopathological analysis of lung tissue

The lungs were fixed in neutral buffered formalin, inserted in paraffin wax and sectioned 4 μ m thick. The lung sections were stained with hematoxylin and eosin (H&E) to evaluate airway inflammation, periodic acid Schiff (PAS) to measure mucus formation and toluidine blue to measure mast cells infiltration. The lung inflammation score was evaluated by using a blind observer by following scale: 0=no inflammation; 1=mild inflammation; 2=moderate inflammation; 3=marked inflammation; and 4=severe inflammation. 0.5 scale can be used when the degree of inflammation falls between two scoring points [34]. The counting of goblet cells in the lung epithelium was implemented by PAS staining. The amount of mucus formation in the airway was calculated and scored by the procedure of Reber et al. [35]. The mast cells present in bronchiolar epithelial tissue was evaluated by toluidine blue staining [36].

Statistical analysis

Results were presented as means $\pm$ SEM. Statistical comparison was carried out by using one-way analysis of variance

(ANOVA), followed by Tukey's multiple comparison tests. Statistical significance was accepted at $p < .05$.

Results

Effect of SG on acute toxicity study

In the acute oral toxicity study, none of the mice has revealed visible signs of toxicity and deaths on a single administration of SG (2000 mg/kg) on day first.

Effect of SG (50 μ g/mice) and SG + FC (25 μ g/mice, each) on total and differential WBC count

Total cells, neutrophils, eosinophils, and monocytes count in OVA-treated mice were significantly increased ($p < .001$), whereas lymphocytes count was decreased significantly in comparison with NC mice ($p < .001$). Treatment of BALB/c mice with SG, FC and SG + FC caused significant decline in total cells, neutrophils, eosinophils, and monocytes cell counts, whereas a significant increase in lymphocytes count when matched to OVA-treated mice (Figure 3).

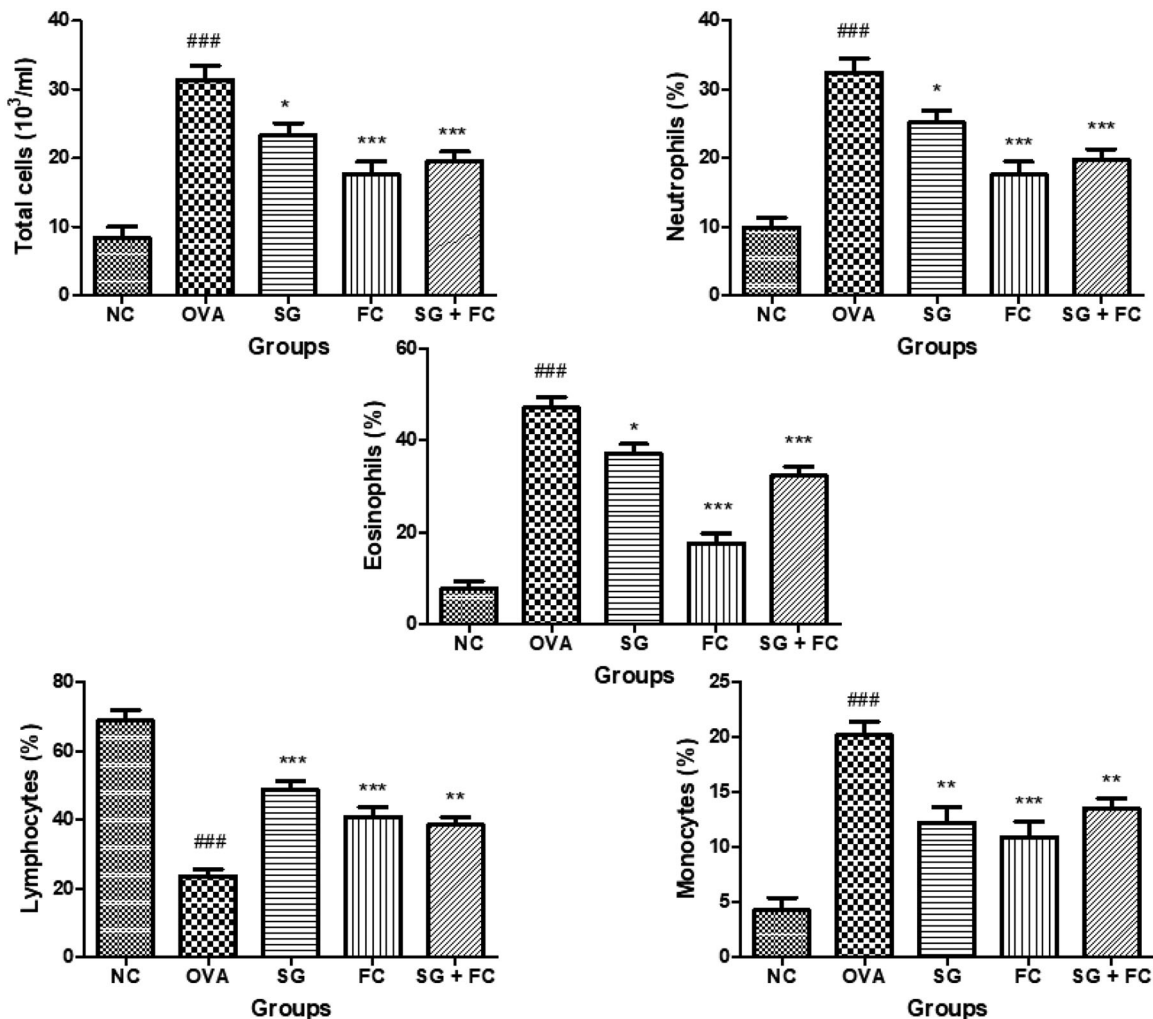


Figure 3. Effect of SG and SG + FC on total and differential cell count in OVA-induced asthma in BALB/c mice. Values expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's Kramer test. ^{###} $p < .001$ represents the significance level as compared to NC group; ^{*} $p < .05$, ^{**} $p < .01$, and ^{***} $p < .001$ as compared to OVA group.

Effect of SG (50 µg/mice) and SG + FC (25 µg/mice, each) on lung weight index

Figure 4 has shown photos of the lung from each treatment group. OVA challenge in BALB/c mice significantly ($p < .001$) improved the lung weight index when compared to NC

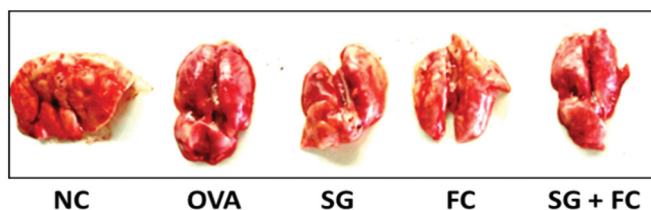


Figure 4. Lung tissue from respective group in OVA-induced asthma in mice.

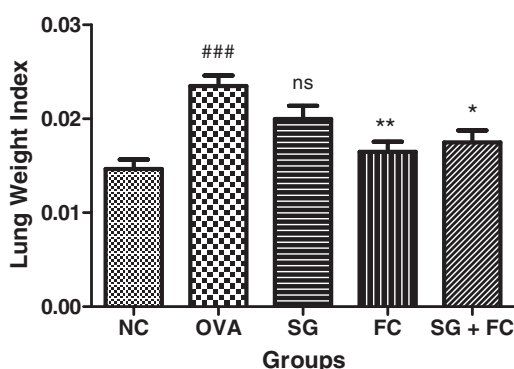
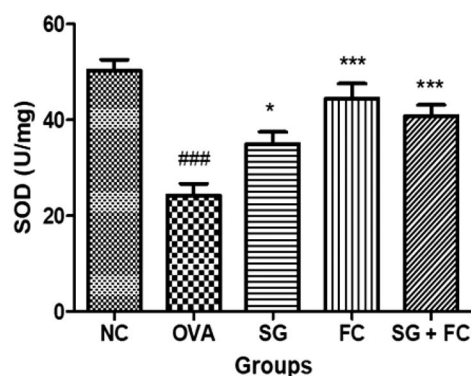
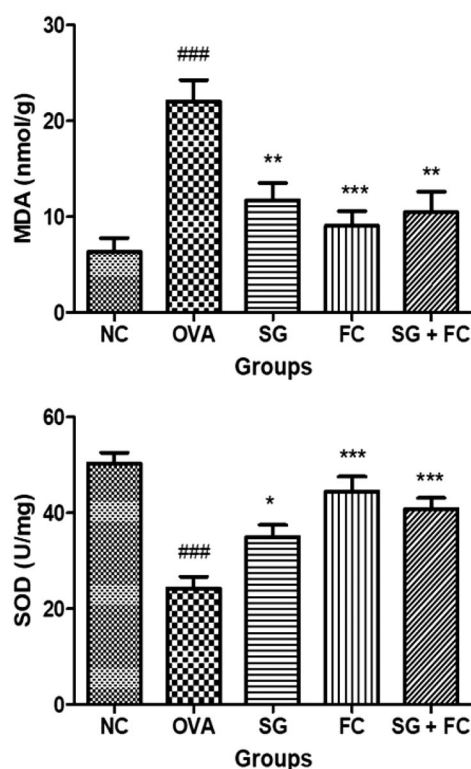


Figure 5. Effect of SG and SG + FC on lung weight index in OVA-induced asthma in BALB/c mice. Values expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's test. ^{###} $p < .001$ represents the significance level when compared with NC group; ^{*} $p < .05$ and ^{**} $p < .01$ when compared with OVA group. ns: non-significant.



mice. SG treatment to mice did not show any significant effect on the lung weight index. FC and SG + FC treatment significantly ($p < .05$ and $p < .01$) decreased the lung weight index as matched to OVA-treated mice (Figure 5).

Effect of SG (50 µg/mice) and SG + FC (25 µg/mice, each) on oxidative stress biomarkers in lung homogenate

The challenge of BALB/c mice with OVA significantly ($p < .001$) improved the MDA and MPO amount in the lung tissue with significant lessening ($p < .001$) in GSH and SOD content in comparison with NC mice. Treatment of BALB/c mice with SG, FC and SG + FC caused a significant decline of MDA ($p < .01$ and $p < .001$) and MPO ($p < .05$, $p < .001$ and $p < .01$) contents with significant increase in GSH ($p < .05$, $p < .001$) and SOD ($p < .05$, $p < .001$) levels when compared to OVA mice (Figure 6).

Effect of SG (50 µg/mice) and SG + FC (25 µg/mice, each) on nitrite/nitrate content in lung

The production of NO in lung tissue was significantly augmented ($p < .001$) when compared with NC mice. The treatment of mice with SG, FC, and SG + FC significantly decreased ($p < .001$) the lung NO level in comparison to OVA-challenged mice (Figure 7).

Effect of SG (50 µg/mice) and SG + FC (25 µg/mice, each) on serum cytokines level

The levels of pro-inflammatory cytokines such as TNF- α , IL-12, IL-6, and TXB₂ were significantly ($p < .001$) elevated in the serum samples of OVA-treated mice when compared to NC

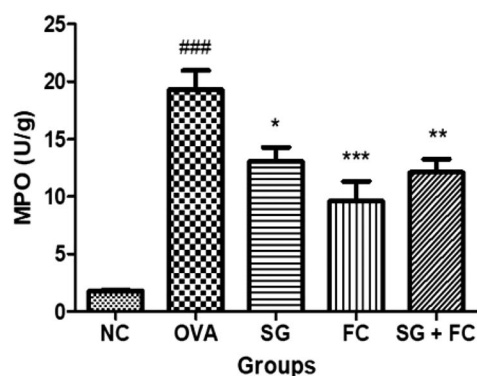
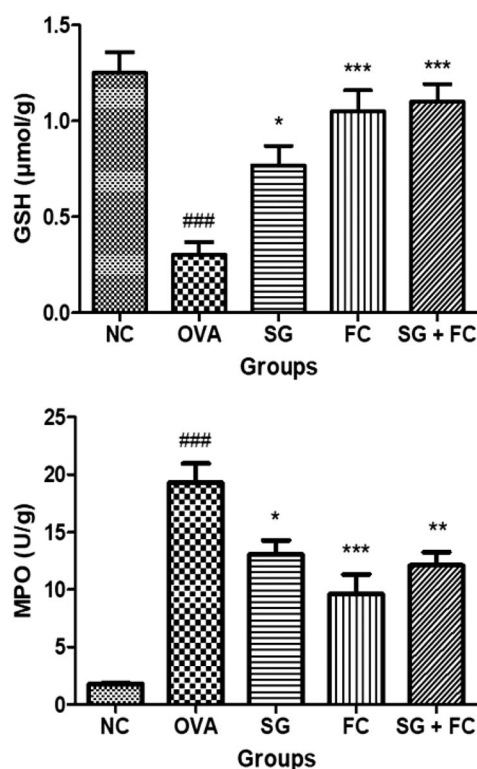


Figure 6. Effect of SG and SG + FC on MDA (nmol/g), GSH (µmol/g), SOD (U/mg), and MPO (U/g) in OVA-induced asthma in BALB/c mice. Values expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's test. ^{###} $p < .001$ represents the significance level when compared with NC group; ^{*} $p < .05$, ^{**} $p < .01$, and ^{***} $p < .001$ when compared with OVA group.

mice. In contrast to that, treatment of mice with SG, FC, and SG + FC showed a significant decline in TNF- α , IL-12, IL-6, and TXB₂ level in comparison to OVA-treated mice (Figure 8).

Effect of SG (50 μ g/mice) and SG + FC (25 μ g/mice, each) on IgE production in serum

Antigen-specific IgE antibodies are produced following antigen-specific Th2 responses. Hence, we examined the effect of SG and SG + FC combination on serum IgE formation in the asthma model. OVA challenge to BALB/c mice significantly ($p < .001$) amplified the IgE level when compared with

NC mice. SG, FC and SG + FC treatment significantly ($p < .01$, $p < .001$) suppressed OVA-induced IgE production when compared with OVA-challenged mice (Figure 9).

Effect of SG (50 μ g/mice) and SG + FC (25 μ g/mice, each) on histopathology of lung tissues

Lung samples isolated from NC group mice revealed normal lung structure without the appearance of bronchial inflammation and goblet cell hyperplasia. The lung sample of OVA-treated mice stained with H&E showed the presence of bronchial airways inflammation, epithelial and goblet cell hyperplasia

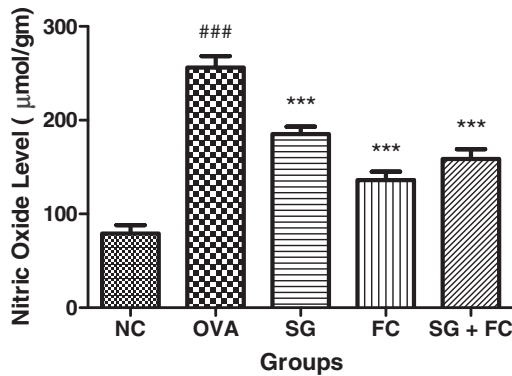


Figure 7. Effect of SG and SG + FC on nitric oxide level (μ mol/gm) in OVA-induced asthma in BALB/c mice. Values expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's test. ### $p < .001$ represents the significance level when compared with NC group; *** $p < .001$ when compared with OVA group.

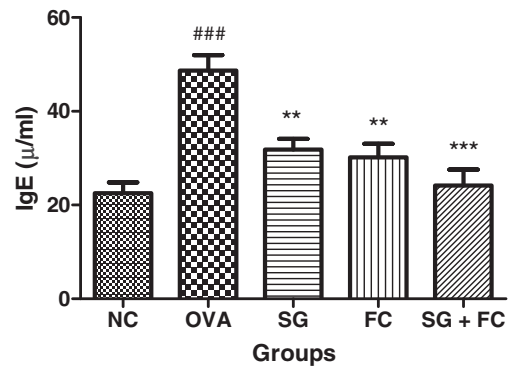


Figure 9. Effect of SG and SG + FC on IgE level (μ g/ml) in OVA-induced asthma in BALB/c mice. Values expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's test. ### $p < .001$ represents the significance level when compared with NC group; ** $p < .01$ and *** $p < .001$ when compared with OVA group.

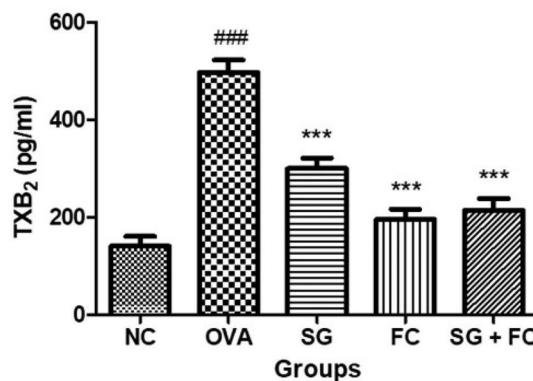
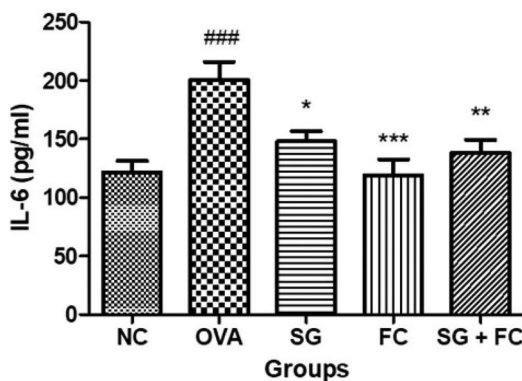
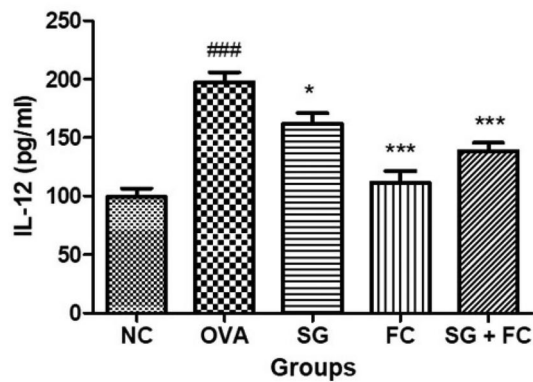
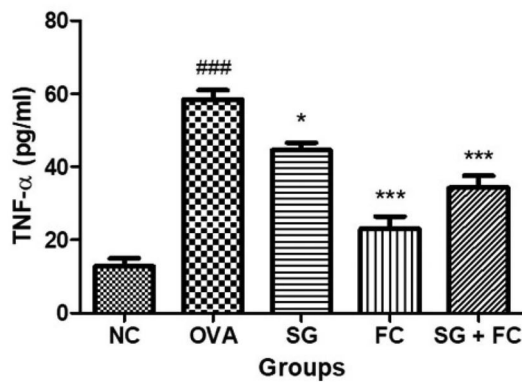


Figure 8. Effect of SG and SG + FC on TNF- α , IL-12, IL-6 and TXB₂ (pg/ml) in OVA-induced asthma in BALB/c mice. Values expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's test. ### $p < .001$ represents the significance level when compared with NC group; * $p < .05$, ** $p < .01$, *** $p < .001$ when compared with OVA group.

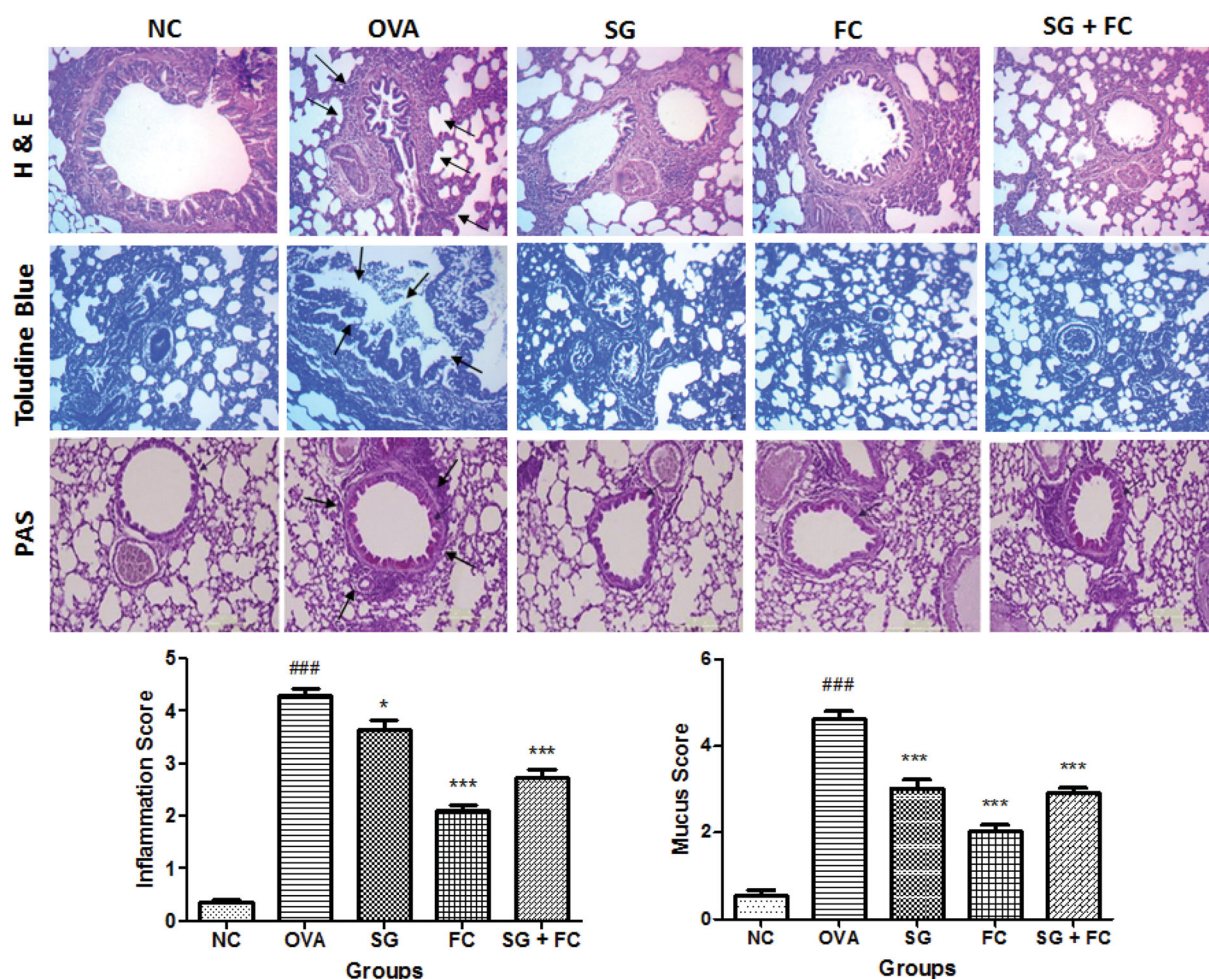


Figure 10. Photomicrograph of transverse sections of lung tissue sensitized and challenged with OVA stained with H&E, toluidine and PAS staining examined under light microscopy ($\times 100$). Arrow indicates the area of inflammation and goblet cell hyperplasia. The shown sections are representative of six animals per group. Graphs represent inflammation score and mucus score. Values expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's test. ### $p < .001$ represents the significance level when compared with NC group; * $p < .05$ and *** $p < .001$ when compared with OVA group.

and evidence of numerous eosinophils at the site of inflammation. However, the staining of OVA-treated mice lung sample with toluidine blue staining showed the trafficking of mast cell in the inflammatory bronchial epithelium and hyperplasia of goblet cells with excessive mucus production in lungs tissue stained with PAS. The treatment of mice with SG and SG + FC showed a significant decrease in bronchial inflammation, epithelial and mucus gland hyperplasia in the bronchiole in comparison to OVA-treated mice in H&E staining. However, there was negligible goblet cell hyperplasia after PAS staining and in toluidine blue staining there was a significant reduction in the inflammatory mast cells count. However, similar observations were observed in FC-treated mice (Figure 10). The inflammation and mucus score was found to be significantly increased ($p < .001$) after the treatment of OVA challenge in BALB/c mice. The elevated inflammation score ($p < .05$ and $p < .001$) and mucus score ($p < .001$) were significantly declined after the treatment of mice with SG, FC, and SG + FC (Figure 10).

Discussion

OVA-induced asthma model was used for the study of bronchial inflammation as it mimics the features of human

asthma [37]. The structural and functional changes allied with allergic asthma have been widely studied by using this model [3,5]. Sensitization and successive challenge with OVA in mice reveals the accumulation of eosinophils and lymphocytes in the BALF and hyperplasia of mucus-producing goblet cells [38]. The probable mechanism behind OVA-induced asthma in mice could be the release of pro-inflammatory cytokines by the activated mast cells, T-lymphocytes and damaged bronchial cells [39]. In this study, we have confirmed the anti-asthmatic activity of SG and SG + FC combination in OVA-induced allergic asthma model by inhibition of serum pro-inflammatory cytokines and restoration of antioxidant stress markers.

In this study, FC was used as a standard anti-inflammatory drug and reported to have protective activity against OVA-induced asthma [40]. Usually, steroidal drugs are effectual in the treatment of chronic inflammatory diseases but are associated with severe side effects such as metabolic disorders, bone marrow suppression, glaucoma, diabetes, ulcers, hypertension, gastric irritation and are observed at high doses [21]. None of the research work was published on the anti-inflammatory effect of SG + FC in OVA-induced bronchial asthma. SG, a steroidal sapogenin was preferred to evaluate

its anti-inflammatory effect with FC with the surplus advantage of being a natural constituent with lesser side effects.

Sensitization and challenge with OVA made significant physiological changes and bronchial airways structure. Biochemically, lung weight to body weight index, inflammatory cell counts in BALF enlarged considerably, with the significant rise in MDA and MPO concentration, cytokines and IgE level. In contrast, SOD and GSH contents reduced significantly on OVA administration indicating bronchial inflammation with disruption of oxidative stress biomarkers in the lungs. In addition to that, on histopathological analysis of lungs tissue, there was evidence of significant bronchial inflammation, epithelial hyperplasia, goblet cells hyperplasia and elevated mast cells scores were reported [41].

The present research has shown that OVA-induced asthma was allied with an influx of neutrophils, eosinophils, monocytes and lymphocytes cells in the lung. Release of cytotoxic proteins from inflammatory cells plays a main character in the progress of asthmatic condition [42]. Challenge of mice with OVA led to a significant rise in inflammatory cell count such as monocytes, neutrophils and eosinophils numbers in BALF samples with a significant decrease in lymphocytes count [43]. The results revealed that administration of SG and SG + FC combination to mice revealed a satisfactory decline in inflammatory cell count in comparison to OVA-challenged mice with an increase in the lymphocyte count. So, it indicated that by decreasing the proliferation and migration of differential blood cells, SG could play an important role in allergic asthma [44,45].

Lung weight index was identified as the best marker of vascular permeability and tissue edema [46]. The challenge of mice with OVA showed elevated microvascular outflow that caused lung edema [47]. In our study, the lung weight index was improved satisfactorily in OVA-treated mice in comparison to NC mice showing lung edema and bronchial inflammation. The treatment of mice with SG and SG + FC showed a satisfactory decline in the lung weight index as matched to OVA-treated mice.

The gathering of inflammatory cells into lung tissue causes successive activation of Th2 cells *via* antigen production indicating the principle mechanism of asthma [48]. Th2 cells perform a significant part in the bronchial airways [5] and Th1 cytokines (TNF- α , IL-6, and IL-12) shows an imperative character in the neutrophils activation that contributes to the asthma pathogenesis [49,50]. The raised level of pro-inflammatory cytokines such as IL-6, IL-12, TNF- α and TXB₂ cytokines in the lung tissue of OVA-challenged mice indicates the stimulation of inflammatory reaction when compared with NC mice. However, the levels of pro-inflammatory cytokines such as IL-6, IL-12, TNF- α , and TXB₂ cytokines were significantly inhibited by treatment of mice with SG and SG + FC would be due to its anti-inflammatory mechanism. This result was in agreement with the earlier research of Ingawale et al. [51] in which hecogenin and hecogenin + FC combination significantly declined the levels of inflammatory cytokines in allergic OVA encouraged asthma in mice.

Overall, B cells encouraged the production of IgE is controlled by Th2 cytokines. Th2 cytokines perform a central

part in the IgE hyperproduction. The instantaneous allergic and prolonged inflammatory response in the lungs was associated with raised levels of IgE [52]. In allergic inflammatory conditions, an increase in the serum IgE concentration encourage the mast cells degranulation through the allergen-specific IgE and considered as an potential target for the formulation of allergic asthma medicines [53]. Treatment of mice with SG and SG + FC combination significantly reduced serum IgE level of OVA-treated mice. Collectively, the above results endorse that SG could inhibit the formation of Th2 cells induced an immune response in an allergic asthma model.

The generation of free radicals by a variety of inflammatory cells (neutrophils, eosinophils and macrophages) of asthmatic patients could contribute to another mechanism of asthma [54]. These reactive oxygen species generates oxidative stress, enhances peroxidation of proteins, lipids, DNA and production of chemoattractant, enhanced bronchial hyperresponsiveness and mucus secretion in asthma patients [55]. The lipids embedded in the plasma membranes were considered as the main goals of oxidative damage in inflammatory disease conditions [56]. Catalytic enzymes like SOD, MDA, catalase, and GSH plays a significant role in allergic asthma by giving protection against oxidative damage by inhibiting the prolonged inflammatory response [57].

MD is produced from prostaglandin synthesis during oxidative stress in cells. It is the key lipid peroxide of oxygen-free radicals that contribute to cell damage [58]. In the current study, the mice challenged with OVA showed significant elevation in MDA contents when matched with NC mice. However, the elevated level of MDA is noticeably reduced with SG and SG + FC treatment in mice in comparison with OVA-challenged mice. GSH is key intracellular redox mediator that purifies lipid hydroperoxides and is associated with vitamin E, vitamin C and NADPH antioxidants protecting against oxidative stress damage [59]. Along with SOD, GSH stimulates the generation of hydrogen peroxide and oxygen radicals from superoxide anion [60]. Significant reduction in SOD and GSH contents were found to be in OVA-challenged mice in comparison to NC mice. The level of lung SOD and GSH gets markedly increased with SG and SG + FC treatment in mice in comparison with OVA-treated mice. The main parameter assessed in the lung homogenate was MPO. The raised MPO contents have been seen in lung tissue of OVA-sensitized mice that was significantly reduced by SG and SG + FC treatment in mice which specifies the cell destruction inside the lung.

Nitric oxide is another main mediator that contributes to the pathophysiology of allergic asthma [61]. Overproduction of inducible nitric oxide synthase (iNOS) in the bronchial airways can participate in the mechanism of asthma. Elevated expression of iNOS indirectly activates Th2 lymphocytes that cause pathophysiological alterations in asthma. The present research showed that lungs NO levels were satisfactory enhanced after OVA challenge in BALB/c mice. The elevated lung NO expressions decreased significantly in SG and SG + FC treatment in mice.

The research study has been further supported by histopathological analysis of lung samples acquired from OVA-

induced asthma. The results showed alterations in the bronchial airways such as bronchial inflammation, epithelial, mucus gland and goblet cells hyperplasia and accumulation of abundant mast cells in OVA-treated mice. The staining of lung tissue samples with H&E, toluidine blue and PAS showed a significant reduction in pleural edema, mucus gland, goblet, epithelial and bronchial cells hyperplasia and influx of eosinophils with an absence of mast cells when compared to OVA-treated lung samples in mice.

Conclusion

In summary, our study results confirmed that the intranasal administration of SG + FC combination at low dose conferred significant protection against OVA-induced allergic asthma in mice. This effect may be due to its capability of reducing the accumulation of inflammatory cells, restoration of antioxidant stress markers, diminished nitric oxide production, reduced serum pro-inflammatory cytokines levels. The research outcomes indicate that SG can serve as a potential beneficial agent for the treatment of allergic asthma management in patients by reducing its dosage in order to reduce the side effects of glucocorticoids.

Disclosure statement

The authors declare that they have no conflict of interest.

References

- [1] Asthma Gf. Global strategy for asthma management and prevention. 2016. Available from: www.ginasthma.org.
- [2] Manuyakorn W, Howarth PH, Holgate ST. Airway remodelling in asthma and novel therapy. *Asian Pac J Allergy Immunol*. 2013; 31(1):3–10.
- [3] Martinez FD. Genes, environments, development and asthma: a reappraisal. *Eur J Biochem*. 2007;29:179–184.
- [4] Lemanske RF, Busse WW. Asthma: clinical expression and molecular mechanisms. *J Allergy Clin Immunol*. 2010;125(2):S95–S102.
- [5] Busse WW, Calhoun WF, Sedgwick JD. Mechanisms of airway inflammation in asthma. *Am Rev Respir Dis*. 1993;147(6_pt_2): S20–S24.
- [6] Rona RJ, Keil T, Summers C, et al. The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol*. 2007;120(3):638–646.
- [7] Foster PS, Maltby S, Rosenberg HF, et al. Modeling TH2 responses and airway inflammation to understand fundamental mechanisms regulating the pathogenesis of asthma. *Immunol Rev*. 2017; 278(1):20–40.
- [8] Matucci A, Vultaggio A, Maggi E, et al. Is IgE or eosinophils the key player in allergic asthma pathogenesis? Are we asking the right question. *Respir Res*. 2018;19(1):10.
- [9] McKenzie GJ, Emson CL, Bell SE, et al. Impaired development of Th2 cells in IL-13-deficient mice. *Immunity*. 1998;9(3):423–432.
- [10] Yang T, Yan L, Zhe L, et al. Characteristics of proinflammatory cytokines and chemokines in airways of asthmatics: relationships with disease severity and infiltration of inflammatory cells. *Chin Med J (Engl)*. 2017;130(17):2033–2040.
- [11] Park CS, Kim TB, Lee KY, et al. Increased oxidative stress in the airway and development of allergic inflammation in a mouse model of asthma. *Ann Allergy Asthma Immunol*. 2009;103(3): 238–247.
- [12] Power FB, Salway AH. Chemical examination of sarsaparilla root. *J Chem Soc Trans*. 1914;105:201–219.
- [13] Hu Y, Xia Z, Sun Q, et al. A new approach to the pharmacological regulation of memory: Sarsasapogenin improves memory by elevating the low muscarinic acetylcholine receptor density in brains of memory-deficit rat models. *Brain Res*. 2005;1060(1–2):26–39.
- [14] Peana AT, Moretti MD, Manconi V, et al. Anti-inflammatory activity of aqueous extracts and steroidal saponins of *Agave americana*. *Planta Med*. 1997;63(03):199–202.
- [15] Thurman FM. The treatment of psoriasis with Sarsaparilla compound. *Pharmacol Res Commun*. 1988;20:59–62.
- [16] Ageel AM, Mossa JS, Al-Yahya MA, et al. Experimental studies on antirheumatic crude drugs used in Saudi traditional medicine. *Drugs Exp Clin Res*. 1989;15(8):369–372.
- [17] Shao B, Guo H, Cui Y, et al. Steroidal saponins from *Smilax china* and their anti-inflammatory activities. *Phytochem*. 2007;68(5): 623–630.
- [18] Liu YW, Hao YC, Chen YJ, et al. Protective effects of Sarsasapogenin against an early stage of diabetic nephropathy in rats: Sarsasapogenin prevents the progression of diabetic nephropathy. *Phytother Res*. 2018;32:1–9.
- [19] Ma D, Zhang J, Sugahara K, et al. Effect of Sarsasapogenin and its derivatives on the stimulus coupled responses of human neutrophils. *Clin Chim Acta*. 2001;314(1–2):107–112.
- [20] Phillipps GH. Structure-activity relationships of topically active steroids: the selection of fluticasone propionate. *Respir Med*. 1990;84:19–23.
- [21] Wolfe MM, Lichtenstein DR, Singh G. Lichtenstein gastrointestinal toxicity of Nonsteroidal anti-inflammatory drugs. *N Engl J Med*. 1999;340(24):1888–1899.
- [22] Barnes PJ, Ito K, Adcock IM. Corticosteroid resistance in chronic obstructive pulmonary disease: inactivation of histone deacetylase. *Lancet*. 2004;363(9410):731–733.
- [23] Baraket M, Oliver BGG, Burgess JK, et al. Is low dose inhaled corticosteroid therapy as effective for inflammation and remodelling in asthma? A randomized, parallel-group study. *Respir Res*. 2012; 13(1):11.
- [24] OECD Guidelines. OECD guidelines for testing of chemicals. Test No. 425. Acute toxic class method. 2008.
- [25] Wang J, Fu Y, Wei Z, et al. Anti-asthmatic activity of osthole in an ovalbumin-induced asthma murine model. *Respir Physiol Neurobiol*. 2017;239:64–69.
- [26] Thompson AB, Teschler H, Wang YM, et al. Preparation of bronchoalveolar lavage fluid with microscope slide smears. *Eur Respir J*. 1996;9(3):603–608.
- [27] Pearce ML, Yamashita J, Beazell J. Measurement of pulmonary oedema. *Circ Res*. 1965;16(5):482–488.
- [28] Daba MH, Abdel-Aziz AA, Moustafa AM, et al. Effects of L-carnitine and *ginkgo biloba* extract (EG b 761) in experimental bleomycin induced lung fibrosis. *Pharmacol Res*. 2002;45(6):461–467.
- [29] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2): 351–358.
- [30] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys*. 1959; 82(1):70–77.
- [31] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*. 1974;47(3):469–474.
- [32] Barone FC, Hillegass LM, Price WJ, et al. Polymorphonuclear leukocyte infiltration into cerebral focal ischemic tissue: myeloperoxidase activity assay and histologic verification. *J Neurosci Res*. 1991;29(3):336–345.
- [33] Green LC, Wagner DA, Glogowski J, et al. Analysis of nitrate, nitrite, and [15 N] nitrate in biological fluids. *Anal Biochem*. 1982; 126(1):131–138.
- [34] McKay A, Leung BP, McInnes IB, et al. A novel anti-inflammatory role of simvastatin in a murine model of allergic asthma. *J Immunol*. 2004;172(5):2903–2908.
- [35] Reber LL, Daubeuf F, Nemska S, et al. The AGC kinase inhibitor H89 attenuates airway inflammation in mouse models of asthma. *PLoS One*. 2012;7(11):e49512.

- [36] Firinci F, Karaman M, Cilaker-Micili S, et al. The effect of atorvastatin on lung histopathology in a murine model of chronic asthma. *Allergol Immunopathol.* 2014;42:355–361.
- [37] Ma Y, Ge A, Zhu W, et al. Morinm attenuates ovalbumin-induced airway inflammation by modulating oxidative stress responsive MAPK signaling. *Oxid Med Cell Longev.* 2016; 2016:1–13.
- [38] Camateros P, Tamaoka M, Hassan M, et al. Chronic asthma-induced airway remodeling is prevented by toll-like receptor-7/8 ligand S28463. *Am J Respir Crit Care Med.* 2007;175(12): 1241–1249.
- [39] Kumar RK, Herbert C, Foster PS. The ‘classical’ ovalbumin challenge model of asthma in mice. *Curr Drug Targets.* 2008;9(6): 485–494.
- [40] Kurai J, Watanabe M, Sano H, et al. A muscarinic antagonist reduces airway inflammation and bronchoconstriction induced by ambient particulate matter in a mouse model of asthma. *IJERPH.* 2018;15(6):1189.
- [41] Tillie-Leblond I, Gosset P, Le Berre R, et al. Keratinocyte growth factor improves alterations of lung permeability and bronchial epithelium in allergic rats. *Eur Respir J.* 2007;30(1):31–39.
- [42] Hamid Q, Tulic MK, Liu MC, et al. Inflammatory cells in asthma: mechanisms and implications for therapy. *J Allergy Clin Immunol.* 2003;111(1):S5–S12.
- [43] Wei DZ, Guo XY, Lin LN, et al. Effects of Angelicin on ovalbumin (OVA)-induced airway inflammation in a mouse model of asthma. *Inflammation.* 2016;39(6):1876–1882.
- [44] Drazen JM, Arm JP, Austen KF. Sorting out the cytokines of asthma. *J Exp Med.* 1996;183(1):1–201.
- [45] Riffo-Vasquez Y, Spina D. Role of cytokines and chemokines in bronchial hyperresponsiveness and air way inflammation. *Pharmacol Ther.* 2002;94(3):185–211.
- [46] Tumes DJ, Cormie J, Calvert MG, et al. Strain-dependent resistance to allergen-induced lung pathophysiology in mice correlates with rate of apoptosis of lung-derived eosinophils. *J Leukoc Biol.* 2007;81:1362.
- [47] Okada S, Kita H, George TJ, et al. Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9. *Am J Respir Cell Mol Biol.* 1997;17(4): 519–528.
- [48] Bousquet J, Chanez P, Lacoste JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med.* 1990;323(15):1033–1039.
- [49] Barnes PJ. Th2 cytokines and asthma: an introduction. *Respir Res.* 2001;2(2):64–65.
- [50] Holgate ST. The airway epithelium is central to the pathogenesis of asthma. *Allergol Int.* 2008;57(1):1–10.
- [51] Ingawale DK, Mandlik SK, Patel SS. Anti-inflammatory potential of hecogenin on atopic dermatitis and airway hyper-responsiveness by regulation of pro-inflammatory cytokines. *Immunopharmacol Immunotoxicol.* 2019;41(2):327–336.
- [52] Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. *Am J Respir Crit Care Med.* 2001;164(supplement_1): S1–S5.
- [53] Lee DS, Park WS, Heo SJ, et al. Polyopes affinis alleviates airway inflammation in a murine model of allergic asthma. *J Biosci.* 2011;36(5):869–877.
- [54] Kongerud J, Crissman K, Hatch G, et al. Ascorbic acid is decreased in induced sputum of mild asthmatics. *Inhal Toxicol.* 2003;15(2): 101–109.
- [55] Barnes PJ. Reactive oxygen species and airway inflammation. *Free Radic Biol Med.* 1990;9(3):235–243.
- [56] Louhelainen N, Myllärniemi M, Rahman I, et al. Airway biomarkers of the oxidant burden in asthma and chronic obstructive pulmonary disease: current and future perspectives. *Int J Chron Obstruct Pulmon Dis.* 2008;3(4):585–603.
- [57] Fatani SH. Biomarkers of oxidative stress in acute and chronic bronchial asthma. *J Asthma.* 2014;51(6):578–584.
- [58] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev.* 2014;2014:1–31.
- [59] Ghezzi P. Role of glutathione in immunity and inflammation in the lung. *Int J Gen Med.* 2011;4:105–113.
- [60] Larkin EK, Gao YT, Gebretsadik T, et al. New risk factors for adult-onset incident asthma. A nested case-control study of host antioxidant defense. *Am J Respir Crit Care Med.* 2015;191(1):45–53.
- [61] Prado CM, Martins MA, Tibério IF. Nitric oxide in asthma pathophysiology. *ISRN Allergy.* 2011;19:832560.