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Original Article

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Modulation of Immunological, Biochemical, and Histopathological Changes of Airway Remodeling by *Withania somnifera* in an Experimental Model of Allergic Asthma in Rats

Nafaa Hasan Ali[1], Sana Rehman[1], Maaz Naqvi[1], Kavita Gulati[2], Arunabha Ray[1]*

[1]Department of Pharmacology, Hamdard Institute of Medical Sciences and Research (HIMSR), Jamia Hamdard University, New Delhi, India
[2]Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India

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***Corresponding Author**
Arunabha Ray
Department of Pharmacology, Hamdard Institute of Medical Sciences and Research (HIMSR), Jamia Hamdard University, New Delhi 110062, India
Tel: +91-981-803-7595
E-mail: arunabha14@yahoo.co.in

Objectives: Airway remodeling in asthma involves chronic inflammation associated with structural changes, which result in severe airflow limitation and very few therapeutic options. Thus, the present study was designed to experimentally evaluate the ameliorative effects of *Withania somnifera* (WS) root extract against Ovalbumin (OVA)-induced airway remodeling in a rat model of asthma.
Methods: Wistar rats were immunized (i.p) and challenged (aerosol) with ovalbumin (OVA), and the effects of WS extract were investigated on the development and progress of airway remodeling by assessing immunological, biochemical, and histological changes in these rats.
Results: OVA-immunization and challenge in rats resulted in significant increases in the levels of IL-13, 8-OhdG, TGF-β, hydroxyproline, and periostin in bronchoalveolar lavage fluid (BALF) and serum/lung homogenate compared to normal control (saline only) rats, and these changes were attenuated after WS extract (200 and 400 mg/kg), as well as dexamethasone (DEX, 1 mg/kg) pretreatments. Further, WS extract attenuated histopathological changes and maintained lung integrity. In herb-drug interactions, sub-threshold doses of WS extract and DEX showed synergistic effects on all parameters studied as compared to either form of monotherapy.
Conclusion: These results indicated that WS exerted significant protective effects against airway remodeling in the experimental model by modulating inflammatory and fibrotic cytokines, and could have the potential for developing a therapeutic alternative/adjunct for the treatment of airway remodeling of bronchial asthma.

Keywords: bronchial asthma, airway remodeling, *Withania somnifera*, cytokines, oxidative stress

INTRODUCTION

Bronchial asthma, a complex syndrome characterized by chronic airway inflammation, presents as several, distinct, clinical phenotypes. The inflammatory process in asthma is characterized by antigen interaction and inflammatory cell infiltration in the airway wall, which subsequently increase the synthesis and release of inflammatory mediators and reactive oxygen species (ROS) [1]. In allergic asthma, Th2 cells release a variety of cytokines, such as IL-4, IL-5, and IL-13, which play significant roles in controlling the production of allergen-specific E, eosinophil recruitment, airway remodeling, and airway hyperresponsiveness [2]. Airway wall damage and tissue repair lead to structural changes in the airways, such as smooth muscle hypertrophy, goblet cell hyperplasia, angiogenesis, and increased collagen deposition, which may cause irreversible

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loss of lung functions. Persistent lung tissue injury, chronic tissue repair, and/or chronic airway inflammation are the main factors linked to airway remodeling [3]. Experimental evidence has shown that a complex network of Th2 cytokines mediates the inflammatory response in the airways. These cytokines, released primarily from monocytes and macrophages, recruit neutrophils and eosinophils into lung tissues, which, in turn, contributes to the development of airway remodeling and lung fibrosis [4]. The severe and intractable forms of asthma are usually associated with airway remodeling, responding poorly to inhaled corticosteroids and bronchodilators, which are the cornerstones of pharmacotherapy, and resulting in increased morbidity and mortality [5, 6]. In view of the lack of effective treatments in contemporary medicine, a potential therapeutic strategy to reverse or control the pathological changes in airway remodeling is of utmost significance and therefore, an unmet need. Medicinal plant-based therapeutic agents have provided good leads for respiratory diseases, and several studies are under way to assess the potential effects of phytotherapeutic agents in the treatment of bronchial asthma and its associated pathophysiology [7]. *Withania somnifera* (WS) is a well-documented medicinal plant in Indian traditional systems of medicine with immense potential due to its anti-inflammatory, immunomodulatory, and antioxidant effects [8]. However, no systematic studies have been conducted to explore the potential role of WS in the airway remodeling of asthma and the related pathophysiology. Therefore, the present study aims to explore the potential effects of WS root extract on OVA-induced animal model of airway remodeling and elucidate the underlying mechanisms.

MATERIALS AND METHODS

1. Drugs and chemicals

The aqueous root extract of WS was procured from Natural Remedies Pvt. Ltd., (Bangalore, India). Ovalbumin (albumin from chicken egg white; OVA grade III) was purchased from Sigma Aldrich Chemical Co., USA. The specific rat enzyme-linked immunosorbent assay (ELISA) kits—TGF-β (Elabscience, USA), 8-hydroxy-2-deoxyguanosine (8-OhdG) and periostin (BT Lab, China), hydroxyproline (Kinesis, USA), and IL-13 (Krishgen Biosystem, India)—were purchased from Amplicon Biotech, India. Dexamethasone was purchased from Abbott Laboratories, India. All the buffers were freshly prepared, and all routine drugs/chemicals needed for the various assays were of high analytical grade and obtained from SRL Labs, New Delhi, India.

2. Phytochemical evaluation

High performance liquid chromatography (HPLC) was used for quantitative analysis of the total withanolide and identification of the phytochemical constituents in the plant extract.

3. Animals

Inbred Wistar albino rats of both sexes weighing 200 ± 20 g were procured from the central animal house facility of Jamia Hamdard, New Delhi, India. The animals were acclimated for 7 days before experimentation to standard laboratory conditions of the light-dark cycle (12 h light-12 h dark), temperature (22 ± 2℃), humidity 45 ± 5%, and had free access to food and water. The study was carried out after receiving ethical approval from the Institutional Animal Ethics Committee (IAEC). Jamia Hamdard (protocol No. 1767).

4. Immunization protocol and sample collection

The immunization of rats with OVA was carried out following the protocol described by Rai et al. [9]. Briefly, on day 1, all rats, except normal controls, were immunized with OVA (50 mg per rat, i.p.) emulsified with 100 mg of aluminum hydroxide $Al(OH)_3$, as an adjuvant, in 0.5 mL of NS. From day 15 to day 21, the rats were challenged by daily exposure to the OVA aerosol (2%) in NS for 30 minutes. In the treatment groups, WS extract and DEX treatment were given 30 min before OVA exposure. The normal control rats were exposed to nebulized NS. The challenge was carried out by exposure of the rats to the OVA aerosol in a closed chamber using an ultrasonic nebulizer (402B, Yuwell, China) at a flow rate of 3 mL/min. After 24 h of the last challenge (day 22), all animals were anesthetized to collect blood and bronchoalveolar lavage fluid (BALF).

After a small incision in the neck, the muscles were retracted and the trachea exposed. A midline slit was made on the trachea and it was cannulated by inserting a polyethylene cannula, which was sutured and fixed. The bronchi and lungs were lavaged three times by injecting with 0.5 mL ice-cold phosphate-buffered saline (PBS) (total volume 1.5 mL) with gentle cardiac massage to increase BALF retrieval. BALF was collected and

centrifuged at 1,500 rpm for 10 min. Blood was collected by cardiac puncture and then centrifuged at 3,500 rpm for 10 min; the supernatant was stored in aliquots at −20℃ for the cytokine assay.

5. Experimental design

The rats were divided into six groups (n = 6/group) as follows: (i) Normal Control (NC): no immunization/challenge; rats were given only 0.5 mL intraperitoneal injections (i.p) of vehicle (saline); (ii) Disease Control (OVA): rats were immunized and challenged with OVA and treated with vehicle; (iii) WS extract (200 mg/kg, p.o.) group: rats were immunized and challenged with OVA and treated orally with WS extract 200 mg/kg; (iv) WS extract (400 mg/kg, p.o.) group: rats were immunized and challenged with OVA and treated orally with WS extract 400 mg/kg; (v) Dexamethasone (DEX) group: rats were immunized and challenged with OVA and treated with DEX 1 mg/kg i.p.; and (vi) DEX + WS 200 group, a combination treatment group: rats were immunized and challenged with OVA and treated with a low dose of combined therapy of WS extract (200 mg/kg, orally) plus a low dose of DEX (0.5 mg/kg, i.p).

6. Preparation of lung tissue homogenate

After the collection of blood and BALF, the animals were sacrificed and the lungs were thoroughly perfused using PBS, then excised, rinsed, weighed, and homogenized as described earlier [10]. The homogenate was centrifuged at 10,000 rpm for 15 min and the supernatant was divided into aliquots, stored at −20℃ and used to determine the levels of cytokines.

7. Histopathological study of lung tissue

The rats were sacrificed by cervical dislocation, and immediately the lungs were removed, cleaned with 0.9% saline, and fixed in 10% neutral formalin (v/v). Sections of the lungs were prepared and stained with hematoxylin and eosin (H&E) for histopathological examination as described earlier [11]. The histopathological changes in the lungs were examined and compared across the various treatment groups by a qualified pathologist.

8. Measurement of cytokine levels using ELISA

The levels of 8-OhdG, IL-13, TGF-β, hydroxyproline, and periostin were quantified by using commercially available ELISA kits according to the manufacturer's instructions.

9. Statistical analysis

The statistical analysis was carried out using the Graph Pad Prism® software, version 8.3.0, San Diego, USA. The results were expressed as mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA), followed by post-hoc Tukey's Multiple Comparison Test. $p < 0.05$ was considered statistically significant.

RESULTS

1. Quantification of bioactive compounds of WS by HPLC analysis

HPLC was used for the quantitative analysis of the total withanolide content and to investigate the presence of the phytoconstituents in the aqueous extract of WS roots. The extract content of the total withanolide was 2% (w/w), which complied with the specifications (≥ 1.5% of total withanolides). The retention times of the peaks of the reference compounds, withanolide A and withanoside IV, in the HPLC chromatogram, were similar to the retention times of the peaks in the sample extract. The HPLC chromatograms of WS extract and the reference marker compounds are shown in Fig. 1.

2. Effect of WS extract on OVA-induced biomarkers of airway inflammation and oxidative stress in lung tissue and BALF

Rats immunized and challenged with OVA showed significant increases in the biomarkers of airway inflammation and oxidative stress as compared to normal controls. Specifically, OVA immunization and challenge resulted in significantly increased levels of IL-13 in the lung homogenate and BALF compared to the corresponding values of the normal control group (OVA-26.56 pg/mL vs. NC-15.28 pg/mL, $p < 0.01$, in lung; and OVA-163.70 pg/mL vs. NC-86.48 pg/mL, $p < 0.01$, in BALF). Pretreatments with both doses of the WS extract at 200 and 400 mg/kg induced dose-dependent reductions in the IL-

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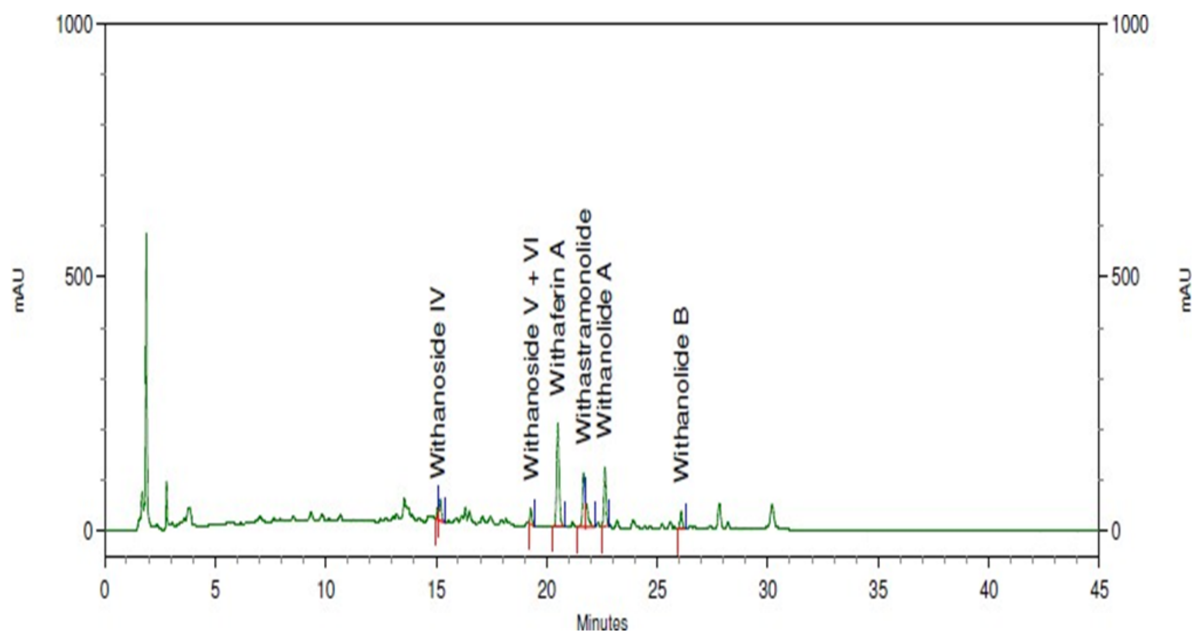

Figure 1. HPLC chromatogram of aqueous WS root extract. The chromatogram of the sample solution shows similar peaks at retention times of Withania somnifera withanolides corresponding to the peaks of standard withanolide A and withanoside IV.

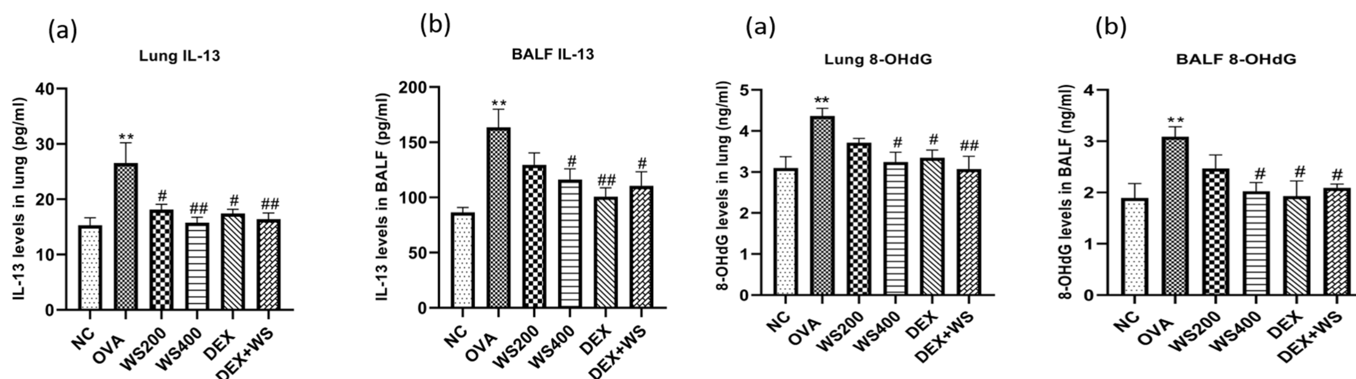

Figure 2. Effects of *WS* extract (200 and 400 mg/kg) on OVA-induced IL-13 levels in the (a) lung tissue and (b) BALF in rats. All data are expressed as mean ± SEM (n = 6/group); NC, normal controls; OVA, Ovalbumin; DEX, dexamethasone; $^{**}p < 0.01$ vs NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs OVA.

Figure 3. Effects of *WS* extract (200 and 400 mg/kg) on OVA-induced 8-OHdG levels in the (a) lung tissue and (b) BALF in rats. All data are expressed as mean ± SEM (n = 6/group); NC, normal controls; OVA, Ovalbumin; DEX, dexamethasone; $^{**}p < 0.01$ vs NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs OVA.

13 levels in the lung homogenates (reduction of 68% in lung homogenate and 40% in BALF). The lower dose of the extract (200 mg/kg) significantly reduced IL-13 levels in only BALF ($p < 0.05$). These WS-induced lowering of IL-13 levels were comparable with the decrease seen with the comparator drug, DEX (1 mg/kg). A combination treatment of DEX (0.5 mg/kg) + WS (200 mg/kg) also induced marked reductions in IL-13 levels in both the BALF and lung homogenates ($p < 0.01$) compared to the OVA group. These results are summarized in Fig. 2.

Similarly, the levels of 8-OHdG, a marker for oxidative DNA damage, were significantly increased in lung homogenates and BALF by 41% and 63%, respectively ($p < 0.05$, for both), after sensitization and challenge with OVA when compared with the normal control group. Pretreatment with the higher dose of WS extract (400 mg/kg) and DEX resulted in a significant reduction of 8-OHdG levels in the lung tissue and BALF ($p < 0.05$ in all cases). In addition, the combination treatment of lower doses of DEX 0.5 mg/kg + WS extract 200 mg/kg markedly attenuated oxidative DNA damage by lowering 8-OHdG levels in lung

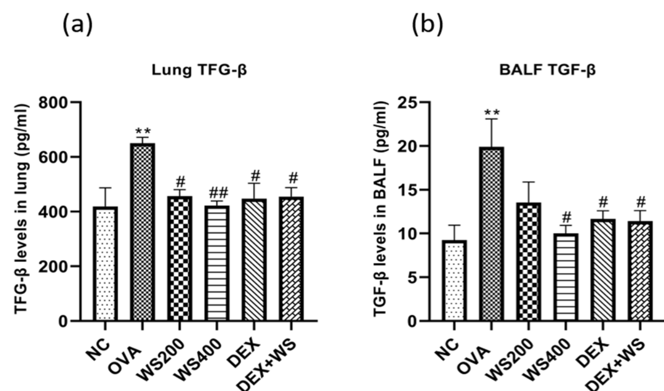

Figure 4. Effects of *WS* extract (WS 200 and 400 mg/kg) on OVA-induced TGF-β levels in the (a) lung tissue and (b) BALF in rats. All data are expressed as mean ± SEM (n=6/group); NC, normal controls; OVA, Ovalbumin; DEX, dexamethasone; $^{**}p < 0.01$ vs NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs OVA.

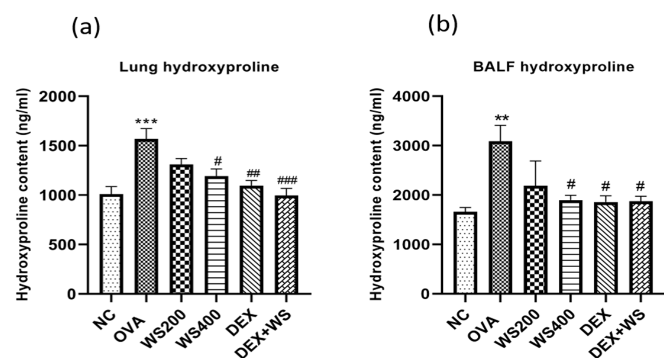

Figure 5. Effects of *WS* extract (200 and 400 mg/kg) on OVA-induced hydroxyproline levels in the (a) lung tissue and (b) BALF in rats. All data are expressed as mean ± SEM (n = 6/group); NC, normal controls; OVA, Ovalbumin; DEX, dexamethasone; $^{**}p < 0.01$, $^{***}p < 0.001$ vs NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs OVA.

tissues ($p < 0.01$) and BALF ($p < 0.05$) compared to the OVA group (Fig. 3).

3. Effect of WS extract on OVA-induced biomarkers of airway remodeling in lung tissue and BALF

OVA sensitization + challenge significantly increased TGF-β expression in both lung homogenates and BALF by 69% and 75%, respectively, as compared to the normal control group ($p < 0.01$ in both cases). Pretreatment with WS extract (200 and 400 mg/kg) significantly reduced the OVA-induced elevations in TGF-β levels in the lung homogenate and BALF in a dose-dependent manner. The reduction in TGF-β levels was significant at the higher dose of WS extract and was comparable with the decrease observed in the DEX and the combination treatment groups ($p < 0.05$) (Fig. 4).

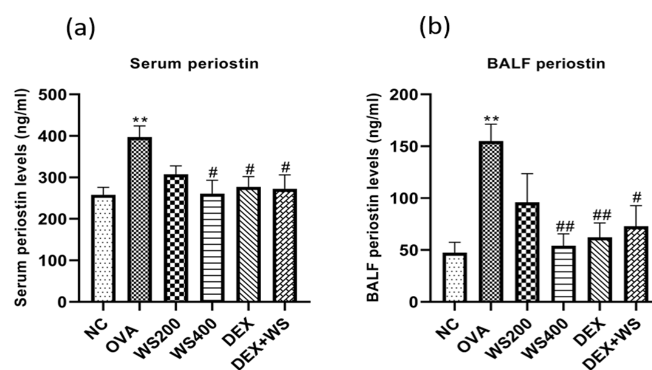

Figure 6. Effects of *WS* extract (200 and 400 mg/kg) on OVA-induced periostin expression in the (a) serum and (b) BALF in rats. All data are expressed as mean ± SEM (n = 6/group); NC, normal controls; OVA, Ovalbumin; DEX, dexamethasone; $^{**}p < 0.01$ vs NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs OVA.

Hydroxyproline is a marker of tissue collagen deposition. The present study showed that OVA-induced asthma in rats was associated with significant increases in lung and BALF hydroxyproline content ($p < 0.01$, $p < 0.001$, respectively) as compared with normal control rats. In contrast, OVA-immunized rats pretreated with WS extract showed a reduction in hydroxyproline levels with marked effects seen with the higher dose of the extract (400 mg/kg). The hydroxyproline levels were reduced by 31.7% and 62.8% in lung homogenates and BALF, respectively, as compared with the OVA group ($p < 0.05$, in both cases), and these changes were similar to those seen with the standard drug, DEX. Further, the combination treatment of DEX + WS (200 mg/kg) caused synergistic reductions in hydroxyproline levels when compared with those observed in DEX monotherapy (Fig. 5).

Similarly, OVA immunization + challenge dramatically increased the expression of periostin, a matrix and fibrotic marker, in blood and BALF ($p < 0.01$ for both) as compared to the normal control. Pretreatment with WS extract significantly decreased OVA-induced periostin levels in both the serum and BALF of asthmatic rats, and the effects were more marked with the higher dose (400 mg/kg) of WS extract ($p < 0.05$ and $p < 0.01$, respectively). Moreover, the combination therapy of low-dose WS + DEX showed significant reductions in periostin levels in blood and BALF, which were comparable with the decrease observed for DEX (1 mg/kg). The low dose of the WS extract (200 mg/kg) also lowered periostin levels in both body

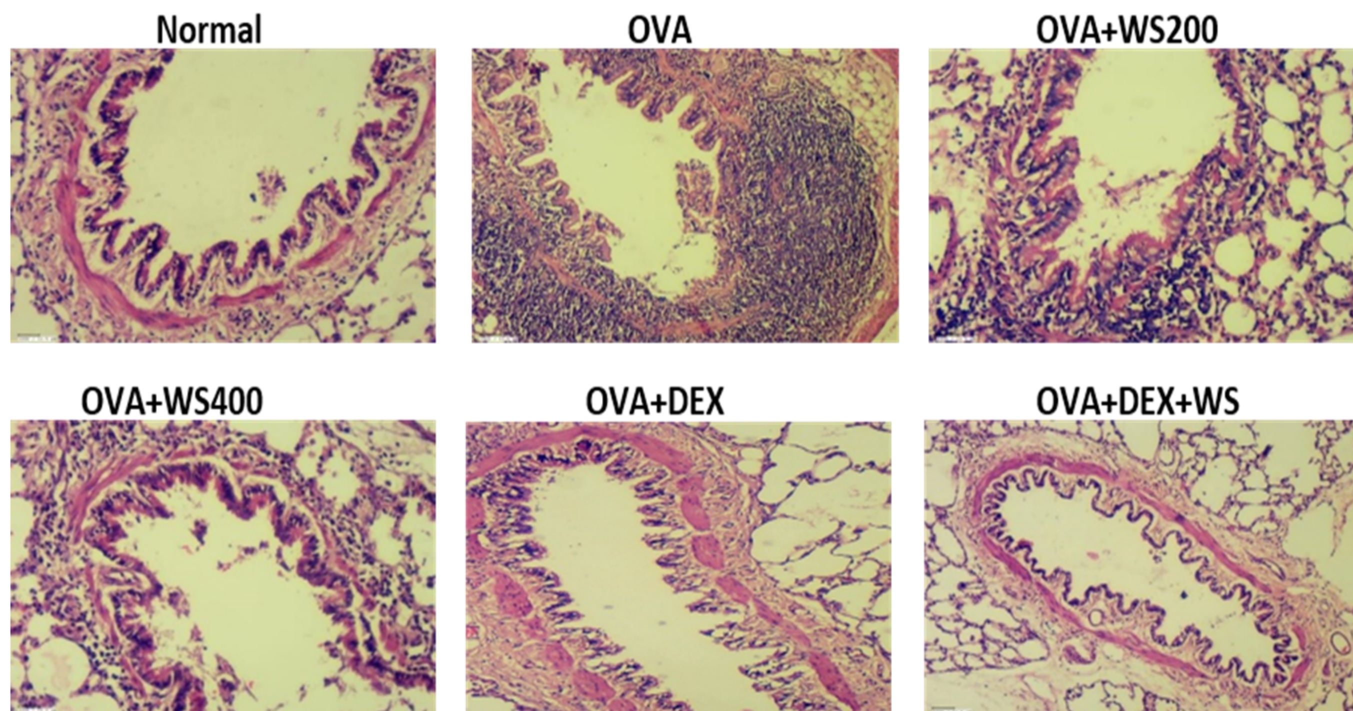

Figure 7. Effects of *WS* extract on OVA-induced changes in lung histology of rats. Hematoxylin and eosin (H&E, 10×.), stained lung tissue sections from rats in the normal control group show normal lung structures. In contrast, Ovalbumin (OVA) group showed more inflammatory cell infiltrate, increased alveolar septae thickness, and goblet cell hyperplasia. Considerable restoration of the lung architecture and modulation of inflammatory responses were observed after treatment with WS extract and DEX. OVA.

fluids, but these changes were not statistically significant (Fig. 6).

4. Effect of WS extract treatment on OVA-induced histopathological changes in lungs

Lung tissue sections from normal rats revealed that bronchioles and lung parenchyma appeared normal; alveolar septae were normal in thickness; and peribronchial inflammatory cell infiltration was not seen. In contrast, OVA-sensitization and challenge induced marked histopathological changes in the lungs, such as the accumulation of intraalveolar fluid, goblet cell hyperplasia, increased alveolar septal thickness, increased subepithelial collagen deposition, and severe peribronchial inflammatory cell infiltration. OVA-immunized rats that were pretreated with WS extract showed mild to moderate inflammatory cell infiltration and marked, dose-dependent reductions in goblet cell hyperplasia, alveolar septae thickness, and interstitial fibrosis. The effects of the higher dose were similar to those seen in the DEX group, while a slight insignificant attenuation in the above histopathological changes was reported in asthmatic rats pretreated with the lower dose of WS extract (200 mg/kg). In addition, the combination therapy of the lower doses of DEX + WS extract showed synergistically increased efficacy in suppressing these changes in lung sections (Fig. 7).

DISCUSSION

Airway remodeling of bronchial asthma results from dysregulated tissue repair after tissue injury and leads to irreversible loss of lung function and refractoriness to pharmacotherapy [12]. Current therapeutic strategies to prevent/treat airway remodeling of asthma are not only far from satisfactory but are also poorly sustainable and compromise patient safety. In the present study, we show that the standardized root extract of the Indian traditional medicinal plant, *Withania somnifera* (WS), protected against the immunological, biochemical, and histopathological changes seen in the experimental model of airway remodeling of asthma. Further, these ameliorating effects of WS were comparable with those seen after the comparator drug, dexamethasone.

Inflammatory cells and structural cells in the bronchial airways secrete several important mediators such as IL-13, IL-5,

and TGF-β, which regulate the process of airway inflammation and remodeling [13]. Yang et al. [14] demonstrated that the administration of an anti-IL-13 monoclonal antibody effectively inhibited airway hyperresponsiveness, inflammatory cell infiltration, and airway remodeling. An earlier study had also revealed that the number of eosinophils in blood smears and BALF was significantly decreased after treatment with WS extract as compared to asthmatic (OVA) mice [15]. Zhao et al. [16] showed that pretreatment with Withaferin A (a bioactive glycowithanolide in WS extract) inhibited OVA-induced lung injury and fibrosis progression in mice through the inhibition of inflammatory cell infiltration and the decrease in pro-inflammatory and profibrotic cytokine expression in BALF and lung tissue specimens. In another study, histopathological changes in the lung tissue were less marked in wild-type animals compared with animals immunized and challenged with aeroallergen and lacking either IL-5 or IL-13 [17]. In consonance with these data, our results showed that WS extract significantly decreased such raised IL-13 levels in the lung homogenate and BALF in OVA-induced asthmatic rats in a dose-dependent manner as compared to the controls. The fact that WS was able to reduce the IL-13 levels in both lung tissue and BALF strongly suggests a pharmacological rationale for the use of this medicinal plant in both airway inflammation and airway remodeling in this allergic model of asthma. TGF-β has been implicated in enhancing fibroblast and smooth muscle proliferation, angiogenesis, and inflammatory and profibrotic cytokine expression [18]. Our results further showed that in the OVA model for airway remodeling, pretreatment with WS extract attenuated the increased expression of TGF-β (a pro-fibrotic biomarker) in lung homogenates in a dose-dependent manner. Comparable results were also seen in the DEX and DEX + WS treatment groups. Attenuations by WS of this fibrotic biomarker further substantiate the protective effects of this plant extract against airway remodeling of asthma. Chronic airway inflammation in asthma is associated with oxidative DNA damage and lipid peroxidation, which is exacerbated by deficient antioxidant defenses in the airways. In a recent clinical study, oral treatment with 250 mg of WS root capsules for 12 weeks in COPD patients significantly decreased systemic inflammation and oxidative stress and improved lung functioning, quality of life, and exercise tolerance as compared with the placebo group [19]. In line with this result, our study findings showed that pretreatment with WS extract protected against oxidative stress-induced DNA damage in the lung tissue as evidenced by lowered 8-OHdG levels (a marker of oxidative stress-induced DNA damage) in lung tissues and BALF in OVA-asthmatic animals. Further, the effects of the combination treatment (DEX + WS) were greater than those with DEX monotherapy alone, indicating the complementary effects of WS extract against oxidative stress-induced DNA damage in the lungs during airway remodeling.

Collagen is one of the important hallmarks of subepithelial fibrosis and airway remodeling in patients with chronic asthma [20]. Previous reports demonstrated higher levels of hydroxyproline in blood and BALF samples in OVA-induced asthma in rats [9, 21]. Similarly, serum periostin is considered one of the novel biomarkers of asthma, especially of the Th2-driven disease phenotype [22]. A study showed that periostin levels were the best predictor for patients who derived maximum benefit after lebrikizumab (a humanized monoclonal antibody) treatment when compared to other inflammatory markers in asthma [23]. In agreement with these observations, our results showed that the increased levels of hydroxyproline and periostin in the blood, BALF, and/or lung tissue seen in the disease control rats were markedly and dose-dependently attenuated after WS administration. Furthermore, when compared with monotherapy, the combination treatment (DEX + WS) showed greater efficacy in reducing these biomarker levels. These results indicated the therapeutic potential of WS in airway remodeling and affirmed the validity of using periostin as an effective biomarker for asthma pathology. In addition, this suggests that WS was also effective in exerting a steroid-sparing effect while preventing airway remodeling. Our histopathological findings showed that WS extract effectively reduced peribronchial inflammatory cell infiltrates, goblet cell hyperplasia, alveolar septae thickness, and subepithelial fibrosis and maintained the structural integrity of the airways. Thus, the immunological and biochemical data of airway remodeling and its modulations by WS and DEX in the literature correlated well with the histopathological data of our experiments.

CONCLUSION

The present study shows that the WS extract induced reductions in IL-13, TGF-β, hydroxyproline, periostin, and 8-OHdG levels in the blood and BALF of OVA-induced airway remodeling in rats. These changes in biomarkers by WS extract were supported by the histopathological findings, which showed reduced inflammatory cells in the airways, goblet cell hyperplasia, and subepithelial fibrosis. The dose-dependent nature of the

WS effect and its comparability with the standard drug DEX validates the pharmacological significance of this finding. The synergy between WS and DEX with respect to various biomarkers and histopathological changes also indicates the possibility of herb-drug interactions and a complementary role of WS if added to standard treatment. These results are of translational significance, as in addition to the proven safety of this medicinal plant extract, they demonstrate that WS could be considered a potential therapeutic agent and/or an effective adjunct to conventional pharmacotherapy in bronchial asthma.

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AUTHORS' CONTRIBUTIONS

N.H.A.: Methodology, investigation, data analysis, writing, and original draft preparation S.R.: initial review and editing. M.N.: Formal analysis and initial reviewing. K.G.: Final review and editing. A.R.: Conceptualization, supervision, critical revision, and approval of the final manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL

The study was conducted after obtaining ethical approval on 05/04/2021 from the Institutional Animal Ethics Committee (IAEC), Jamia Hamdard (protocol No. 1767).

CONFLICTS OF INTEREST

No conflict of interest.

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ORCID

Nafaa Hasan Ali, https://orcid.org/0000-0003-0524-9107
Sana Rehman, https://orcid.org/0000-0001-8183-1778
Maaz Naqvi, https://orcid.org/0000-0002-2349-3771
Kavita Gulati, https://orcid.org/0000-0002-8815-1879
Arunabha Ray, https://orcid.org/0000-0003-0886-7875

REFERENCES

1. Chung F. Anti-inflammatory cytokines in asthma and allergy: interleukin-10, interleukin-12, interferon-gamma. Mediators Inflamm. 2001;10(2):51-9.
2. Fehrenbach H, Wagner C, Wegmann M. Airway remodeling in asthma: what really matters. Cell Tissue Res. 2017;367(3):551-69.
3. Chetta A, Zanini A, Foresi A, Del Donno M, Castagnaro A, D'Ippolito R, et al. Vascular component of airway remodeling in asthma is reduced by high dose of fluticasone. Am J Respir Crit Care Med. 2003;167(5):751-7.
4. Denis O, van den Brûle S, Heymans J, Havaux X, Rochard C, Huaux F, et al. Chronic intranasal administration of mould spores or extracts to unsensitized mice leads to lung allergic inflammation, hyper-reactivity and remodelling. Immunology. 2007;122(2):268-78.
5. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med. 2012;18(5):716-25.
6. Alzobaidi N, Rehman S, Naqvi M, Gulati K, Ray A. Periostin: a potential biomarker and therapeutic target in pulmonary diseases. J Pharm Pharm Sci. 2022;25:137-48.
7. Gulati K, Rai N, Ray A. Status of research in respiratory pharmacology in India during the last five years (2012-2017). Proc Indian Natl Sci Acad. 2018;84(1):55-72.
8. Ahmad M, Dar NJ. *Withania somnifera*: ethnobotany, pharmacology, and therapeutic functions. In: Bagchi D, editor. Sustained energy for enhanced human functions and activity. London: Academic Press; 2017. p. 137-54.
9. Rai N, Gulati K, Rahman RU, Ray A. Effects of a polyherbal agent on structural changes and biochemical markers during airway remodelling in experimental model of bronchial asthma. EC Pharmacol Toxicol. 2016;2(2):99-107.
10. Nair P, Prabhavalkar K. Anti-asthmatic effects of saffron extract and salbutamol in an ovalbumin-induced airway model of allergic asthma. Sinusitis. 2021;5(1):17-31.
11. Kim DI, Song MK, Lee K. Comparison of asthma phenotypes in OVA-induced mice challenged via inhaled and intranasal routes. BMC Pulm Med. 2019;19(1):241.
12. Mukherjee AA, Kandhare AD, Rojatkar SR, Bodhankar SL. Ameliorative effects of Artemisia pallens in a murine model of ovalbumin-induced allergic asthma via modulation of biochemical perturbations. Biomed Pharmacother. 2017;94:880-9.
13. Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. Immunity. 2019;50(4):975-91.

14. Yang G, Volk A, Petley T, Emmell E, Giles-Komar J, Shang X, et al. Anti-IL-13 monoclonal antibody inhibits airway hyperresponsiveness, inflammation and airway remodeling. Cytokine. 2004;28(6):224-32.
15. Oberholtzer HM, Pretorius E, Smir E, Ekpo OE, Humphries P, Auer RE, et al. Investigating the effect of Withania somnifera, selenium and hydrocortisone on blood count and bronchial lavage of experimental asthmatic BALB/c mice. Scand J Lab Anim Sci. 2008;35(4):239-48.
16. Zhao HM, Gao ZW, Xie SX, Han X, Sun QS. Withaferin A attenuates ovalbumin induced airway inflammation. Front Biosci (Landmark Ed). 2019;24(3):576-96.
17. Cho JY, Miller M, Baek KJ, Han JW, Nayar J, Lee SY, et al. Inhibition of airway remodeling in IL-5-deficient mice. J Clin Invest. 2004;113(4):551-60.
18. Yan Y, Liu L, Dou Z, Xu Y, Yan X. Soufeng Yuchuan decoction mitigates the ovalbumin-induced lung damage in a rat model of asthma. Biomed Pharmacother. 2020;125:109933.
19. Singh P, Salman KA, Shameem M, Warsi MS. Withania somnifera (L.) dunal as add-on therapy for COPD patients: a randomized, placebo-controlled, double-blind study. Front Pharmacol. 2022;13:901710.
20. Eap R, Jacques E, Semlali A, Plante S, Chakir J. Cysteinyl leukotrienes regulate TGF-β(1) and collagen production by bronchial fibroblasts obtained from asthmatic subjects. Prostaglandins Leukot Essent Fatty Acids. 2012;86(3):127-33.
21. Chauhan PS, Dash D, Singh R. Intranasal curcumin inhibits pulmonary fibrosis by modulating matrix metalloproteinase-9 (MMP-9) in ovalbumin-induced chronic asthma. Inflammation. 2017;40(1):248-58.
22. Izuhara K, Matsumoto H, Ohta S, Ono J, Arima K, Ogawa M. Recent developments regarding periostin in bronchial asthma. Allergol Int. 2015;64 Suppl:S3-10.
23. Scheerens H, Arron JR, Zheng Y, Putnam WS, Erickson RW, Choy DF, et al. The effects of lebrikizumab in patients with mild asthma following whole lung allergen challenge. Clin Exp Allergy. 2014;44(1):38-46.