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Original Article

Lycorine Attenuates Airway Inflammation in an OVA induced Allergic Asthma Model

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ABSTRACT

Background: Asthma is one of the most prevalent, complex, and severe respiratory disorders, with rising rates of occurrence and death in both children and adults. An alkaloid derived from plants, lycorine possesses several pharmacological effects, primarily anti-inflammatory and antioxidative properties. **Materials and Methods:** In the current study, the anti-asthmatic potential of lycorine against ovalbumin (OVA)-induced asthma mice model has been examined. The animals were categorized into five groups. The group I mice received saline while the group II mice received OVA. Groups III mice were provided with OVA + Lycorine in low dose whereas group IV mice were given OVA + Lycorine in high dose. Group V animals received OVA + Dexamethasone, which was used as the positive control. The bronchoalveolar lavage fluid isolated from the lungs was utilized to estimate various parameters such as total and differential immune cells (eosinophils, neutrophils, lymphocytes, macrophages), secreted inflammatory cytokines levels, and oxidative stress markers. The lungs were subjected to histological examination and respiratory mechanics examination. **Results:** Lycorine treatment significantly suppressed the total and differential immune cells, IgE, IL-4, IL-5, IL-13, and TNF-α cytokines, MDA, NO_2 and NO_3 levels and enhanced the IFN-γ cytokine level along with the SOD, CAT, and GSH enzyme activities. Further, the histopathological study revealed that lycorine was able to decrease the thickness of the columnar epithelial cells in the airways. Thus, lycorine suppressed the ROS generation that in turn reduced the inflammation in the airways of the lungs by reducing Th2 cytokines and augmenting the levels of Th1 cells. **Conclusion:** These findings indicate that lycorine was able to regulate the Th1/Th2 balance OVA-challenged asthmatic mice, signifying its protective impact that may be valuable in the management of allergic asthma.

Keywords: Asthma, Ovalbumin, Lycorine, Inflammatory cytokines, Oxidative stress.

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INTRODUCTION

Asthma is a chronic inflammatory disease of the airways, accompanied with symptoms such as blockage of airflow, hyperresponsive airways, abundant T cell-mediated cytokine production, hyper-secretion of mucus, wheezing, chest stiffness and broncho-constriction.[1-3] The immune system is crucially involved in the regulation of inflammatory diseases, particularly asthma. Normally, for proper immune functioning, the balance between T helper type 1 (Th1) as well as T helper type 2 (Th2) cells is essential.[4] A disproportion in the levels of Th1 and Th2 can lead to inhibition of Interleukin (IL)-12 and γ-interferon (γ-IFN) levels, and elevation of the levels of IL-4, IL-6, and IL-13 proteins.[5]

Numerous immune cells including mast cells, eosinophils, T-helper lymphocytes (Th2), basophils, macrophages, dendritic cells, and neutrophils are triggered and matured during the progression of asthma.[6,7] Specifically, the stimulation of immunoglobulin E (IgE) production by activated Th2 CD4+ cells is triggered by the release of type 2 cytokines, such as IL-4, IL-5, and IL-13. Asthmatic inflammation is mediated by IgE that causes promotion of mast cell activation, goblet cell hypergenesis, excessive mucus secretion, infiltration of eosinophils, and hyperresponsiveness of the airways.[8,9] Ovalbumin (OVA) has been employed as an experimental trigger for asthma in animal models. The main mechanisms associated with OVA-stimulated asthma involve overexpression of nitric oxide (NO) and Th2 cytokines (IL-4, IL-5, and IL-13).[10]

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As one of the most commonly occurring ailments, asthma negatively impacts both human health and quality of life. In spite of considerable progress in diagnosis and treatment, asthma continues to be a major global health issue, and many patients are still unable to control it properly. Anti-inflammatory corticosteroids and bronchodilators are employed presently in therapeutic approaches.[11] However, as a consequence of long-term continuous use of these combination therapies, some patients do not respond or experience undesirable side effects.[12] To counteract this trend, recent studies have sought out complementary therapies, such as phytotherapy, and efficient treatments that can help control airway remodelling.[13] A large number of evidence suggests that plant-based immunomodulatory agents are an effective, safe, and accessible alternative to existing therapies to combat inflammation and allergy symptoms.[14,15] Lycorine, a naturally occurring alkaloid, is found in several plants in the Amaryllidaceae family.[16] Its anti-inflammatory, antitumor, antioxidant, antimalarial, antiaging, antiviral, antibacterial, antifungal, anti-plasmodial, and insect repellent properties are among its many benefits.[17-20] Moreover, inflammatory conditions of the bronchioles and lungs have been reported to be treated effectively with lycorine.[21]

The primary objective of the current investigation is to analyze the anti-asthmatic potential of Lycorine against ovalbumin-induced asthma mice model. The effect of lycorine on several parameters including the total and differential immune cells, IgE and secreted inflammatory cytokines levels, oxidative stress markers, and respiratory mechanics was examined.

MATERIALS AND METHODS

Chemicals and Reagents

Lycorine, OVA, Dexamethasone, Griess reagent, thiobarbituric acid (TBA), trichloroacetic acid (TCA), Ellman's reagent and pyrogallol were procured from Sigma-Aldrich (USA). Enzyme-linked immunosorbent assay (ELISA) kits of mouse IL-6, TNF-α, IgE, and IL-12 were purchased from Krishgen Biosystems (Mumbai, India). Additional reagents and chemicals utilized in the study were analytical quality.

Animals and Treatment

The experimental study was conducted on BALB/c mice aged 6-8 weeks. The mice were housed in a pathogen-free surrounding at 24°C with a 12-hr light/dark cycle. All animal experiments were conducted in accordance with the protocol approved by the ethical committee. The animals were categorized into five groups. The group I mice received saline while the group II mice received OVA. Groups III mice were provided with OVA + Lycorine in low dose whereas group IV mice were given OVA + Lycorine in high dose. Group V animals received OVA + 1mg/kg of Dexamethasone, which was used as the positive control. Group II, III, IV, and V mice were injected with 10 mg ovalbumin mixed with 1 mg aluminium hydroxide on 0^{th} and 5^{th} day to trigger asthma.

Estimation of Total and Differential Immune Cells

On the final day of the experiment, the animals were sacrificed. An endotracheal tube was used to collect the bronchoalveolar lavage fluid (BALF) from the left lung. After centrifugation (1600 rpm, 4°C, 10 min), a cell pellet was dispersed in saline and analyzed by Wright-Giemsa staining for differential cell counts. Using a haemocytometer, total and differential inflammatory cells (eosinophils, neutrophils, lymphocytes, macrophages) were measured.

Estimation of IgE Levels

The blood was subjected to centrifugation following BALF collection. The serum was then separated and kept at -80°C. The IgE levels of all the samples were then quantified with the help of ELISA kits, as per the manufacturer's protocol.

Measurement of Secreted Inflammatory Cytokines

The levels of secreted inflammatory cytokines such as γ-IFN, TNF-α, IL-4, IL-5, and IL-13 in the BALF were estimated with the corresponding ELISA kits, by following the manufacturer's instructions.

Assessment of Respiratory Mechanics

Mice were anesthetized intraperitoneally (10 mg/kg xylazine and 100 mg/kg ketamine) and a tracheal cannula was implanted 24 hr after the last trial. Mice were then attached to a tiny ventilator and ventilated at 150 breaths per minute with a 3 cm H_2O positive end-expiratory pressure (PEEP). The animal was then given pancuronium bromide and airway hyperresponsiveness (AHR) was assessed both at baseline and after increasing doses of methacholine supplied using an ultrasonic nebulizer. The forced oscillation approach was used to quantify respiratory mechanics, with the single compartment and constant phase model, a mathematical model that permits changes to be partitioned into major airway and peripheral sections. The impedance data were fitted using a model that included an airway resistance (Raw) and inertance, as well as a constant-phase model encompassing tissue damping (G) and elastance (H). The hysteresivity of respiratory tissue (η) was computed as G/H.[22,23]

Effect of Lycorine on the Antioxidant Markers

In the BALF of mice, the levels of antioxidant enzymes including SOD (superoxide dismutase), CAT (catalase), and GSH (glutathione) were measured biochemically.[24] Following the procedure explained by Marefati *et al.* (2019),[25] the levels of nitrite (NO_2), nitrate (NO_3), and malonaldehyde (MDA) were assessed.

Histopathological analysis of lungs

Tissues were fixed for 24 hr in 10% neural formalin solution. Following the dehydration step, the tissues were fixed in paraffin, dissected into 5 m thick slices, and stained with haematoxylin and eosin (H&E). Digital pictures were acquired using LAS microscope software to identify histopathologic characteristics (Leica Microsystems, Germany).

Statistical Analysis

In this investigation, all data are reported as the mean ± SD of at least three replicates. The statistical program SPSS (version 15.0) was used for all statistical analyses. The data was evaluated using one-way ANOVA and Bonferroni's test. $p < 0.05$ was regarded as significant.

RESULTS

Effect of lycorine on the total and differential immune cell count in OVA-challenged mice

As demonstrated in Figure 1, asthmatic mice exhibited a higher total leukocyte count than control group mice. In OVA-induced mice, the number of neutrophils, eosinophils, and macrophages elevated considerably. In OVA-triggered mice, lycorine and dexamethasone treatment reduced the total and differential inflammatory cell count in a dose-dependent fashion, indicating its immunomodulating effect.

Impact of lycorine on the IgE levels in OVA-triggered mice

When compared to control group mice, the IgE levels were considerably higher in the OVA-challenged group. Lycorine and dexamethasone treatment dramatically reduced OVA-specific IgE levels in a dose-dependent approach (Figure 2), suggesting the inflammation alleviation potential of lycorine.

Effect of lycorine on the levels of secreted inflammatory cytokines in OVA-induced mice

The Th1 related inflammatory cytokines such as γ-IFN, TNF-α, and Th2 related cytokines such as IL-4, IL-5, and IL-13 were measured in the BALF and the results are illustrated in Figure 3. Higher levels of IL-4, IL-5, IL-13, and TNF-α and lower level of γ-IFN were observed in the OVA-challenged animals when compared to the control group. In contrast, lycorine treatment downregulated the levels of IL-4, IL-5, IL-13, and TNF-α and upregulated the level of γ-IFN in a concentration-dependent approach. Moreover, the suppression of IL-5, IL-13, and TNF- α treated with the highest dose of lycorine was comparable to the dexamethasone treatment.

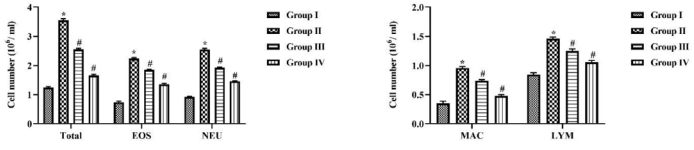

Figure 1: Effect of Lycorine and dexamethasone on total and differential cell count in OVA-challenged asthma in BALB/c mice model.
The data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Bonferroni's test. #*p*<.001 compared to control group I; **p*<.001 compared to group II.

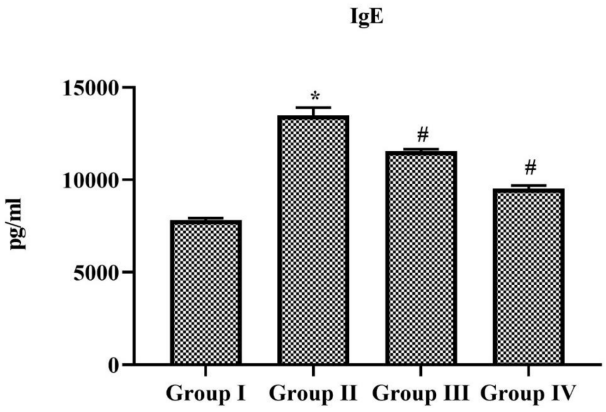

Figure 2: Effect of Lycorine and dexamethasone on Immunoglobulin E (IgE) levels in OVA-stimulated asthma in BALB/c mice model.
The data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Bonferroni's test. #*p*<.001 compared to control group I; **p*<.001 compared to group II.

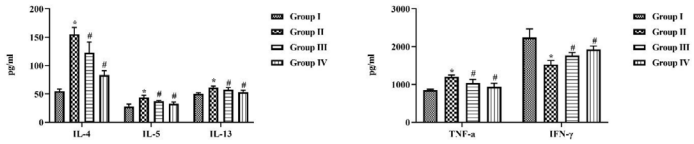

Figure 3: Effect of Lycorine and dexamethasone on IL-4, IL-5, IL-13, TNF-α, and IFN-γ levels in OVA-stimulated asthma in BALB/c mice model.
The data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Bonferroni's test. #*p*<.001 compared to control group I; **p*<.001 compared to group II.

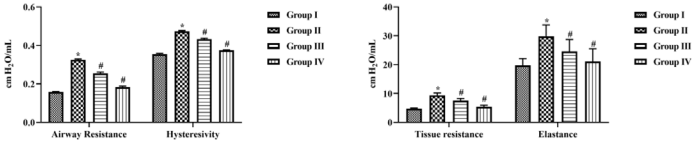

Figure 4: Effect of Lycorine and dexamethasone on airway resistance, hysterivity, tissue resistance, and elastance in OVA-challenged asthma in BALB/c mice model.
The data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Bonferroni's test. #*p*<.001 compared to control group I; **p*<.001 compared to group II.

Effect of lycorine on the respiratory parameters in OVA-triggered mice

All the respiratory parameters including the airway resistance, hysterivity, tissue resistance, and elastance were enhanced significantly in the asthmatic mice whereas these parameters

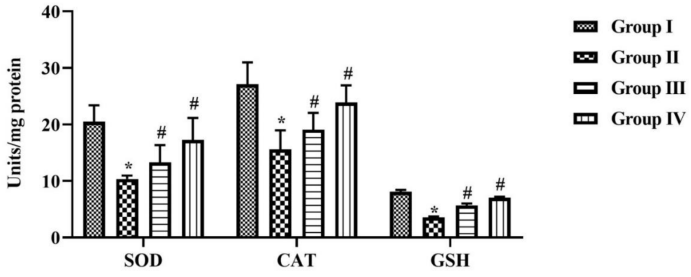

Figure 5: Effect of Lycorine and dexamethasone on SOD, GSH, and CAT enzyme levels in OVA-challenged asthma in BALB/c mice model.
The data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Bonferroni's test. #p<.001 compared to control group I; *p<.001 compared to group II.

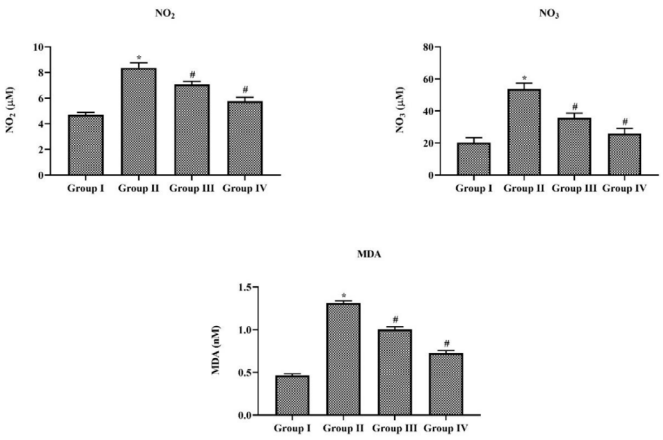

Figure 6: Effect of Lycorine and dexamethasone on MDA, NO_2, and NO_3 levels in OVA-stimulated asthma in BALB/c mice model.
The data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Bonferroni's test. #p<.001 compared to control group I; *p<.001 compared to group II.

seemed to decrease remarkably upon dexamethasone and lycorine treatment in a dose-dependent trend (Figure 4).

Effect of lycorine on the antioxidant enzyme levels in OVA-challenged mice

The level of antioxidant enzymes SOD, CAT, and GSH was found to reduce considerably upon OVA induction, as evident from Figure 5. Contrastingly, lycorine and dexamethasone treatment reversed this effect by increasing the levels of antioxidant enzymes with increasing concentration in a significant manner.

Impact of lycorine on the oxidative stress markers in OVA-stimulated mice

The MDA, NO_2, and NO_3 concentrations in the lung tissue of BALB/c mice improved dramatically after they received OVA (Figure 6). However, lycorine administration in BALB/c mice resulted in a considerable reduction in these levels, indicating their anti-oxidative potential. Moreover, the suppression of the

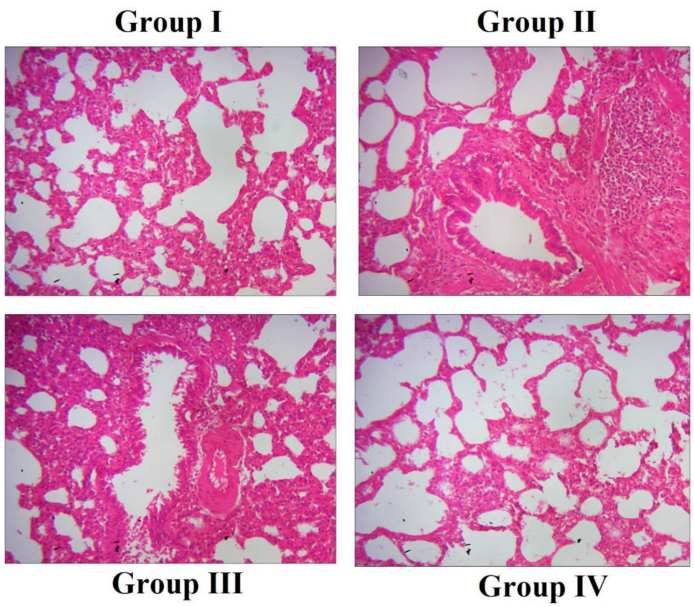

Figure 7: Photographs of transverse sections of mice lung tissue challenged with OVA, lycorine and dexamethasone followed by staining with H&E and examination under light microscopy (X100).
The OVA-mice demonstrated extensive inflammation and the lycorine treatment effectively ameliorated the inflammatory response in the lung tissues of OVA-mice.

oxidative stress markers treated with the highest dose of lycorine was comparable to the dexamethasone treatment.

Effect of lycorine on the histopathological analysis of lung tissues

According to the histological analysis, in comparison to normal mice, OVA-induced animals exhibited high infiltrating inflammatory cells in the airways and thicker columnar epithelium airway walls. Interestingly, lycorine treatment efficiently reversed this phenomenon by decreasing the infiltration and thickening of the walls with several air sacs. The treatment with dexamethasone showed almost complete recovery of the lung tissue and resembled like the normal control group (Figure 7).

DISCUSSION

Allergic asthma is one of the most common and complex inflammatory diseases, hugely impacting people of all ages. Incidence rate, hospitalization frequency, and death have all escalated dramatically in the last 50 years. Corticosteroids, leukotriene modifiers, and anti-cholinergic drugs have been used to treat asthma for decades, with the objective of lowering inflammation and bronchoconstriction in the respiratory system. However, owing to their negative consequences, there is an increasing interest in establishing phytomolecules rich plant-based approach since they are safe, available, and readily accessible. Thus, plant derived compounds are being advocated

as valuable treatment options in effectively treating inflammatory illnesses including asthma.[26,27]

Lycorine, a plant-based alkaloid, is widely known for possessing excellent anti-inflammatory and anti-oxidant properties. Moreover, earlier research has demonstrated a critical role of lycorine as a potent attenuator of acute lung injury and pulmonary fibrosis in animal model.[28,29] OVA is commonly utilized in animal studies for induction of asthma.[30] The OVA-challenged asthma model was chosen to examine bronchial inflammation because it closely resembles the symptoms of human asthma. This model has been used to investigate the morphological and functional alterations associated with allergic asthma.[31] In this study, the OVA-induced mouse model demonstrated increased allergy and asthmatic symptoms, indicating an appropriately generated airway inflammation in the animal.

The prevalence of inflammatory cells in the lungs (Neutrophils, eosinophils, and macrophages) is an indication of inflammation and asthma.[32] Macrophages are important players in inflammation because their activation triggers a cascade of inflammatory responses, which include the production of inflammatory mediators and cytokines.[33] Eosinophil infiltration into the bronchial tubes of lungs is a hallmark of asthma, and a higher number of such cells has been observed in BALF.[34] In OVA-triggered group II mice, we discovered that the total and differential inflammatory cell counts were enhanced, and that lycorine considerably reduced these inflammatory cell counts. These findings reveal the potential anti-asthmatic action of lycorine in the animal model.

IgE is one of the most critical components in the course of allergic responses by recruiting effector cells such as eosinophils, basophils, and mast cells in allergy, and is thus an important target for developing anti-asthmatic approaches.[15] Our findings reveal that the IgE expression was elevated in OVA-challenged group II mice and lycorine treatment effectively reduced the IgE levels significantly, suggesting its inhibitory role in the allergic inflammatory responses. These findings are consistent with prior research that found *Scrophularia buergeriana* and emodin to be effective in reducing OVA-LPS challenged allergic inflammation by lowering IgE levels.[35]

The progression of asthma is linked to the differentiation of CD4+ T cells, comprising Th1 and Th2. In particular, the Th2 immune pathway is especially significant in the onset and establishment of asthma. Clinical manifestations of this allergic disease are the result of an imbalance between Th1/Th2 cells.[36] The Th1-related cytokine such as IFN-γ cytokine can be associated with potent anti-allergic properties of drugs, since it has been demonstrated to prevent the migration of eosinophils to the lungs, block the production of IgE, and the transformation of precursor cells to Th2 cells. It also contributes significantly in preventing airway smooth muscles constriction.[37] TNF-α is a prototypical pro-inflammatory cytokine released by Th1 cells that has pleiotropic potential in a wide range of cells and is involved in both acute and chronic inflammatory diseases. As a result, therapeutic TNF-α blockade is extremely efficacious in the treatment of such inflammatory conditions.[38]

Contrarily, Th2 cells generate IL-4 and IL-5 cytokines and aid B cells, and are considered to make a significant contribution in the pathogenesis of allergic disorder. The proliferation and differentiation of eosinophils are regulated by IL-4 and IL-5, which also serve as an important signal for their migration into inflammatory tissue in response to antigen stimulation.[39] IL-13 is an effective therapeutic strategy for treating asthma, as it triggers airway hyperresponsiveness (AHR) and various structural changes in chronic asthma, such as goblet cell hyperplasia and fibrosis of subepithelial cells.[40]

When compared to the levels in OVA-stimulated mice, Lycorine effectively reduced the concentration of secreted pro-inflammatory cytokines such as IFN-γ, TNF-α, IL-4, IL-5, and IL-13. Notably, lycorine had a larger inhibitory impact on Th2 cell-specific cytokines (IL-4, IL-5, and IL-13) than on Th1 cell-specific cytokine (TNF-α). Lycorine has earlier been demonstrated to decreased the levels of TNF-α in ISO-induced animal model.[41,42] The respiratory parameters such as airway resistance, tissue resistance hysteresis, and elastance in the OVA-challenged group II mice was augmented and lycorine treatment aided in lowering these lung function parameters in a concentration-dependent trend, which is concomitant with earlier investigations.[43]

Oxidative stress is a crucial contributory factor to inflammation and is linked to the emergence of chronic asthma due to AHR caused by direct contracting of bronchial smooth muscle. Activated inflammatory cells including eosinophils, neutrophils, monocytes, and macrophages can generate reactive oxygen species (ROS), which play a central role in pathogenesis of asthma.[44] Another important mediator in the pathogenesis of allergic asthma is nitric oxide (NO), that leads to oxidative damage to the cells. The results showed that OVA-challenged animals were under extreme oxidative stress, as evidenced by the elevated MDA, NO_2, and NO_3 levels and reduced antioxidant enzyme levels. Furthermore, the concentrations of pro-inflammatory cytokines were enhanced in the OVA-induced asthmatic rats, suggesting that oxidative stress contributed to the advancement of inflammation. These results are in line with earlier reports demonstrating that plant-derived compounds such as vanillic acid, and *Phyllanthus amarus* phytoconstituents attenuate immunological response as well as oxidative stress in OVA-challenged asthma.[45,46] Lycorine has earlier been demonstrated to effectively inhibit oxidative stress in an LPS-stimulated lung injury mice model.[28]

SOD, catalase, and GSH are antioxidant enzymes that play a pivotal role in asthma by protecting against oxidative damage and decreasing the persistent inflammatory response.[47] ROS has the capability to interact with GSH, which leads to exhaustion of the antioxidant and ultimately creating an oxidative stress condition.[48] In our investigation, the antioxidant enzyme concentration was restored upon lycorine treatment, suggesting their remarkable anti-oxidant potential. In an earlier investigation, lycorine was found to lower the MDA levels and increase the SOD activity in an ISO-triggered mice model, confirming its anti-oxidant property.[42] Overall, elevated ROS increases inflammation in the airways of the lungs by overexpressing Th2 cytokines and suppressing the levels of Th1 cells.

Asthma is marked by thickened airway wall owing to inflammation and increased mucus production as a result of inflammatory cells penetrating the airway wall, which is evident from the histopathological examination of lung tissues of OVA-induced group II mice. These aberrant characteristics of the airway lumen obstruct the airway channels and generate excessive surface tension, resulting in airway obstruction.[43] This is attributed to activation of mucous glands as well as hyperplasia of goblet cells in the airway epithelium.[49] However, lycorine treatment led to the reduction in the columnar epithelium thickness, implying its excellent protective ability against airway resistance by OVA-stimulated asthma.

CONCLUSION

In summary, lycorine treatment conferred remarkable protection against asthmatic symptoms in OVA-induced mice. This impact might be attributed to its capacity to minimize inflammatory cell build-up, restore antioxidant enzyme activity, diminish reactive species generation, and lower pro-inflammatory cytokines levels. Thus, lycorine suppressed the ROS generation that in turn reduced the inflammation in the airways of the lungs by reducing Th2 cytokines and augmenting the levels of Th1 cells. The findings of the study indicate that lycorine might be valuable in the treatment and management of allergic asthma.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

OVA: ovalbumin; **Th2:** T helper type 2; **IL:** Interleukin; **γ-IFN:** γ-interferon; **NO:** Nitric oxide; **TBA:** Thiobarbituric acid; **TCA:** Trichloroacetic acid.

SUMMARY

- Asthma is one of the most prevalent, complex, and severe respiratory disorders, with rising rates of occurrence and death in both children and adults
- Lycorine treatment significantly suppressed the total and differential immune cells, and cytokines.
- Lycorine suppressed the ROS generation that in turn reduced the inflammation in the airways of the lungs by reducing Th2 cytokines and augmenting the levels of Th1 cells

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