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Original Article

Berbamine Ameliorates LPS-Induced Inflammation in RAW264.7 Cells and Ovalbumin-Induced Allergic Asthma in Mice

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ABSTRACT

Background: Asthma is a common and long-lasting respiratory disorder affecting more than 300 million individuals worldwide. Berbamine is a natural and highly active pharmacological compound extracted from the plant Berberis amurensis. **Objectives:** In our study, we evaluated the potency of berbamine against asthma, a non-communicable chronic lung disease that affects approximately 300 million individuals and is a foremost cause of mortality associated with long-lasting respiratory ailment. **Materials and Methods:** An *in vitro* method using RAW264.7 macrophage cells was employed, where allergic conditions were simulated through LPS administration. Before LPS treatment, the cytotoxic effect of berbamine on RAW264.7 cells was examined. Following LPS stimulation in berbamine-treated cells NO, TNF-α, and IL-6 levels were quantified to assess inflammation. Additionally, an allergic asthma model in animals was created through ovalbumin sensitization and treated these animals with berbamine. **Results:** Eotaxin levels and ovalbumin-specific IgE antibodies were quantified to evaluate the induction of asthma resulting from ovalbumin exposure. Bronchoalveolar lavage fluid was collected to examine the infiltration of total and differential leukocyte counts. Lung weight index, NO, and myeloperoxidase levels in the experimental animals were analyzed to assess berbamine's efficacy against ovalbumin-induced allergic reactions. Oxidative stress and antioxidant levels in the berbamine-treated asthmatic mice was measured. The inflammation-reducing effects of berbamine during ovalbumin sensitization were assessed by quantifying levels of TNF-α, INF-γ, TXB2, and the proinflammatory cytokines IL-6 and IL-12. Furthermore, we explored the efficacy of berbamine in type 2 inflammation by measuring IL-4, IL-5, and IL-13 cytokines. Our results demonstrate that berbamine exhibits a potent anti-inflammatory effect in both asthma models. It effectively attenuated inflammation triggered by LPS stimulation and ovalbumin sensitization, inhibiting cytokines associated with type 2 inflammation. **Conclusion:** Thus, our findings confirm that berbamine not only reduces inflammatory responses but also mitigates inflammation in allergic asthmatic conditions.

Keywords: Allergic asthma, Phytoalkaloids, Berbamine, LPS stimulation *in vitro* model, Ovalbumin sensitization *in vivo* model, Type 2 inflammation.

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INTRODUCTION

Asthma is a common long-lasting respiratory disorder affecting more than 300 million individuals globally,[1,2] with prevalence rates varying between 1% and 29% across different countries.[3,4] About 9.1% of children, 11.0% of adolescents, and 6.6% of adults were reported with asthma, underscoring a significant public health issue.[5] The condition is marked by rhonchi, dyspnea, expectoration, and it carries serious health risks due to chronic airway inflammation.[6] This inflammation results in airflow obstruction and increased airway hyper responsiveness, contributing to about 2.5 lakh mortalities per year globally. Additionally, asthma's impact goes beyond physical symptoms; it adversely affects the well-being of patients and their families, leading to psychological, medical, and financial burdens.[7] The disease has become especially prevalent in wealthier nations, with its incidence rising significantly in the last three decades.[8]

Type 2 inflammation is a key contributor to the pathophysiology of specific bronchial hyper-reactivity phenotypes, particularly allergic asthma. This immune response is driven by T-helper 2 (Th2) cytokines, including IL-4, IL-5, and IL-13, which facilitate the activation and mobilization of eosinophils, mast cells, and basophils.[9,10] Additionally, elevated levels of allergen-specific Immunoglobulin E (IgE) exacerbate the inflammatory cascade, leading to increased airway hyper responsiveness and

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obstruction.[11,12] Studies indicate that type 2 inflammation not only plays a role in acute asthmatic episodes but also contributes to chronic disease progression and associated complications.[13]

Persistent inflammation and impaired tissue repair mechanisms result in airway remodeling, which manifests in clinical symptoms over time.[14] While no definitive treatment for asthma presently exists, conventional treatments primarily targets to control symptoms through the use of leukotriene receptor antagonists, inhaled corticosteroids and long-acting β2-agonists.[15,16] However, these therapies do not consistently modify disease progression across all asthma subtypes. The development of targeted biologic therapies that inhibit interleukin 4,5 and 13 signaling, has demonstrated significant benefits in managing severe asthma and improving patient outcomes. These advancements underscore the critical function of type 2 inflammation in guiding therapeutic strategies for asthma management.[17]

Bisbenzylisoquinoline alkaloids exhibit neuroprotectant and inflammatory inhibiting properties.[18,19] Berbamine, is one such bisbenzylisoquinoline alkaloid extracted from Berberis amurensis,[20,21] is a promising novel compound which has garnered interest for its therapeutic potential across different diseases.[22-24] Studies have shown that berbamine displays considerable anti-tumor effects across multiple cancer types by suppressing cell growth and triggering apoptosis in cancer cells.[25,26] Its diverse effects indicate that berbamine may be a highly promising therapeutic compound. In our study we examined the ameliorative efficacy of berbamine against the allergic asthma in *in vitro* and animal model.

MATERIALS AND METHODS

Chemical

Berbamine and OVA was commercially procured from Sigma Aldrich, USA. To assess the biochemical markers, the assay kits were obtained from BioLegend, Abcam, and Elabscience, USA, respectively.

Cell culture

The RAW264.7 macrophage cell line was maintained in a high-glucose Dulbecco's Modified Eagle Medium enriched with 10% inactivated FBS at 37°C within a 5% CO2 atmosphere. A total of 5,000 cells per well were plated in a 96-well plate and incubated for 12 hr. After incubation, the cells were exposed to 1 µg/mL Lipopolysaccharide (LPS) for 24 hr to induce an inflammatory response.

Cell viability assay

For the evaluation of berbamine on survival of cells, the RAW264.7 cells were cultured for 24 hr and subjected to berbamine (1-50 µM) concentration treatment for 24 hr. Upon completion of treatment period 10 mL of MTT chemical was added incubated for 4 hr and the crystals formed was dissolved with DMSO. The OD values were then quantified at 570 nm, with the optical density of the control group (untreated cells) set as 100% viability.

Quantification of NO levels

The measurement of Nitric Oxide (NO) levels in the cell supernatant was quantified with Griess reagent. RAW264.7 cells were seeded at 5000 cells per well in 96-well plates and incubated for 12 hr. Afterward, they were treated with varying concentrations of berbamine (1-50 µM) and 1 µg/mL of Lipopolysaccharide (LPS) for 24 hr. 50 µL of supernatant from each well was mixed with 50 µL of Griess Reagents I and II, incubated for 10 min at room temperature, and the optical density was measured at 540 nm to evaluate nitric oxide levels.

Quantification of TNF-α and IL-6 levels

RAW264.7 cells were various concentrations of berbamine (1-50 µM) and 1 µg/mL of Lipopolysaccharide (LPS) for 24 hr. After treatment, supernatants were collected, and levels of Tumor Necrosis Factor α and Interleukin-6 (IL-6) were determined using ELISA kits from Abcam, following the manufacturer's instructions.

Experimental Animals

Female BALB/c mice (6-8 weeks old, 20-25 g) were housed under a 12-hr light/dark cycle at 23±2°C with free access to standard chow and water. They were acclimated for one week before experiments began.

Treatment Regimen

The animals were assorted to four treatment clusters: normal control, ovalbumin-sensitized, ovalbumin-sensitized with berbamine treatment, and ovalbumin-sensitized with dexamethasone treatment.

Control Group: Mice in this group received oral saline daily as a negative control.

OVA-Sensitized Group: These mice were sensitized with ovalbumin by receiving intraperitoneal injections of 10 mg/kg of ovalbumin combined with 1 mg/kg of aluminum hydroxide on the 0th and 5th days of treatment.

Berbamine Group: Mice in this group underwent the same sensitization and challenge procedure as the OVA-sensitized group, in addition to receiving an oral dose of 20 mg/kg of berbamine daily for 21 days.

Dexamethasone (Positive Control) Group: Similar to the berbamine group, these mice were sensitized and challenged according to the OVA protocol, and they received oral administration of 2 mg/kg of dexamethasone daily for 21 days.

The mice were sacrificed 24 hr following the final treatment, and blood samples along with lung tissue were collected for subsequent analysis. Body weight of mice were measured before

the euthanization and the lung tissues extracted were weighed to measure the lung weight index.

BALF Collection and Leukocyte count

24 hr after the final treatment, bronchoalveolar lavage was performed by cannulating the trachea. The lungs were flushed twice with 0.7 mL PBS to collect Bronchoalveolar Lavage Fluid (BALF), which was then centrifuged at 1500× g for 10 min. The supernatant was stored at -80ºC for cytokine analysis, while the cell pellets were resuspended in 100 µL PBS. Leukocyte counts and differential analysis were conducted using the ProCyte Dx® Hematology Analyzer.

Quantification of Eotaxin and Ova specific IgE

Eotaxin and Ova specific IgE antibodies were quantified in the experimental mice using the Mouse Eotaxin ELISA Kit from Invitrogen and OVA Specific IgE ELISA Kit from BioLegend. The test were conducted in triplicates as the per the guidelines provided in the kit manual.

Quantification of NO and MPO

Nitric oxide and the myeloperoxidase levels in the ova sensitized untreated and drugs treated animals were quantified using the colorimetric assay kits procured from Elabscience and Merck respectively. The protocol prescribed by the manufacturer were followed to perform the analysis and the tests were performed in triplicates.

Quantification of Oxidative stress

Oxidative stress induced by OVA sensitization was evaluated in the experimental animals. Superoxide Dismutase (SOD) activity was quantified following the protocol established by Minami and Yoshikawa (1979).[27] Catalase activity was assessed using Aebi's method, where Hydrogen Peroxide (H_2O_2) served as the substrate, and absorbance changes were monitored at 240 nm over a duration of 2 min.[28] Glutathione levels were determined using the protocol developed by Ellman (1959).[29] Additionally, Malondialdehyde (MDA) levels were quantified using a colorimetric kit from the Lipid Peroxidation (MDA) Assay Kit.

Evaluation of inflammation

The inflammatory response induced due to Ova sensitization and the anti-inflammatory effect of berbamine was evaluated via quantifying various inflammation stimulating cytokines and proteins. TNF α, IL-12, IL-6, TXB2, IL-4, IL-5, IL-13, IFN γ were measured in the experimental animals using ELISA kits purchased from Abcam and Elabscience. The experiments were performed accordance to the protocol instructed in the kit and experiments were done in triads to avoid false results. Optical Density (OD) is measured using a spectrophotometer set to a 450 nm.

Statistics

Data are presented as the Mean±SEM and analyzed using GraphPad Prism software (version 5.0, USA). A *p*-value below 0.05 was considered statistically significant. One-way ANOVA followed by Tukey's *post hoc* test was used to evaluate statistical significance.

RESULTS

Berbamine doesn't rendered cytotoxicity on RAW264.7 cells

The cytotoxic effect of berbamine on RAW264.7 macrophage cells were quantified with cell viability assay and the results were tabulated in Figure 1. Treatment with varied concentrations of berbamine in the absence of LPS treatment doesn't rendered any significant cell death in RAW264.7 cells. Even the highest dose 50 µM berbamine treated cells exhibited less than 10% of dead cells with 24 hr incubation.

Berbamine attenuated inflammation in LPS induced RAW264.7 cells

The inflammatory response triggered by the LPS and the anti-inflammatory potency of berbamine against LPS triggered inflammation was assessed and the results were illustrated in Figure 2. Anti-inflammatory response of berbamine with varied concentrations against LPS triggered inflammation was measured via quantifying the NO, TNFα and interleukin 6 in the LPS triggered RAW264.7 cells treated with berbamine. LPS stimulation had significantly increased the levels of inflammatory stimulators NO, TNFα and IL-6 in RAW264.7 cells than the normal control cells. Treatment with berbamine along with LPS stimulation were significantly reduced NO, TNFα and IL-6 levels.

Berbamine suppressed Eotaxin and Ova specific IgE levels in OVA sensitized mice

Eosinophilic leukocytes are found in elevated quantities in the lungs of individuals with asthma. In asthmatic lungs, eotaxin, a strong eosinophil chemoattractant, is produced, contributing to this accumulation was quantified in Ovalbumin sensitized alone and drug treated animals. Treatment with berbamine and dexamethasone in Ova sensitized animals had attenuated the levels of exotoxin to 1850±12 pg/mL and 1690±10pg/mL respectively. Comparatively Ovalbumin alone sensitized mice shown significantly increased levels of eotaxin 2100±22 pg/mL and the control animals exhibited 1250±15 pg/mL of eotaxin (Figure 3A). Figure 3B illustrates Ova specific IgE antibodies levels in the drugs administered with and without Ovalbumin sensitized animals. Ovalbumin sensitization increased the Ova specific IgE levels to 390±7ng/mL whereas it is 235±9ng/mL in normal control animals. Treatment with berbamine and dexamethasone had decreased the levels of Ova specific IgE levels to 335±4ng/mL and 287±12ng/mL respectively.

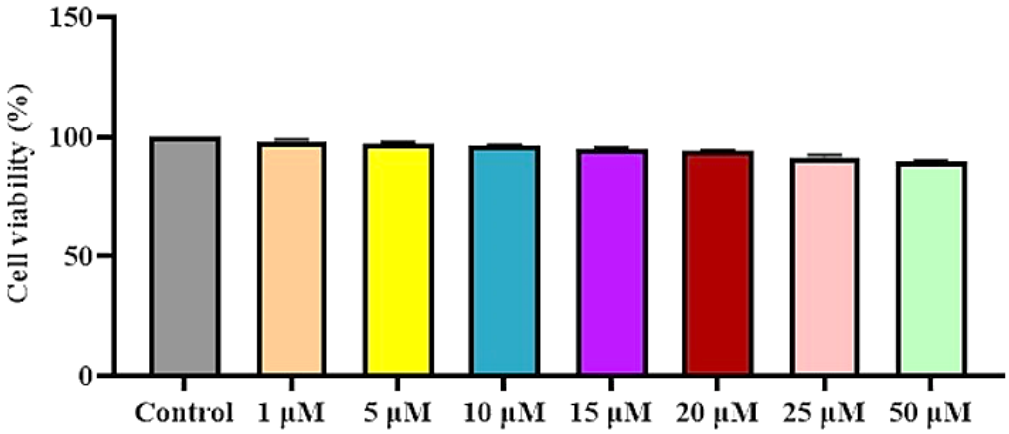

Figure 1: Berbamine doesn't rendered cytotoxicity on RAW264.7 cells. RAW264.7 macrophage cells were treated with various concentrations of berbamine (0, 5, 10, 25, and 50 µM) for 24 hr. Cytotoxicity of berbamine was assessed with cell viability assay. Results were analyzed with one Way ANOVA with Tukey's *post hoc* test and presented as the Means±SD. Each experiment was repeated in triplicate.

Berbamine inhibited leukocyte infiltration in BALF of OVA sensitized mice

Leukocyte infiltration is a key factor in asthma pathophysiology, actively driving inflammation hence the total leukocytes and the differential count in the BALF of experimental animals were quantified (Figure 4). Ovalbumin sensitization in the experimental animals had considerably increased the leukocyte infiltration into the lungs. Animals received only Ovalbumin sensitization exhibited about 48±3x10^{5}cells/mL whereas the control group animals shown 17±3x10^{5}cells/mL. Treatment with berbamine and dexamethasone had considerably decreased the leukocyte count to 37±2x10^{5}cells/mL and 29±3x10^{5}cells/mL respectively. Infiltration of eosinophils were considerably enhanced compared to the macrophages, monocytes and the neutrophils in the Ovalbumin alone sensitized animals. Berbamine treatment had considerably attenuated the infiltration of eosinophils in the Ovalbumin sensitized animals.

Berbamine decreased NO and MPO levels in OVA sensitized mice

Figure 5A illustrates the lung weight index of the Ovalbumin sensitized berbamine treated animals. Sensitization with ovalbumin had enhanced the lung weight index in the animals untreated with drugs. Berbamine and dexamethasone administration had decreased the lung weight index. Treatment with berbamine and dexamethasone in ovalbumin sensitized mice were also decreased the level of nitric oxide as 284±7 µmol/g, 267±6 µmol/g respectively and myeloperoxidase to 54±0.6 u/g, 46±0.5 u/g respectively. Ovalbumin sensitized untreated animals exhibited 312±6 µmol/g of NO and 62±0.4 u/g of myeloperoxidase whereas the levels were 148±8 µmol/g and 37±0.9U/g respectively in the control animals.

Berbamine scavenged oxidative stress in OVA sensitized mice

Enhanced free radical generation and depletion of antioxidant triggers asthmatic attack further hence we quantified the levels of MDA and antioxidants levels in the experimental mice. Ovalbumin sensitization had significantly enhanced the lipid peroxidation which is exhibited with increased MDA levels in the Ovalbumin sensitized untreated animals. It also reduced antioxidants SOD, CAT and the glutathione. Treatment with berbamine had enhanced antioxidants and the MDA levels were significantly decreased (Figure 6).

Berbamine attenuated inflammation response in OVA sensitized mice

Figure 7 presents the inflammatory stimulators levels quantified in the Ovalbumin sensitized untreated and drugs treated animals. Sensitization with ovalbumin in the experiment animals had enhanced TNF-α, INF-γ, TXB2 and the proinflammatory cytokines IL-6 and IL-12 levels. Berbamine administration in the Ova sensitized animals had decreased the inflammatory stimulators and the inflammation stimulating cytokines. Compared to TNF-α, the levels of INF-γ and TXB2 were considerably increased with Ovalbumin sensitization.

Berbamine attenuated type 2 inflammation in OVA sensitized mice

Type 2 inflammation, driven by both adaptive and innate immune mechanisms, plays a critical role in the pathogenesis of chronic airway diseases, including asthma. Therefore, we quantified the pro-inflammatory cytokines involved in the type 2 inflammation and the results were illustrated in Figure 8. Berbamine and dexamethasone treatment reduced the levels of inflammatory stimulator cytokines interleukins 4, 5 and 13 in Ovalbumin

sensitized animals. Sensitization with ovalbumin significantly enhanced the inflammation stimulating cytokines involved in type-2 inflammation than control animals.

DISCUSSION

Asthma is a long-lasting inflammatory airway disease that impacts nearly 350 million people worldwide.[30] It is characterized by persistent symptoms that remain uncontrolled despite standard therapy, often due to inadequate access to specialist care and insufficient treatment optimization.[2,5] A hallmark of asthma is airway inflammation, which contributes to key pathological features, including hyper responsiveness, obstruction and remodeling of airway.[31] One of the primary challenges in managing severe asthma is its heterogeneity, as patients often exhibit dynamic inflammatory triggers influenced by factors such as environmental exposures, aging, and therapeutic interventions. Furthermore, variability in treatment responses and the existence of accompanying medical conditions add to the complexity of asthma management, necessitating a personalized approach to optimize disease control.[32]

Currently, primary ailments for allergic asthma are calcineurin inhibitors, corticosteroids and antihistamines. However, these drugs are linked to multiple adverse effects, such as hypertension, growth retardation, osteoporosis, tachycardia.[33,34] As a result, there is a pressing need for the development of safer and more effective therapies. In recent decades, numerous studies have emphasized the protective benefits of natural compounds, following successful preclinical trials.[35,36] We explored the pharmaceutical efficacy of one such natural bioactive alkaloid berbamine against inflammatory response stimulated by allergic asthmatic.

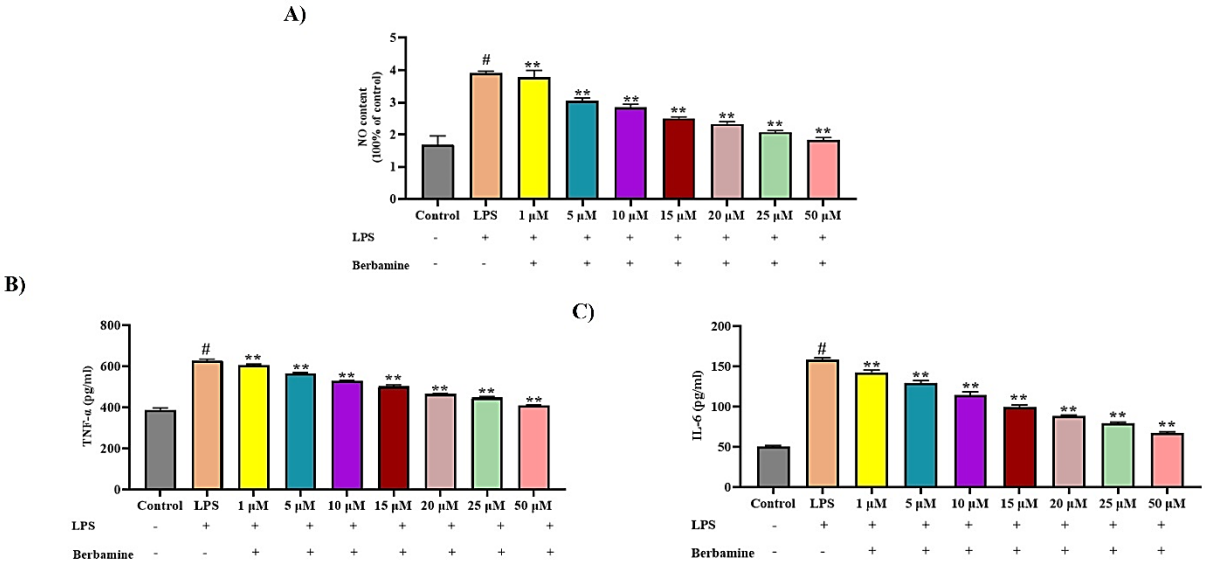

Figure 2: Berbamine attenuated inflammation in LPS induced RAW264.7 cells. RAW264.7 macrophage cells were treated with various concentrations of berbamine (0, 5, 10, 25, and 50 µM) for 24 hr with and without LPS stimulation. A) Nitric oxide levels were quantified with Griess reagent method, B) Tumor Necrosis Factor α (TNFα) and Interleukin-6 (IL-6) levels were measured using ELISA kits. Results were analyzed with one Way ANOVA with Tukey's *post hoc* test and presented as the Means±SD. Each experiment was repeated in triplicate.

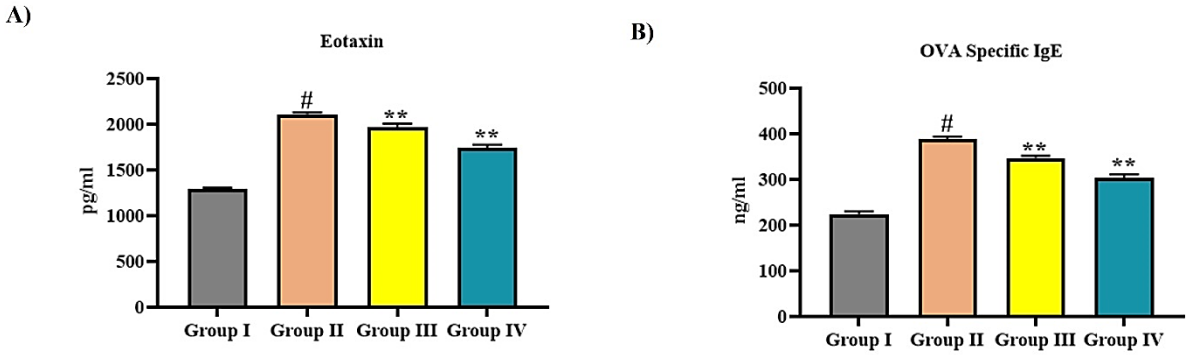

Figure 3: Berbamine suppressed Eotaxin and Ova specific IgE levels in OVA sensitized mice. A) Eotaxin, B) Ova specific IgE antibodies were quantified in the control, Ovalbumin sensitized untreated, ovalbumin sensitized berbamine treated and Ovalbumin sensitized dexamethasone treated animals using the ELISA kits. Results were analyzed with one Way ANOVA with Tukey's *post hoc* test and presented as the Means±SD. Each experiment was repeated in triplicate.

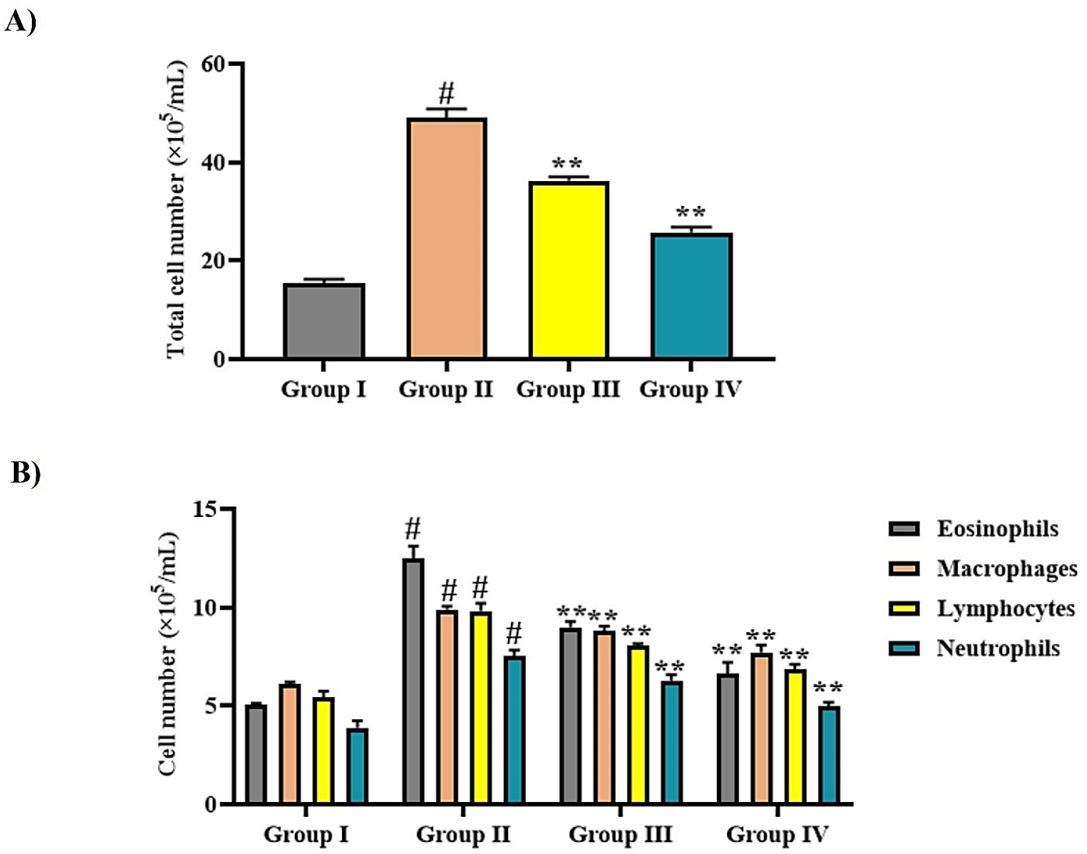

Figure 4: Berbamine inhibited leukocyte infiltration in BALF of OVA sensitized mice. A) Total leukocyte infiltration B) Differential count in the BALF of control, Ovalbumin sensitized untreated, ovalbumin sensitized berbamine treated and Ovalbumin sensitized dexamethasone treated animals were quantified. Results were analyzed with one Way ANOVA with Tukey's *post hoc* test and presented as the Means±SD. Each experiment was repeated in triplicate.

Lipopolysaccharide (LPS) is a crucial lipid component found in the outer membrane of Gram-negative bacteria, known for its ability to activate host immune responses.[37] It interacts with specific receptors on macrophages, causing proinflammatory cytokines release. While these cytokines are vital for normal immune function, their excessive production can result in an uncontrolled inflammatory response, making LPS a significant mediator in inflammatory processes particularly sepsis and related pulmonary complications. *In vitro* models often utilize LPS-treated macrophages, such as the RAW264.7 murine cell line, to study endotoxin-induced inflammation.[38,39] Treatment with varied concentration of berbamine doesn't rendered any cytotoxic effect in RAW264.7 macrophages but attenuated inflammation triggered by LPS stimulation. The nitric oxide levels and the TNF-α, IL-6 levels were reduced upon administration of berbamine in LPS administered RAW264.7 macrophages which confirms the inflammation attenuating effect of berbamine against the LPS induced inflammatory response.

Allergic and autoimmune inflammatory reactions play significant roles in the asthma disease progression. Individuals with asthma often exhibit increased Immunoglobulin E (IgE), which is crucial for the allergic response. IgE binds to allergens, triggering the discharge of inflammation stimulators that cause inflammation of airways and bronchoconstriction.[40] Higher concentration of eosinophils is typically found in the asthma patients and these eosinophils release inflammatory agents that also play a role in airway inflammation and structural alterations.[41] During inflammation, increased NO is synthesized by various airway cells, comprising eosinophils.[42] Eotaxin, a small protein found in asthmatic individual's lungs, acts as a powerful eosinophils attractant. It is classified as a CC chemokine and works by binding to the chemokine receptor CCR3 on the surface of leukocytes, facilitating the eosinophils migration from small blood vessels into the lung tissue, thereby exacerbating the inflammatory response in asthma.[43,44]

In our analysis Ovalbumin sensitization in mice triggered the allergic response enhanced the eotaxin and Ova specific IgE this may be the cause of enhanced leukocyte infiltration in the BALF of Ovalbumin sensitized mice. The enhanced eosinophil infiltration further enhanced the levels of nitric oxide and the myeloperoxidase levels thereby triggered inflammatory response in Ovalbumin sensitized animals. Treatment with berbamine alleviated the allergic response induced by the Ovalbumin sensitization in the experimental animals via attenuating the

synthesis of eotaxin and Ova specific IgE antibodies. Decrease in nitric oxide and myeloperoxidase levels of the berbamine treated Ova sensitized mice confirmed the anti-inflammatory potency of berbamine.

Oxidative stress is a key factor in asthma pathophysiology, resulting from an imbalance between Reactive Oxygen Species (ROS) generation and the body's antioxidant defenses. Studies have demonstrated that immune cells are the prime sources of ROS during inflammation of airways in asthma.[45-47] These cells generate ROS in response to various stimuli, which can lead to tissue damage and exacerbate inflammatory processes.[48] This oxidative damage is implicated in the remodeling of airway tissues, increased bronchial hyperreactivity, and diminished lung function associated with asthma. The accumulation of ROS also triggers the inflammatory stimulating cytokines synthesis, further sustaining the inflammatory cycle and worsening asthma symptoms.[49] Therapeutic strategies targeting oxidative stress, such as the use of antioxidants, have shown promise in mitigating airway inflammation and improving clinical outcomes in asthma patients.[50] Berbamine treatment in Ovalbumin sensitized animals scavenged free radicals, increased the antioxidants levels thereby prevented lipid peroxidation and lung tissue damage.

The early asthmatic response is primarily triggered by bronchoconstrictor mediators synthesis, which contribute to

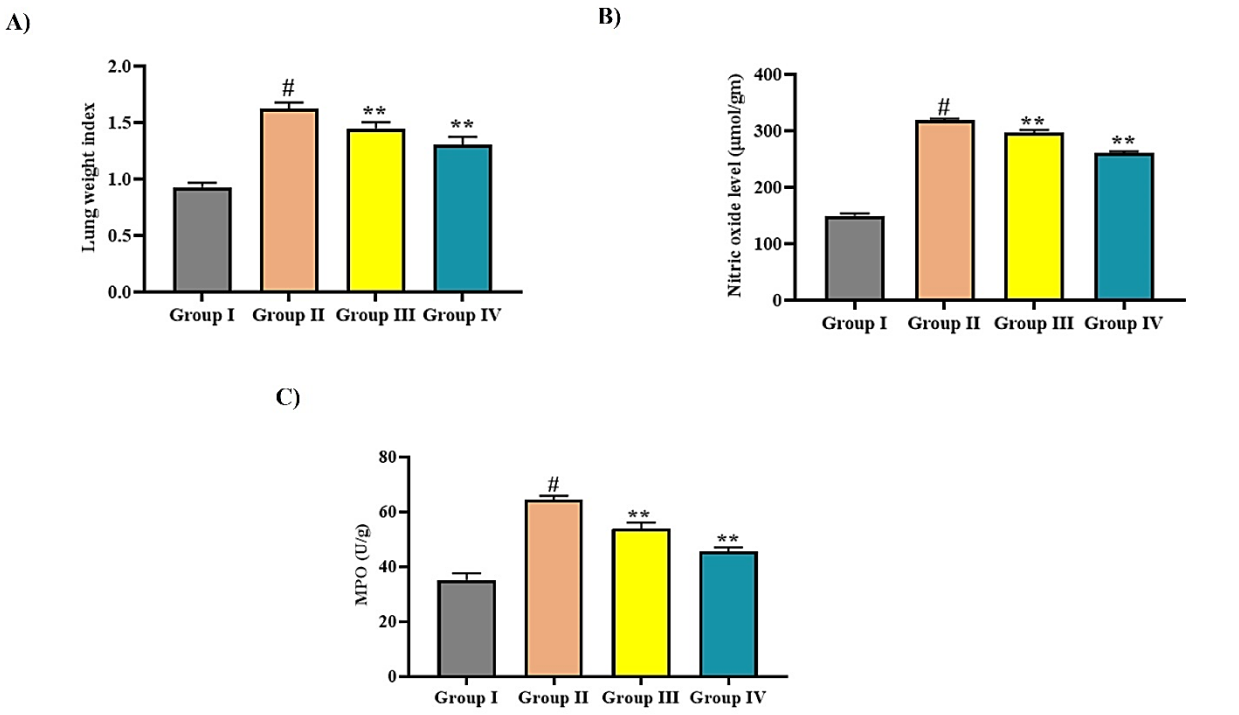

Figure 5: Berbamine decreased NO and MPO levels in OVA sensitized mice. A) Lung weight index, B) Nitric oxide C) Myeloperoxidase levels control, in the Ovalbumin sensitized untreated, ovalbumin sensitized berbamine treated and Ovalbumin sensitized dexamethasone treated animals using the colorimetric assay kits. Results were analyzed with one Way ANOVA with Tukey's *post hoc* test and presented as the Means±SD. Each experiment was repeated in triplicate.

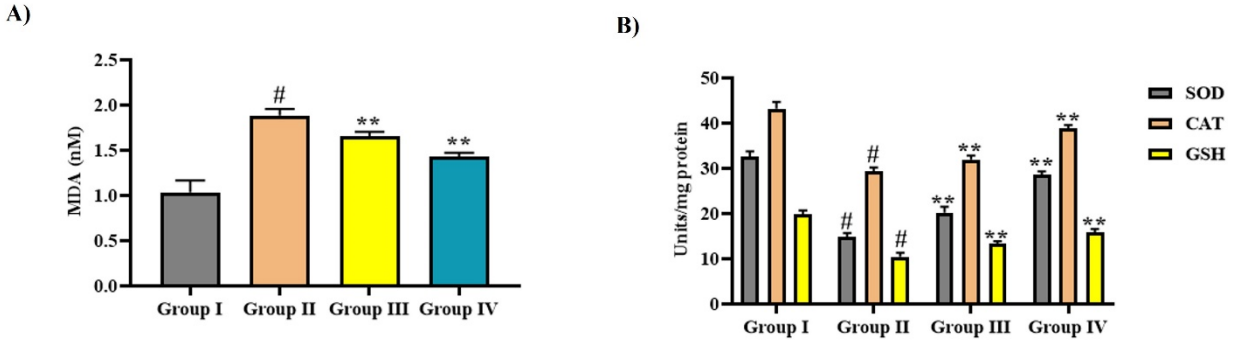

Figure 6: Berbamine scavenged oxidative stress in OVA sensitized mice. A) Malondialdehyde, B) Antioxidants - Superoxide dismutase, Catalase, Glutathione levels in the control, Ovalbumin sensitized untreated, ovalbumin sensitized berbamine treated and Ovalbumin sensitized dexamethasone treated animals were quantified. Results were analyzed with one Way ANOVA with Tukey's *post hoc* test and presented as the Means±SD. Each experiment was repeated in triplicate.

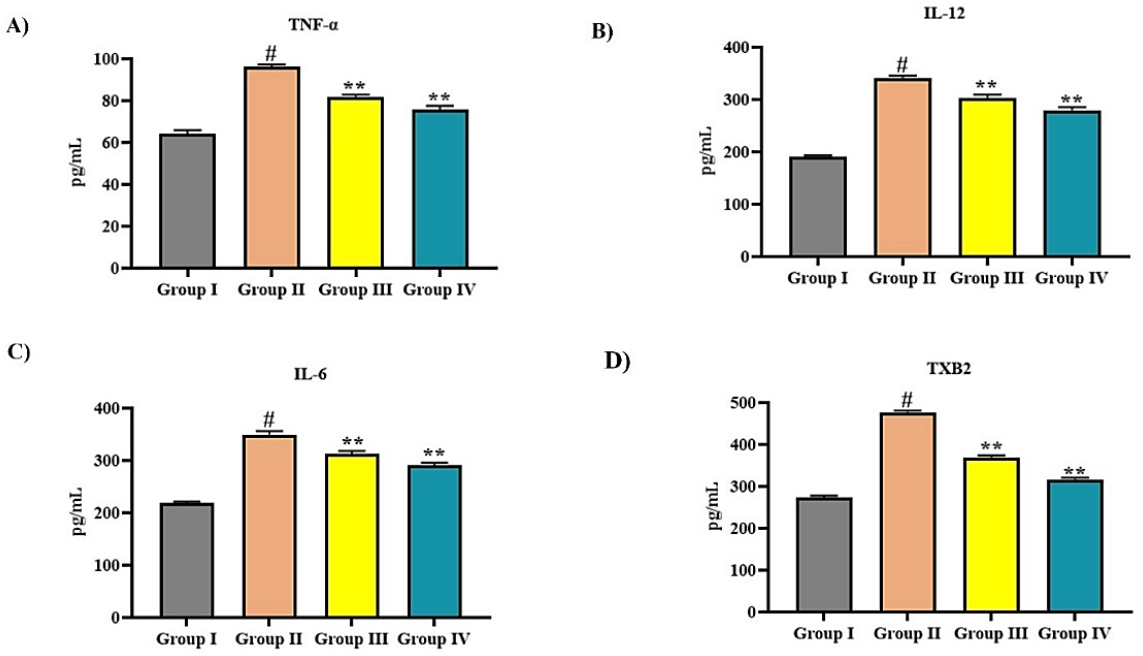

Figure 7: Berbamine attenuated inflammation response in OVA sensitized mice. A) Tumor Necrosis Factor Alpha (TNF-α), B) Interferon gamma (INF-γ), C) Thromboxane B2 (TXB2), D) Interleukin 6 (IL-6), E) Interleukin 12 (IL-12) levels in the control, Ovalbumin sensitized untreated, ovalbumin sensitized berbamine treated and Ovalbumin sensitized dexamethasone treated animals were quantified. Results were analyzed with one Way ANOVA with Tukey's *post hoc* test and presented as the Means±SD. Each experiment was repeated in triplicate.

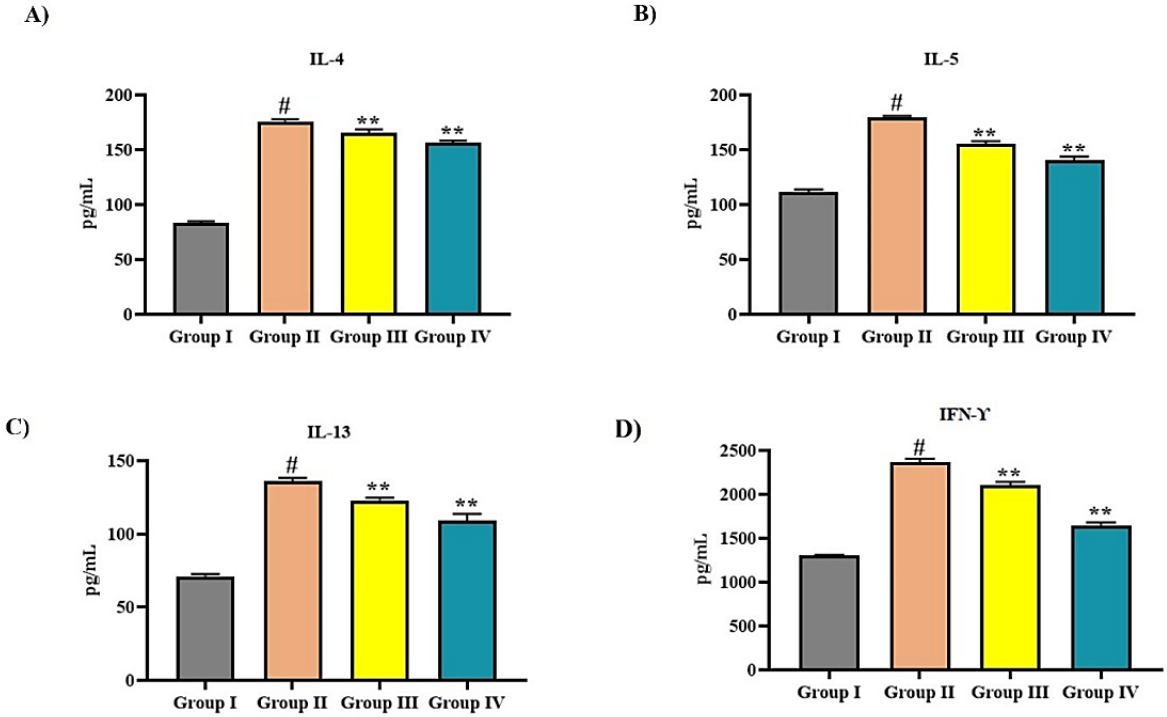

Figure 8: Berbamine attenuated type 2 inflammation in OVA sensitized mice. A) Interleukin-4 (IL-4), B) Interleukin 5 (IL-5), C) Interleukin 13 (IL-13) levels in the control, Ovalbumin sensitized untreated, ovalbumin sensitized berbamine treated and Ovalbumin sensitized dexamethasone treated animals were quantified. Results were analyzed with one Way ANOVA with Tukey's *post hoc* test and presented as the Means±SD. Each experiment was repeated in triplicate.

immediate bronchoconstriction. In contrast, late asthmatic reaction is presented with acute inflammation, marked by inflammatory cells influx, particularly eosinophils, into the airways.[51] Therefore in our study we examined the levels of thromboxane B2 and the inflammatory mediators in the berbamine treated Ovalbumin sensitized animals. Berbamine administration considerably decreased TXB2 and inflammatory mediator TNF-α which is a prime cytokine triggers innate immune response, with significant roles in host defense against infections and in the inflammatory processes associated with asthma.[52,53] Elevated levels of TNF-α have been observed in the asthmatic patients airways, and the inhalation of recombinant TNF-α has been demonstrated to trigger airway hyperreactivity and neutrophilia in normal individuals.[54,55] This correlates with our study Ovalbumin sensitization enhanced the TNF-α levels whereas treatment with berbamine attenuated the inflammatory response.

Interferon-gamma (IFN-γ) has a multifaceted action in asthma; it can reduce the recruitment of lymphocytes and eosinophils, inhibiting airway hyperresponsiveness and mucus production while promoting airway neutrophils and overall lung inflammation.[56] Additionally, IFN-γ may affect eosinophil behavior by influencing their activation, lifespan, or apoptosis.[57] Ovalbumin sensitization enhance IFN-γ levels and the inflammatory cytokines IL-6 and IL-12 levels in the experimental animals whereas treatment with berbamine attenuated IFN-γ production and decreased IL-6 and IL-12 levels.

Type 2 airway inflammation, driven by eosinophilic activity, play a prime role in over half of asthma cases.[58] Upon allergen exposure, antigen-presenting cells activate Th2 cells, which synthesis cytokines such as Interleukin 4, 5 and 13. Interleukin 4, 5 and 13 trigger immunoglobulin E production by B cells, which then adheres to mast cells and triggers the release of mediators that cause bronchoconstriction.[59,60] IL-5 is vital for eosinophil development, recruitment, and activation in the lungs, where they release Major Basic Protein (MBP), further stimulating mast cell degranulation and inhibiting M2 receptors, leading to increased acetylcholine release and bronchospasm.[61] Additionally, IL-13 triggers contraction of smooth muscle causing obstruction of airways, enhancing mucus production, and promoting airway fibrosis.[62] Therefore targeting the Type 2 airway inflammation with a phytochemical ameliorate the asthmatic attack effectively. Berbamine treatment in ovalbumin sensitization animals decreased the Type 2 inflammatory mediators interleukins 4,5 and 13 proving its efficacy in attenuating asthmatic attack in Ovalbumin sensitized animals.

CONCLUSION

In summary, this study demonstrates the significant inflammation attenuating effects of berbamine, a natural alkaloid extracted from Berberis amurensis, in the context of asthma. The results indicate that berbamine effectively attenuates inflammation in both *in vitro* and *in vivo* asthma models, as evidenced by the reduction of inflammatory mediators. Furthermore, berbamine was effective in alleviating ovalbumin-induced allergic responses, highlighting its potential as a therapeutic candidate for asthma and other inflammatory respiratory disorders. These findings warrant further investigation into berbamine's underlying mechanisms and its clinical applicability in asthma management and respiratory health improvement.

ACKNOWLEDGEMENT

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ABBREVIATIONS

LPS: Lipopolysaccharide; **CO2:** Carbon dioxide; **NO:** Nitric oxide; **TNF-α:** Tumor Necrosis Factor alpha; **BALF:** Bronchoalveolar Lavage Fluid; **MTT:** 3-[4,5-Dimethylthiazol-2-yl]-2,5 Diphenyl Tetrazolium Bromide; **SOD:** Superoxide Dismutase; **CAT:** Catalase; **GSH:** Glutathione; **MDA:** Malondialdehyde; **IL:** Interleukins; **TXB2:** Thromboxane B 2; **IFN γ:** Interferon gamma; **OD:** Optical Density; **ROS:** Reactive Oxygen Species; **OVA:** Ovalbumin; **IgE:** Immunoglobulin E; **ICS:** Inhaled Corticosteroids; **MBP:** Major Basic Protein; **LPS:** Lipopolysaccharides; **AHR:** Airway Hyperresponsiveness; **ELISA:** Enzyme Linked Immunosorbent Assay; **ANOVA:** Analysis of Variance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

This work has approved by the institutional animal ethical committee by Wushan Traditional Chinese Medicine Hospital, Wushan, Chongqing, 404700, China.

SUMMARY

Type 2 inflammation is a key contributor to the pathophysiology of specific bronchial hyper-reactivity phenotypes, particularly allergic asthma. We explored the pharmaceutical efficacy of one such natural bioactive alkaloid berbamine against inflammatory response stimulated by allergic asthmatic. Ovalbumin sensitization in mice triggered the allergic response enhanced the eotaxin and Ova specific IgE this may be the cause of enhanced leukocyte infiltration in the BALF of Ovalbumin sensitized mice. Berbamine was effective in alleviating ovalbumin-induced allergic responses, highlighting its potential as a therapeutic candidate for asthma and other inflammatory respiratory disorders

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