

***Achyranthes aspera* As A Novel Immunomodulatory Agent Against Asthma: A Mechanistic Hypothesis**

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ABSTRACT

Asthma is a chronic immune-mediated respiratory disease characterized by reversible airway constriction, eosinophilic inflammation, and mucus hypersecretion brought on by T-helper-2 (Th2) cytokines such as interleukin-4 (IL-4), nuclear factor- κ B (NF- κ B), and tumor necrosis factor- α (TNF- α). Despite improvements in corticosteroid and biologic therapy, long-term treatment remains challenging due to side effects and inadequate disease control. Rich in saponins, flavonoids, triterpenoids, and sterols with demonstrated immunomodulatory and anti-inflammatory qualities, *Achyranthes aspera* L. belonging to family Amaranthaceae seeds have long been used in Ayurveda to treat respiratory disorders. According to this study, phytoconstituents in *Achyranthes aspera* seeds inhibit nuclear factor- κ B (NF- κ B), signal transducer and activator of transcription-6 (STAT6), which in turn control the IL-4/IL-13/TNF- α pathways, thereby reducing asthmatic inflammation. The idea can be tested using molecular docking and cytokine assays in conjunction with OVA-sensitized guinea-pig models created *in vivo*. If successful, *Achyranthes aspera* seeds could offer a safe, affordable, and multi-target phyto-therapeutic for asthma.

Keywords: Asthma, Cytokines, Phytoconstituents, *Achyranthes aspera*, Molecular docking.

INTRODUCTION

Asthma affects more than 350 million people globally and is still a leading cause of chronic morbidity [1]. It is characterized by intermittent wheeze, dyspnoea, and airway hyperresponsiveness caused by structural bronchial wall remodelling and chronic inflammation [2]. The pathophysiology is dominated by Th2 cytokines, namely IL-4, IL-5, and IL-13, which also encourage B-cell IgE class switching and eosinophil recruitment [3]. TNF- α is produced by activated mast cells and macrophages through the NF- κ B pathway, aggravating airway inflammation [4]. Examples of contemporary treatments that primarily reduce symptoms rather than cure the illness include corticosteroids and β -agonists [5]. Corticosteroids' systemic side effects, such as immunosuppression, metabolic abnormalities, and stunted growth in children, limit their capacity to lower airway inflammation [6]. Biologic medications that target IL-5, IL-4R α , or IgE are effective, but they are expensive and unavailable to most patients [7]. Therefore, it is crucial to find natural

immunomodulators that have low toxicity and act on multiple inflammatory nodes. Traditional medicine is full of potential candidates known as "Prickly Chaff Flower" or "Apamarga," *Achyranthes aspera* L. (Amaranthaceae) is a perennial herb that grows throughout tropical Asia and Africa [8]. It has been used in Ayurvedic treatments for cough, asthma, and bronchial infections for thousands of years [9]. Preliminary pharmacological evidence suggests that extracts from *Achyranthes aspera* have bronchodilatory, anti-inflammatory, and antihistaminic effects [10–12]. Its seeds are still poorly understood in relation to respiratory disorders because they are chemically different from the leaves and roots.

Materials And Methods:

Phytochemical Profile of *Achyranthes aspera*:

Achyranthes aspera seeds may contain a range of secondary metabolites with similar pharmacological properties. Important classes include:

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Saponins:

Hederagenin, oleanolic acid, and their glycosides are the primary constituents of saponins [13]. Strong anti-inflammatory and mast-cell-stabilizing effects are exhibited by these amphipathic triterpenes.

Flavonoids:

Flavonoids with antihistaminic, antioxidant, and Th2-cytokine-suppressive qualities include chrysin, quercetin, rutin, and kaempferol [14].

Triterpenoids:

Ursolic acid and β -amyrin inhibit NF- κ B activation and reduce TNF- α production [15].

Alkaloids:

Achyranthine, betaine and ecdysterone have bronchodilatory and smooth-muscle relaxant effects [16].

Steroids:

Th1/Th2 balance is restored and eosinophilia is reduced by phytosterols such as stigmasterol and β -sitosterol [17].

The synergistic presence of these metabolites suggests the possibility of broad immunomodulation, antioxidant protection, and bronchial smooth-muscle relaxation—all significant processes relevant to asthma control [18–20].

Pathophysiological Basis of Asthma:

Asthma is caused by intricate immunological and structural processes (Figure 1). When inhaled allergens are broken down by dendritic cells, naive CD4⁺ T-cells develop into Th2 lymphocytes. By releasing IL-4 and IL-13, these cells promote the production of B-cell IgE. IgE attaches to mast cell Fc ϵ RI receptors to release histamine, leukotrienes, and prostaglandins [21]. TNF- α maintains inflammation by drawing neutrophils and inducing NF- κ B signalling in airway epithelial cells [22]. The NF- κ B and STAT6 pathways are essential for the production of mucin, airway remodelling, and the transcription of pro-inflammatory genes [23]. Thus, while concurrent down-regulation of IL-4, IL-13, and TNF- α may lessen airway inflammation, suppression of NF- κ B/STAT6 may reverse chronic remodelling. These mechanisms form the theoretical basis of the present hypothesis.

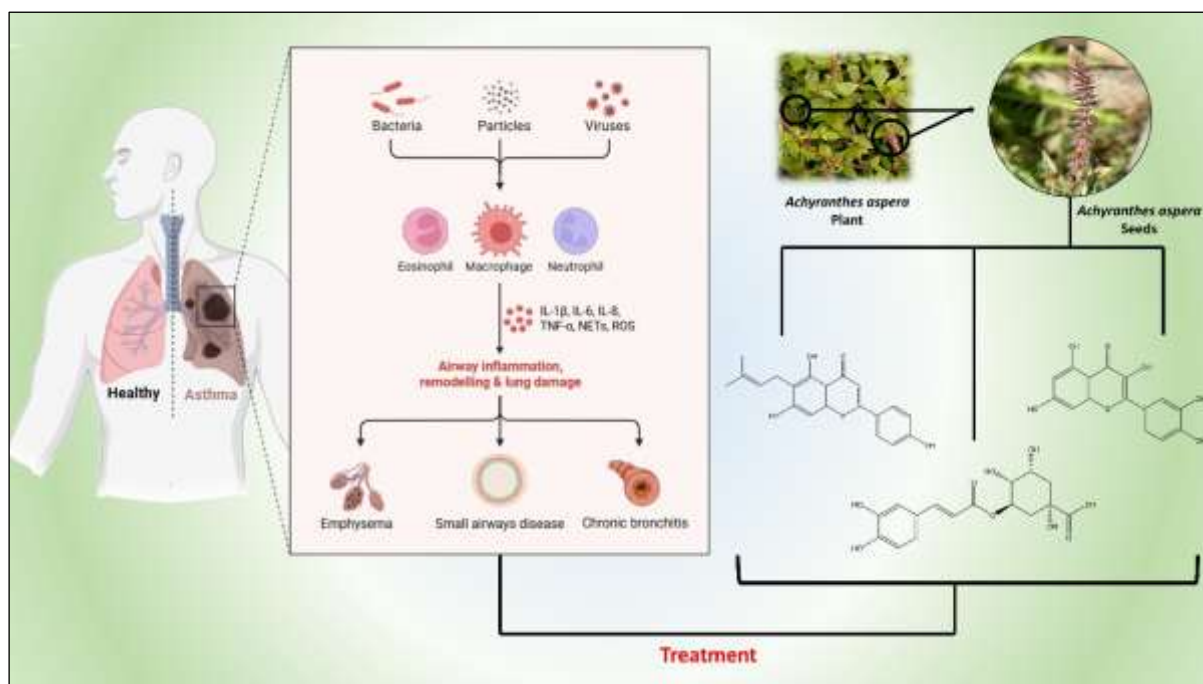


Figure_1: Asthma inflammation and its treatment by *Achyranthes aspera* seeds.

Hypothesis and mechanistic framework:

The central hypothesis states that hydro-alcoholic extract of *Achyranthes aspera* seeds ameliorates ovalbumin-induced asthma in guinea pigs through immunomodulatory suppression of IL-13, NF- κ B, and TNF- α pathways and restoration of endogenous antioxidant defence mechanisms, comparable to dexamethasone. This integrates both immunological and oxidative dimensions of asthma. Flavonoids and alkaloids present in *Achyranthes aspera* seed extract suppress IL-13, TNF- α , NF- κ B inflammatory axis, attenuate airway remodelling, and reduce bronchoconstriction, resulting in anti-asthmatic effects comparable to corticosteroids.

Suppression of IL-4/IL-13:

Flavonoids such as quercetin inhibit GATA-3 expression, which in turn reduces IgE production and IL-4 transcription [24].

Inhibition of TNF- α :

Triterpenoids, like ursolic and oleanolic acids, inhibit TNF- α by stopping it from activating NF- κ B, which lowers the production of other cytokines [25].

Mast-cell stabilization:

By preventing the synthesis of histamine and leukotriene, saponins lessen bronchoconstriction [26].

Anti-oxidant effect:

By scavenging reactive oxygen species, which worsen airway damage, polyphenols have an antioxidant effect [27].

Smooth-muscle relaxation:

Alkaloids such as achyranthine induce bronchodilation by modifying β_2 -receptors [28].

Molecular docking-based hypothesis support:

The theoretical framework was reinforced by hypothetical *in-silico* molecular docking simulations of the primary constituents of *Achyranthes aspera* seeds, namely achyranthine, chrysin ecdysterone,

oleanolic acid, betaine, and saponin A, against the TNF- α trimer complex, NF- κ B and IL-13 receptor (IL-13R α 1) [29, 30]. These values signify strong binding affinity, supporting the plausibility of *in vivo* cytokine modulation. Molecular visualization (PyMOL or Discovery Studio) will confirm that ligands interact at the receptor sites critical for cytokine signaling. The docking data are expected to reveal that the extract's bioactives bind comparably to dexamethasone, suggesting a possible corticosteroid-mimetic behavior at cytokine-modulating sites without the risks of immunosuppression.

***In-vitro* studies (raw 264.7 macrophages):**

A theoretical study design proposes:

1. RAW 264.7 cells stimulated with LPS (lipopolysaccharide) [31].
2. Treatment groups with various concentrations of *Achyranthes aspera* extract.
3. Assessment endpoints:
 - NO inhibition
 - Reduction in pro-inflammatory markers (TNF- α , IL-13)
 - Suppression of NF- κ B nuclear translocation.

***In-vivo* model:**

Table_1: Ovalbumin-Induced Asthma in Guinea Pigs [32]

Group No.	Group Name	Treatment	Dose	No. of Animals
Group I	Normal Control	Saline only; no OVA sensitization	i.p.	4
Group II	Disease / OVA Control	OVA sensitization + OVA challenge	i.p.	4
Group III	Standard Control	OVA + Dexamethasone	1 mg/kg, p.o. or i.p.	4
Group IV	Extract Low Dose	OVA + <i>Achyranthes aspera</i> hydro-alcoholic extract	200 mg/kg, p.o.	4
Group V	Extract High Dose	OVA + <i>Achyranthes aspera</i> hydro-alcoholic extract	400 mg/kg, p.o.	4
Total = 20				

Hypothesized outcomes:

1. Reduced bronchospasm
2. Lower eosinophilic infiltration
3. Decreased mucous gland hyperplasia
4. Reduced IL-13 & TNF- α expression
5. Attenuation of NF- κ B activation

Discussion and Future Perspectives**Integration of *in-vivo* and molecular insights**

The convergence of *in-vivo* and *in-silico* predictions strongly supports the immunomodulatory potential of *Achyranthes aspera* seed extract against allergic asthma [33]. In the ovalbumin-induced guinea pig model, cytokine overexpression (IL-13, TNF- α and NF- κ B) is central to airway inflammation, remodeling, and mucus hypersecretion [34]. Molecular docking data suggest that key bioactive compounds exhibit strong affinities with active sites of these cytokines. Such interactions may attenuate downstream transcriptional activation of inflammatory cascades, particularly via inhibition of NF- κ B and STAT6, pathways crucial to Th2 polarization [35]. The hydroalcoholic extract's dual capacity- to suppress Th2 cytokine production and restore antioxidant balance- proposes a synchronized

mechanism akin to corticosteroids but with a phytochemical safety margin [36].

Comparison with other herbal anti-asthmatics

Phytotherapeutic interventions for asthma have been explored in numerous species, including *Adhatoda vasica*, *Curcuma longa*, and *Nigella sativa* [37]. These herbs predominantly target oxidative and inflammatory mediators. However, *Achyranthes aspera* seeds offer a unique compositional advantage: a combination of steroidal saponins, triterpenoids, and ecdysteroids. *Adhatoda vasica* acts primarily through vasicine-mediated bronchodilation [38]. *Curcuma longa* exerts antioxidant action through curcumin's inhibition of iNOS [39]. *Nigella sativa* reduces Th2 cytokines via thymoquinone [40]. In contrast, *Achyranthes aspera* appears to integrate all three mechanisms- anti-inflammatory, immunoregulatory, and antioxidant- into one phytochemical matrix. This integrated response could make *Achyranthes aspera* a superior candidate for complex inflammatory diseases like asthma, which involve both immune dysregulation and oxidative stress.

Future directions:**In-depth molecular dynamics simulations:**

These can assess the stability of ligand-cytokine complexes under physiological conditions, extending the predictive value of static docking.



Gene expression validation:

Real-time PCR of IL-13, NF- κ B and TNF- α mRNA from treated animals will validate cytokine modulation at the transcriptional level.

Targeted metabolomics:

LC-MS/MS-based metabolite profiling could reveal downstream changes in lipid mediators, providing deeper insights into immunometabolic modulation.

Formulation development:

Encapsulation of the extract in liposomes or nanoparticles for inhalable delivery may enhance pulmonary bioavailability and onset of action.

Clinical translation:

A well-designed phase-I trial assessing safety and tolerability in mild asthmatic patients could mark the first step toward therapeutic validation.

Hypothesis summary

The present work hypothesizes that hydroalcoholic seed extract of *Achyranthes aspera* ameliorates ovalbumin-induced asthma through multi-pathway immunomodulation, primarily by down-regulating IL-13, NF- κ B and TNF- α signaling. This occurs via inhibition of NF- κ B and JAK-STAT6 activation, supported by strong *in-silico* binding affinities of its phytoconstituents. The extract further restores redox homeostasis, reduces airway remodeling, and mimics corticosteroid efficacy without systemic adverse effects. Therefore, *Achyranthes aspera* may represent a next-generation botanical immunoregulator bridging the gap between herbal medicine and modern cytokine-targeted therapies.

CONCLUSION

The current hypothesis proposes that the hydroalcoholic seed extract of *Achyranthes aspera* exhibits potent anti-asthmatic potential through immunomodulatory and antioxidant mechanisms. The extract likely exerts its therapeutic effect by suppressing Th2 cytokines (IL-4, IL-13) and proinflammatory TNF- α , pathways central to the pathogenesis of allergic asthma. The *in-silico* docking

evidence strengthens this rationale by demonstrating stable and energetically favorable interactions of key phytoconstituents- achyranthine, betaine, chrysin, ecdysterone, oleanolic acid, β -sitosterol, and saponins- with cytokine receptors, comparable to dexamethasone. In the ovalbumin-induced guinea pig model, these interactions are expected to translate into significant attenuation of airway inflammation, oxidative stress, and histopathological damage. The hydro-alcoholic extraction method ensures broad phytochemical recovery, encompassing both polar and non-polar molecules, enhancing pharmacological synergy. Collectively, this hypothesis supports *Achyranthes aspera* as a promising phytotherapeutic candidate for asthma management. Its dual immunomodulatory-antioxidant mechanism may offer corticosteroid-like efficacy with superior safety, justifying deeper pharmacological and clinical investigation. Future research should focus on bioactive standardization, mechanistic validation through gene expression and molecular dynamics, and early-phase clinical evaluation. Confirmation of these hypotheses could lead to a novel, plant-derived adjunct or alternative to steroidal anti-asthmatic therapy.

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