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RESEARCH ARTICLE

Therapeutic Efficacy of an Herbal Dry Powder Inhaler in Alleviating Ovalbumin-induced Lung Inflammation and Restoring Hemostatic Balance in a Rat Asthma Model

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Abstract

Background: Asthma is a chronic inflammatory airway disease characterised by immune dysregulation and coagulation abnormalities. Current treatments primarily focus on symptom management but are often accompanied by a range of side effects. Herbal formulations have shown promise in modulating inflammation and coagulation in respiratory disorders. **Aim:** This study aimed to evaluate the protective effects of a Herbal Dry Powder Inhaler in an ovalbumin-induced asthma rat model by assessing its impact on inflammation and coagulation parameters. **Methods:** Male Wistar rats were divided into experimental groups and sensitised with ovalbumin via intraperitoneal injection on days 0, 7, and 14, followed by an intratracheal ovalbumin challenge on day 21. From days 15 to 20, sensitised rats received randomised treatments, including a placebo, Herbal Dry Powder Inhaler, dexamethasone, or a combination of both. Bronchoalveolar Lavage Fluid (BALF) was collected to assess inflammatory cell infiltration. Histopathological analysis of lung tissue was performed, and coagulation markers, including Prothrombin Time (PT) and Thrombin Time (TT), were evaluated. **Results:** The Herbal Dry Powder Inhaler significantly reduced inflammatory cell infiltration in BALF and lung tissue, demonstrating anti-inflammatory effects comparable to dexamethasone. Furthermore, it effectively corrected coagulation abnormalities, improving PT ($p<0.01$) and TT ($p<0.05$) in asthmatic rats. Histopathological analysis confirmed reduced airway inflammation and tissue damage in treated groups. **Conclusion:** The findings suggest that the Herbal Dry Powder Inhaler exerts protective effects in allergic airway disease by mitigating inflammation and normalizing coagulation abnormalities. This herbal formulation holds potential as a complementary therapy for asthma management, warranting further investigation in clinical settings. **Major Findings:** The herbal dry powder inhaler significantly reduced airway inflammation and corrected coagulation abnormalities in an ovalbumin-induced asthma rat model. It improved prothrombin time ($p<0.01$) and thrombin time ($p<0.05$), showing effects comparable to dexamethasone. These findings suggest its potential as a natural alternative or adjunct therapy for asthma management.

Keywords: Asthma, Ovalbumin, Rat Model, Sulphur Containing Components, Terpens

1. Introduction

Asthma, a persistent respiratory condition characterised by airway inflammation and hyperresponsiveness, presents a substantial challenge to effective long-term management[1-3]. Despite being incurable, it can be effectively controlled. The development of asthma is influenced significantly by exposure to indoor allergens during childhood and a family history of asthma or allergies. Long-term allopathic treatments, while providing relief, often bring about a range of side effects. For example, β2 agonists may cause fine tremors, nervousness, headaches, muscle cramps, and palpitations. Antimuscarinic agents can result in dry

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mouth, gastrointestinal motility disturbances, cough, and headaches. Corticosteroids are linked to adverse effects such as osteoporosis, hypertension, diabetes, weight gain, cataracts, glaucoma, skin thinning, easy bruising, and muscle weakness. Additionally, leukotriene inhibitors and xanthines have their own distinct side effect profiles[4-6].

To mitigate these side effects, herbal medicine stands out as a prominent alternative to conventional pharmaceutical treatments[7]. Herbal remedies aim to relieve infections, boost immunity using immuno-stimulants and anti-infectives, reduce swelling and obstruction through anti-inflammatories, and promote respiratory muscle relaxation, increased mucus elimination, and improved circulation using circulatory tonics[8] Traditional remedies like ajwain, garlic, and clove have been historically utilised for asthma treatment[8,9]. In this study, we explore a novel herbal formulation in the form of a nanodry powder inhaler, incorporating essential oils of *Ajwain*, *Garlic*, and *Clove*.

Thymol, a crucial component of this formulation, has been shown to reduce hyperresponsiveness and allergic airway inflammation by preventing inflammatory cell infiltration, downregulating Th2 cytokines, and decreasing ovalbumin-specific IgE levels[9]. Additionally, it mitigates pathological changes by inhibiting NF-κB activation. Allicin and Diallyldisulphide exhibit anti-inflammatory effects, reducing nitric oxide production and interleukin levels[10]. Eugenol, another constituent, inhibits leukotriene formation, offering a potential avenue for therapeutic intervention[11].

These findings highlight the potential of herbal formulations as alternatives for asthma management, providing insights into their mechanisms of action and paving the way for further exploration in clinical applications[11].

Clinical investigations of asthma are unable to address all aspects of the disease's pathophysiology. To gain insights into these pathways and evaluate the efficacy of treatments before human trials, researchers have developed animal models. Various species, including drosophila, rodents, guinea pigs, kittens, goats, pigs, monkeys, and equines, have been employed in laboratory asthma models. Among these, rodents, particularly BALB/c mice, have been the focus of extensive study in the past two decades. The aim of animal asthma models is to mimic the pathophysiological aspects of the human condition, traditionally characterised by two phases: sensitization and challenge[12,13].

Common methods of sensitization involve intraperitoneal and subcutaneous injections, though intranasal instillation of allergens is gaining popularity due to its relevance to human asthma caused by allergen inhalation. Various paths of sensitization and challenge have been explored, with limited trials comparing their efficacy. A crucial consideration in establishing a robust animal model lies in selecting the causative allergen. While Ovalbumin (OVA) is a widely used allergen derived from chicken eggs and mass-produced for affordability, its suitability as an asthma allergen for replicating airway inflammation in humans has been debated. Conversely, House Dust Mites (HDM) have been widely utilised in animal models due to their inherent enzymatic activity, strong immunogenicity, and ability to directly activate innate immune cells via the Dectin-2 receptor, thereby driving allergic inflammation[13].

The routes of sensitization and challenge have been studied extensively to enhance both acute and chronic animal models of asthma. Intraperitoneal (IP) administration, prevalent since the 1980s, has been a popular method for inducing sensitization. This typically involves two IP injections separated by 7–14 days, followed by a challenge with the allergen one week later.

In terms of pulmonary inflammation, experimental asthma models reveal an increase in eosinophils, lymphocytes, macrophages, and neutrophils, particularly in the airways, peribronchial space, and parenchyma. Eosinophil counts rise in both blood and Bronchoalveolar Lavage (BAL). Additionally, dendritic cells may migrate into ganglia from external sources, interacting with sensory neurons and influencing allergic airway inflammation.

This comprehensive approach to animal models provides valuable insights into the intricate aspects of asthma pathophysiology, contributing to the refinement of research methodologies and the development of more effective treatments for this complex respiratory condition[12,13].

2. Materials and Method[13]

The animal study protocol was reviewed and approved by the Institutional Animal Ethics Committee of Parul University (Protocol No. CPCSEA 921/PO/ReBi/S/05/CPCSEA/PIPH 24/19) under the title "Development and Evaluation of Polyherbal Formulation for the Treatment of Asthma"[13].

2.1 *In Vivo* Pharmacokinetic Studies

2.1.1 Animal Procurement and Maintenance

Healthy Wistar rats, weighing between 250g and 400g, were procured from the central animal facility of the Parul Institute of Pharmacy[13]. The animals were housed under controlled temperature and humidity conditions, provided with a standard diet, and given ad libitum access to water. All animal care and handling procedures strictly followed the guidelines set by The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was duly approved by the Institutional Animal Ethics Committee (IAEC)[2,13].

2.1.2 Saline Solution

To prepare normal saline (0.9% NaCl) for animal studies, weigh 9 grams of sodium chloride and dissolve it in approximately 800 mL of distilled water with constant stirring before adjusting the volume to 1 l. The solution is then sterilised either by passing it through a 0.22 µm filter or by autoclaving at 121°C for 15-20 min. Store the sterile solution in clearly labeled containers at room temperature or in a refrigerator until use. During administration, use sterile syringes for intraperitoneal injection or oral gavage, ensuring proper dosing and aseptic techniques, while monitoring the animals for any adverse reactions.

2.1.3 Formulation

Dry Powder Inhaler sample prepared by dissolving in the distilled water and supplied as per Kg body weight through nebuliser to pulmonary route of inhalation.

2.1.4 Standard/Control Sample

Dexamethasone Sodium Phosphate Injection USP (2mg/mL) procured from the market.

2.2 Experimental Study Design

Healthy male Wistar rats were organised into different groups and sensitised intraperitoneally on days 0, 7, and 14. On day 21, the rats were intratracheally challenged with ovalbumin. The sensitised groups were subjected to random treatment assignment from days 15 to 20, with the following categories as mentioned in Table 1[14].

Saline (Group I - Asthma group)

Formulation (Group II)

Standard (dexamethasone – Group III)

Formulation combined with dexamethasone (Group IV)

Normal group (Group V)[15]

On day 21, the animals were sacrificed, and observations were recorded, including cell counts in

Table 1. Animal experimental design

S. No.	Group	Animals used	Disease induction	Treatment and dose	Treatment duration
1	Asthma Group I	6 rats	Intraperitoneally on days 0, 7, and 14 and challenged to ovalbumin intratracheally on day 21.	1mL NS Oral (0.9%) + 1mL NS i.p.	6 days
2	Formulation Group II	6 rats		Per Kg dose oral Inh + 1mL NS i.p	
3	Formulation + Standard Group III	6 rats		Per Kg dose oral Inh +Dexamethasone (0.5 mg/Kg) i.p.	
4	Standard group IV	6 rats		1mL NS Oral (0.9%) +Dexamethasone (0.5 mg/Kg) i.p.	
5	Normal group V	6 rats	-	1mL NS Oral (0.9%)	
	Total	**30**			
	Parameters of evaluation	Cell counts in BALF, histopathology of rat lungs, and coagulation parameters, such as Prothrombin test and the activity of coagulation factors APTT.			

BALF, histopathological examinations, and assessments of coagulation parameters such as the Prothrombin test and the activity of coagulation factors APTT.

2.3 Inflammatory Cell Counts in Bronchoalveolar Lavage Fluid (BALF)

After euthanasia and opening of the chest cavity, the rat lungs were carefully examined for morphological lesions. A tracheal cannula was then inserted in situ, and the left lung was lavaged three times with 2.5 mL of normal saline, with the collected fluid being gently aspirated. The BALF was centrifuged, and the resulting cell pellet was resuspended in 1 mL of normal saline. Leukocyte counts were determined using a Neubauer counting chamber[13,15].

2.4 Blood Coagulation Test (Prothrombin Time - PT, Activated Partial Thromboplastin Time - APTT)

For coagulation analysis, 2.7 mL of rat blood was collected in a vacuum tube containing sodium citrate as an anticoagulant. Platelet-deficient plasma was prepared by centrifugation at 3000 rpm for 10 min. The coagulation tests, including Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT), were conducted using the STA-R Evolution automatic blood coagulation analyser (Stago Inc., France), following the manufacturer's guidelines. All tests were performed within 2 h of sample collection. Additionally, one animal from each group was selected for an acute toxicity study[15,16].

2.5 Lung Tissue Histopathology

Lungs from one animal per group were isolated post-drug administration and preserved in 10% formalin for 24 h. The preserved lung tissues were then sectioned into 4 µm slices and stained using H and E stain (Mayer's hematoxylin and eosin). Histopathological evaluation was conducted under a microscope to assess tissue lesions and inflammatory cell infiltration. Specific pathological markers, including Peribronchial Infiltration (PBI), Hyperplasia of Lung tissues (HP), and Interalveolar Septal Thickening (IST), were examined to determine potential signs of toxicity and structural alterations[13,16].

2.6 Statistical Analysis

The results were presented as mean ± SEM, with 'n' representing the number of rats in each group. Statistical comparisons between two means were performed using an unpaired Student's t-test, while ANOVA for repeated measurements was used for comparisons across multiple groups, followed by the Dunnett's t-test. Statistical analysis was conducted using GraphPad Prism 4.0 and GraphPad InStat software. A $p<0.05$ was considered statistically significant for all comparisons[14,15].

3. Results

On day 21, one animal from each group was sacrificed, and their lungs were isolated according to the specified procedure for cell count, coagulation studies, and histopathological examinations. The graphical results are well explanatory as depicted in Figure 1[9,14].

The formulation significantly reduced leukocyte influx into BALF and lowered the eosinophil percentage compared to the asthma group[9]. Dexamethasone and the combination treatment exhibited comparable improvements[4,11].

Regarding coagulation, treatment with the formulation reduced PT and APTT significantly compared to the disease control group[15]. When combined with dexamethasone, the effects on coagulation parameters were more pronounced[16].

3.1 Effect of the Formulation on OVA-Induced Eosinophilia in BALF

As shown in Figure 1 and Table 2, the asthma group demonstrated a significant increase in leukocyte influx into BALF compared to the normal control rats (2.8 ± 0.37 × 10^5/mL versus 14 ± 1.45 × 10^5/mL, $p<0.01$), with eosinophils comprising over 50% of the total leukocytes. Rats treated with the formulation exhibited a significant reduction in cell migration, resulting in a substantial decrease in total leukocytes (10.74 ± 1.16 × 10^5/mL versus 7.21 ± 0.91 × 10^5/mL, $p<0.01$) and a marked decrease in eosinophil percentage (31.97 ± 4.04% versus 13.76 ± 1.23%, $p<0.01$) compared to the asthma group. The positive control, dexamethasone, also showed a similar suppressive effect on leukocyte influx into BALF.

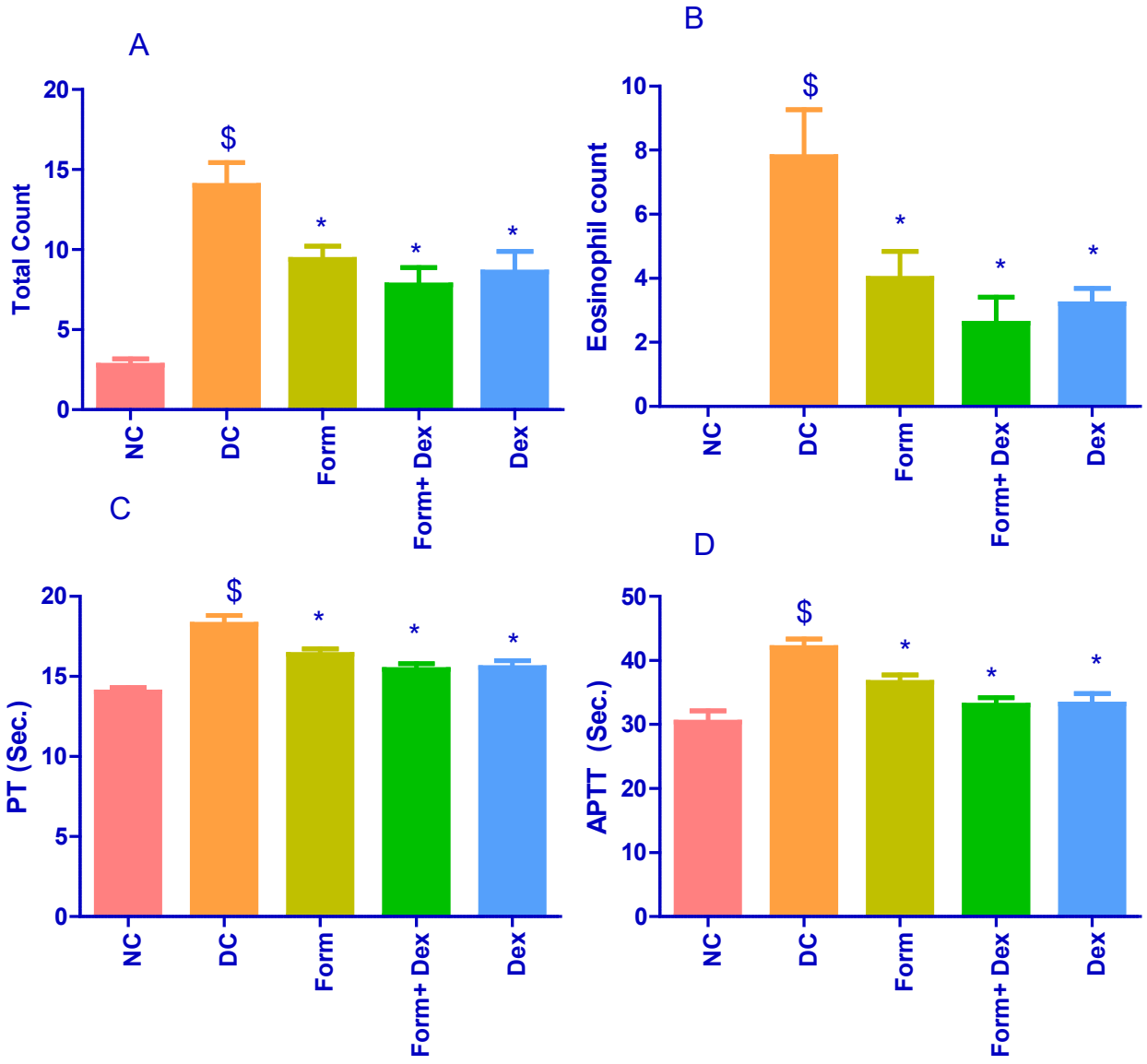


Figure 1 **(A-D).** Impact of the Formulation on **(A)** Total Count, **(B)** Eosinophil Count, **(C)** Prothrombin Time (PT), and **(D)** Activated Partial Thromboplastin Time (APTT) in Experimental Rats. The data were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Values significantly differ from the Disease Control (DC) group ($p<0.05$), and $ values significantly differ from the Normal Control (NC) group ($p<0.001$). Each point represents the Mean±SEM of six experiments. NC: Normal Control group, DC: Disease Control group, Form: Formulation Control group, Form+Dex: Formulation with Dexamethasone Control group, Dex: Dexamethasone Control group.

3.2 Effect of the Formulation on PT and APTT for Blood Coagulation

As shown in Figure 1 and Table 2, the normal control group exhibited significantly lower Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) compared to the asthma group ($p<0.01$). Treatment with the formulation significantly reduced

Table 2. Results of Total count, Eosinophil count, PT and APTT

Tests	NC G-5	DC –G1	Form G-2	Form+ Dex G-3	Dex G-4
Total Count	2.8±0.37	14±1.45$	9.4±0.81*	7.8±1.06*	8.6±1.28*
Eosinophil count	0±0	7.8±1.46$	4±0.83*	2.6±0.81*	3.2±0.48*
PT (Sec.)	14.02±0.28	18.26±0.55$	16.38±0.34*	15.44±0.35*	15.56±0.41*
APTT (Sec.)	30.4±1.75	42±1.34$	36.6±1.12*	33.0±1.18*	33.2±1.59*

Values are presented as Mean ± SEM, with N=6[14]. NC: Normal control, DC: disease control, Form: Formulation group, Form+Dex: Formulation with Standard Dexamethasone group, Dex: Standard Dexamethasone group. $: Significant difference from the corresponding normal control group ($p<0.01$). *: Significantly different from the disease control ($p<0.05$).

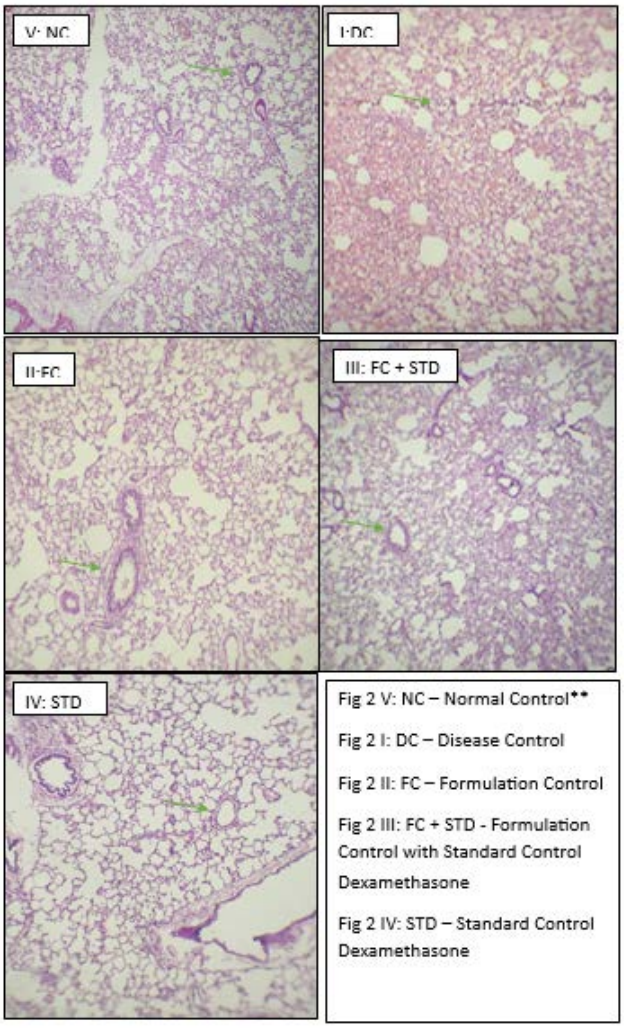


Figure 2 (I-V). Histopathology pictures of rat lungs.

both PT ($p<0.01$) and APTT ($p<0.01$). However, when the formulation was combined with dexamethasone, it demonstrated a more pronounced effect on these coagulation parameters.

3.3 Rat Lungs Tissues Comparative Examination (Histopathology)

Figure 2 illustrates the comparative histopathological sections of rat lung tissues. The normal control group showed no signs of toxicity, whereas the asthma group displayed severe interalveolar septal thickening, peribronchial infiltration, and hyperplasia[13]. Treated groups demonstrated attenuated inflammation, supporting the safety and efficacy of the NP DPI formulation[17].

3.4 Attenuated Inflammation in the Lungs of Asthmatic Rats

The histological changes in lung tissues for each group were assessed using H&E staining (Hematoxylin and Eosin), with arrows highlighting inflammation and mucus secretion alterations. The scale was set at 100 µm (magnification 200×). Statistical comparisons are as follows: $p<0.01$ vs. NC group; # $p<0.05$ and ## $p<0.01$ vs. DC group.

Figure 2-V shows the histopathological section of lungs from the untreated normal control group, with no signs of toxicity. Group-III (Figure 2-III) showed negligible toxicity. In Group-II (Figure 2-II), mild PBI and HP were observed. Group-I (Figure 2-I) displayed severe IST, PBI, and HP, indicating significant inflammation. These findings reinforce the safety of the NP DPI formulation.

4. Discussion

Asthma is a chronic respiratory condition characterised by airway inflammation, which results in symptoms like paroxysmal airflow obstruction, airway

hyperresponsiveness, wheezing, chest tightness, and coughing[1]. The condition is associated with allergic inflammation, marked by the infiltration of mast cells, eosinophils, and lymphocytes into the airway walls, leading to mucus hypersecretion[9]. Histological analysis of the lungs showed varying degrees of inflammation in all OVA-treated groups[11,12].

Notably, rats in the asthma group (G-1) exhibited more severe inflammation and greater inflammatory cell influx compared to the treatment groups. Among the treatment groups, the formulation group generally exhibited more pronounced inflammation than the dexamethasone or formulation combined with dexamethasone groups.

However, rats treated with the formulation demonstrated a significant reduction in both inflammatory cell infiltration and mucus production compared to the asthmatic rats[11,12]. Additionally, the total cell count in the BALF of the asthmatic rats was significantly higher than in the other groups. Drug-treated rats showed a substantial decrease in cell count compared to their asthmatic counterparts[9-11]. These findings suggest that the formulation effectively attenuates one of the key features of allergic asthma, highlighting its potential as a therapeutic option for asthma management[13,17].

5. Conclusion

In conclusion, the herbal dry powder inhaler demonstrated significant effectiveness in suppressing airway inflammation and restoring hemostatic balance in a rat asthma model[9-11]. Its efficacy was notably comparable to dexamethasone while potentially avoiding many adverse effects associated with long-term steroid use[4,5]. These promising results encourage further clinical investigation into this novel formulation as a complementary therapy for asthma[16,17].

6. Acknowledgement

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7. Author Contribution

Avani Khristi – Complete research coordination, formulation development, analytical, sample preparation and execution of animal study, Dr. Archana Navale - Designing animal study protocol, Ruchi Yadav - Animal study assistance like in dosing and blood sample collection, Dr. Lalit Lata Jha - guidance for the animal study also designing statistical check with software, Dr. Abhay Dharamsi - guidance for formulation development for animal studies. All authors have read and agreed to the published version of the manuscript.

8. References

1. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020; 396(10258):1204-1222. https://doi.org/10.1016/S0140-6736(20)30925-9 PMid:33069326
2. World Health Organization. Assessing national capacity for the prevention and control of noncommunicable diseases: Report of the 2019 global survey. Geneva: World Health Organization; 2020.
3. National Center for Health Statistics, CDC. National Health Interview Survey, Compiled 12/7/2020. 2019 National Health Interview Survey (NHIS) Data. National Center for Environmental Health; 2021.
4. Heffler E, Madeira LNG, Ferrando M, Malvezzi L, Passalacqua G, Canonica GW, *et al*. Inhaled corticosteroids safety and adverse effects in patients with asthma. J Allergy Clin Immunol Pract. 2018; 6(3):776-781. https://doi.org/10.1016/j.jaip.2018.01.025 PMid:29408385
5. Broadbent C, Pfeffer P, Steed L, Walker S. Patient-reported side effects of oral corticosteroids. Eur Respir J. 2018; 52:PA3144. https://doi.org/10.1183/13993003.congress-2018. PA3144
6. Erdem SB, Nacaroglu HT, Karkiner CSU, Gunay I, Can D. Side effects of leukotriene receptor antagonists in asthmatic children. Iran J Pediatr. 2015; 25(5). https://doi.org/10.5812/ijp.3313
7. Nadjib BM. Successful antiviral activity against coronaviruses by essential oils and their characteristic terpenes: An update. J Pharmacol Clin Toxicol. 2020; 8(1):1138.
8. Bui TP, Tran TA, Nguyen TTH. Investigation into SARS-CoV 2 resistance of compounds in garlic essential oil. ACS Omega. 2020; 5:8312-8320.

9. Meeran MFN, Javed H, Al Taee H, Azimullah S, Ojha SK. Pharmacological properties and molecular mechanisms of thymol: Prospects for its therapeutic potential and pharmaceutical development. Front Pharmacol. 2017; 8:380. https://doi.org/10.3389/fphar.2017.00380 PMid:28694777 PMCid:PMC5483461
10. Kim SR, Jung YR, An HJ, Kim DH, Jang EJ, Choi YJ, *et al*. Anti-wrinkle and anti-inflammatory effects of active garlic components and the inhibition of MMPs via NF-**κB** signaling. PLoS One. 2013; 8(9). https://doi.org/10.1371/journal.pone.0073877 PMid:24066081 PMCid:PMC3774756
11. Pan C, Dong Z. Antiasthmatic effects of eugenol in a mouse model of allergic asthma by regulation of vitamin D3 upregulated protein 1/NF-**κB** pathway. Inflammation. 2015; 38(4):1385-1393. https://doi.org/10.1007/s10753-015-0110-8 PMid:25588851
12. Aun MV, Bonamichi-Santos R, Arantes-Costa FM, Kalil J, Giavina-Bianchi P. Animal models of asthma: Utility and limitations. J Asthma Allergy. 2017; 10:293-301. https://doi.org/10.2147/JAA.S121092 PMid:29158683 PMCid:PMC5683778
13. Dong F, Wang C. Puerarin attenuates ovalbumin-induced lung inflammation and hemostatic unbalance in rat asthma model. Evid Based Complement Alternat Med. 2014; 2014:726740. https://doi.org/10.1155/2014/726740 PMid:24523826 PMCid:PMC3910132
14. Bisht J, Khristi A. Development and validation of novel high-performance liquid chromatography method for simultaneous estimation of p-Cymene and Aloe-emodin. J Nat Remedies. 2023; 23(1). https://doi.org/10.18311/jnr/2023/31612
15. Khristi A, Patel S, Wagh Y. Development and validation of an analytical method for the simultaneous estimation of azadirachtin and camphor in a new herbal formulation. Int J Pharm Qual Assur. 2022; 13(4):1-3. https://doi.org/10.25258/ijpqa.13.4.17
16. Khristi A, Jha L, Dharamsi A. RP-HPLC method development and validation for simultaneous estimation of thymol, eugenol and alliin in bulk and novel nanoformulation. Res J Pharm Technol. 2022; 15(12). https://doi.org/10.52711/0974-360X.2022.00972
17. Patel L, Shah D, Chandpa H, Khristi A, Bhavsar P. Inhalation therapy with *Clerodendrum serratum* Linn. extract: An experimental study on ovalbumin-induced asthma in rats. J Nat Remedies. 2024; 24(1). https://doi.org/10.18311/jnr/2024/34453