



**EVALUATION OF ANTI-ASTHMATIC POTENTIAL OF TRADITIONAL SIDDHA FORMULATION PIPPALYATHI MATHIRAI IN MILK INDUCED LEUKOCYTOSIS IN MICE**

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**ABSTRACT**

Asthma is a chronic inflammatory illness of the lungs characterized by increased leukocyte infiltration into the airways, particularly eosinophils, and decreased respiratory function. Bronchoconstriction, increased airway hyperresponsiveness (AHR), and mucus production are all caused by inflammation. Steroids and anti-allergic agents becomes the first line drug of choice for managing asthma offers undesirable side effects. Pippalyathi Mathirai (PM) is a novel siddha formulation referred for clinical management of allergic asthma condition. Hence the aim of the present study is to explore the anti-asthmatic activity of the formulation PM against milk induced leukocytosis in mice. Result analysis of the present investigation signifies that there was a significant increase in the level of Eosinophil, Neutrophil, WBC and monocytes count in mice belongs to group II which was challenged with milk. Treatment with trial drug PM at both the dose level has shown significant decrease in the Eosinophils, WBC, lymphocyte and monocyte level. Further Procalcitonin (PCT) which is an actual index of inflammation and allergy was found decreased in PM treated animals. It was concluded from the datas that trial drug PM possess promising anti-allergic and bronchodilator property. In conclusion the siddha formulation PM reveals potential anti-asthmatic property through bioactive chemicals derived from the herbal ingredients.

**KEY WORDS:** *Asthma, Allergy, Siddha, Pippalyathi Mathirai, Milk induced leucocytosis, Steroids, Side effects*

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## 1. Introduction

Asthma is a chronic inflammatory illness of the airways that manifests itself through a variety of varied and recurrent symptoms, as well as airflow restriction and bronchospasm. Wheezing, coughing, chest tightness, and shortness of breath are all common symptoms of asthma. According to a World Health Organization estimate, about 300 million people were newly diagnosed with asthma each year, and around 250,000 people died as a result of the condition [1].

Several medications are presently available to treat asthma. Corticosteroids, the most effective nonspecific anti-inflammatory drugs available, are extensively utilized to enhance lung function in asthma patients. However, it is well established that inhaled corticosteroids have a limited ability to influence airway remodelling [2]. As a result, interest in developing natural medications with fewer adverse effects than presently utilized treatments has grown [3].

Medicinal plants are the world's oldest source of pharmacology. Over the millennia, a large variety of traditional medical systems (folk medicine) have arisen under various cultural settings. Even today, the majority of people in less developed nations rely on herbal treatments as their primary source of health care. The alternatives for producing high-quality herbal medical goods have greatly improved in recent decades as a result of scientific and technological advancement. Phytotherapy is widely accepted as a "natural and gentle alternative" to synthetic pharmaceuticals among the general population in industrialised nations, and global sales of herbal medicines are steadily increasing [4].

The Siddha system of medicine is a well-known ancient practise that has survived and thrived for millennia. With extensive knowledge of medicinal herbs and associated supplements, the link between the physiology of biological systems and their importance and coordination in individual well-being was well known. A number of features of siddha remain unknown. Pippalyathi Mathirai is a traditional siddha formulation indicated for the clinical management of allergic and asthma condition as listed in the siddha literature

## 2. Materials and Methods

### 2.1. Experimental Animals

Healthy Balb-C male mice weighing between 20-25 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between  $22 \pm 2^\circ \text{C}$  and relative humidity 60–65%. They were provided with food (Sai feeds, Bangalore, India) and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India. SU/CLATR/IAEC/XVII/175/2021

### 2.2. Experimental Methodology [5]

Animals were randomly divided in four group of 6 mice each (one normal control, second milk intoxicated, three and four are drug treatment groups). Animal belongs to group I received normal saline 0.1ml. Group II mice received boiled and cooled milk (4 mL/kg, s.c.) from day 1 to 5. Animal belongs to group III received milk (4 mL/kg, s.c.) and treated with 250mg/kg dose of Pippalyathi Mathirai (PM) (p.o) 1 hr before milk injection for five days. Animal belongs to group IV received milk (4 mL/kg, s.c.) and treated with 500mg/kg dose of Pippalyathi Mathirai (PM) (p.o) before milk injection for five days

### 2.3. Induction of Leukocytosis [6]

Balb-C mice were used for this study in which boiled and cooled milk (4 mL/kg, s.c.) was injected to the mice results in abnormal increase in Total WBC, Procalcitonin, eosinophil count.

### 2.4. Blood collection [7]

At the end of the study after overnight fast all mice were anesthetized by intra muscular injection with pentobarbital sodium. Blood collected by ocular puncture for biochemical estimations of Total WBC, Procalcitonin, eosinophil count.

### 2.5. Histopathology [8]

At the ends of the study all the mice were sacrificed and lung was harvested and stored in the fixative solution (10% formalin) and cut into 10  $\mu\text{m}$  thickness. Staining was done by using hematoxylin and eosin

**2.6. Statistical Method**

The statistical analysis was carried by one-way analysis of variance ANOVA. Results are expressed as ±SEM. The data were statistically analyzed by ONE WAY ANOVA followed by Dunnett’s multiple comparison test. Probability P values < 0.05 were considered as significant.

**3. Results**

**3.1. Effect of PM on Haematology profile of mice challenged against milk induced eosinophilia**

From the result analysis of the present investigation it was evident that there was a significant increase in the level of Eosinophil, Neutrophil, WBC and monocytes count in mice belongs to group II which was challenged with milk. Treatment with trial drug PM at both the dose level has shown significant decrease in the Eosinophils, WBC, lymphocyte and monocyte level. Further Procalcitonin (PCT) which is an actual index of inflammation and allergy was found decreased in PM treated animals. It was concluded from the datas that trial drug PM possess promising anti-allergic and bronchodilator property. As shown in Table 1.

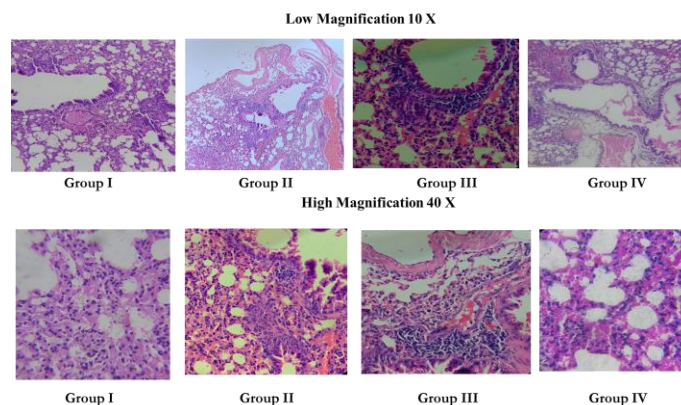
**Table 1: Effect of PM on Haematology profile of mice challenged against milk induced eosinophilia**

| Group                          | WBC count (×10 <sup>3</sup> µl) | Eosinophils (%) | Neutrophils 10 <sup>3</sup> /mm <sup>3</sup> | Lymph (%)   | Mon (%)     | PC T (%)    | Lung Weight in Gms |
|--------------------------------|---------------------------------|-----------------|--|-------------|-------------|-------------|--------------------|
| Group I - Control              | 4.267 ± 0.31                    | 1.583 ± 0.07    | 2.737 ± 0.19                                 | 65.6 ± 1.40 | 4.4 ± 0.41  | 5.4 ± 0.034 | 6.4 ± 0.01         |
| Group II - Milk 4 mL/kg, s.c   | 16.48 ± 1.08                    | 6.883 ± 0.48    | 7.883 ± 0.17                                 | 88.0 ± 2.86 | 8.35 ± 0.32 | 6.43 ± 0.30 | 0.6867 ± 0.01      |
| GROUP III – Milk + 250mg/kg PM | 10.08 ± 0.308                   | 3.783 ± 0.46    | 4.4 ± 0.35                                   | 78.6 ± 1.08 | 7.63 ± 0.33 | 3.66 ± 0.18 | 0.4033 ± 0.02      |
| GROUP III – Milk + 500mg/kg PM | 7.95 ± 0.66                     | 2.333 ± 0.21    | 3.583 ± 0.25                                 | 73.6 ± 2.04 | 5.7 ± 0.18  | 2.64 ± 0.07 | 0.355 ± 0.01       |

**3.2. Effect of PM on Histopathology of Mice lung**

Inter alveoli septum and bronchioles appears normal in sample belongs to group I and further perivascular region appears normal, alveolar septa and wall appeared widen and normal in sample belongs to group I. Severe signs of inflammation with increased migration of inflammatory cells were observed in the

sample belongs to group II further evidence on migration of eosinophils around the airway, blood vessels were observed in group II sample. Moderate dilatation with mild aggregation of inflammatory cells were observed in sample belongs to group III mice. Opening of bronchi and lumen of blood vessels appears widen with no invasion of inflammatory cells in sample belongs to group IV mice. As shown in Fig 1.



**Figure 1: Histopathology of Mice Lung**

**4. Discussion**

Allergic inflammation in conjunction with hyperactivity of the airways is the primary characteristic of allergic asthma. The inflammatory response is characterized by an increase in eosinophils and mast cells, increased mucus production, and T cell activation. Numerous studies have demonstrated that T-helper type (Th2) cells play a critical role in initiating and maintaining allergic airway inflammation and asthma via their enhanced production of Th2-type cytokines (IL-4, IL-5, and IL-13) [9]. These pro-inflammatory cytokines, which are also generated by mast cells, alveolar macrophages, and epithelial cells in the bronchial tissue, play a critical role in the pathophysiology of airway inflammation.

Milk-induced leukocytosis and eosinophilia in mice serves as a model for stress-induced asthma evaluation. Milk is a significant allergen that causes the formation of eosinophils in rats. Eosinophilia is a condition in which the peripheral eosinophil count exceeds 4% of total leukocytes. Eosinophils act as an inflammatory cell in the late stage, particularly during the development of allergic asthma. Eosinophils secrete mediators such as eosinophil cationic protein

(ECP), eosinophil-derived neurotoxin (EDNT), granulocyte macrophage colony stimulating factor (GM-CSF), tumor necrosis factor (TNF), and prostaglandin (PG), all of which cause epithelial shedding, bronchoconstriction, and inflammation in the respiratory tract [10]. Result analysis of the present investigation signifies that there was a significant increase in the level of Eosinophil, Neutrophil, WBC and monocytes count in mice belongs to group II which was challenged with milk. Treatment with trial drug PM at both the dose level has shown significant decrease in the Eosinophils, WBC, lymphocyte and monocyte level. Further Procalcitonin (PCT) which is an actual index of inflammation and allergy was found decreased in PM treated animals. It was concluded from the datas that trial drug PM possess promising anti-allergic and bronchodilator property.

Phytochemicals are plant-derived chemicals that are frequently used to explain the huge variety of secondary metabolic products present in plants. The phytochemical screening assay is a simple, rapid, and low-cost process that provides researchers with a quick answer to the various types of phytochemicals in a combination and is an essential tool in bioactive compound analysis [11].

Asthma is a chronic obstructive pulmonary illness caused by inflammation that affects millions of individuals of all ages [12]. Cough with wheezing, shortness of breath, and/or chest tightness are the primary symptoms, which intensify at night and/or in the early morning [13,14]. Bronchial asthma is defined by reversible restriction of airflow, chronic hyperactivity of the airways, and airway remodelling [15]. Inflammatory cell infiltration is a feature of asthma's immunohistopathology (in sudden-onset) [16]. Although asthma has been recognized for decades, it continues to rank highly on the list of incurable disorders. Histopathological finding of the study advocates that inter alveoli septum and bronchioles appears normal in sample belongs to group I and further perivascular region appears normal, alveolar septa and wall appeared widen and normal in sample belongs to group I. Severe signs of inflammation with increased migration of inflammatory cells were observed in the sample belongs to group II further evidence on migration of eosinophils around the airway, blood vessels were

observed in group II sample. Moderate dilatation with mild aggregation of inflammatory cells were observed in sample belongs to group III mice. Opening of bronchi and lumen of blood vessels appears widen with no invasion of inflammatory cells in sample belongs to group IV mice.

## 5. Conclusion

Asthma is a serious public health disease that affects individuals globally; its incidence is constantly growing, resulting in significant healthcare costs. Long-acting beta2 sympathomimetic drugs (LABA), such as formoterol and salmeterol, should never be used alone in the treatment of asthma, but must always be combined with appropriate dosages of ICS. By contrast, worry regarding the systemic effects of inhaled corticosteroids is growing. In conclusion the siddha formulation Pippalyathi Mathirai reveals potential anti-asthmatic property through bioactive chemicals derived from the herbal ingredients and may be clinically recommended for the management of allergic asthma.

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