Research Article

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PRE-CLINICAL EVALUATION OF BRONCHODILATORY POTENTIAL OF THE NOVEL SIDDHA FORMULATION THOOTHULA PAZHA CHOORANAM ON MILK-INDUCED LEUKOCYTOSIS IN ALBINO MICE

S. Arul Jothi [*1], K.Dhivyalakshmi [2], N.Anbu [3], D.Sivaraman [4]

*[*1&2] P.G Scholar, Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India.*

[3] Head, Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India.

[4] Scientist, Centre for Laboratory Animal Technology and Research, Col.Dr.Jeppiaar Research Park, Sathyabama Institute of Science and Technology , Jeppiaar Nagar, Rajiv Gandhi road, Chennai - 600 119,Tamil Nadu, India.

ABSTRACT

Asthma is an inflammatory disease of the lungs characterized by increased infiltration of leukocytes, especially eosinophils, into the airways, and reduced respiratory function. The inflammation leads to bronchoconstriction, increased airway hyper-responsiveness and mucus production. The treatment of asthma in the modern medicine is based on the use of beta agonists, leukotriene modifiers and inhaled corticosteroids that allowed an acceptable control of the main symptoms. However, this therapy offers numerous side effects and other limitations such as cost, treatment compliance, discontinuation of the therapy etc. Current asthma therapy lacks satisfactory success due to adverse effect, hence patients are seeking complementary and alternative medicine to treat their asthma. Siddha system of traditional medicines pioneering the asthma therapy by offering potential preparation that tend to cause bronchodilation and also minimize the symptoms that aggravate asthma in both children's and elderly peoples. Siddha preparation thoothula pazha chooranam (TTPC) is one such novel formulation indicated for treating bronchial asthma as per the standard literature reference. Hence the main aim of the present study is to evaluate bronchodilatory potential of the formulation TTPC on milk-induced leukocytosis in albino mice. It was observed from the results of the study that there was an abnormal increase in the level of Eosinophils, WBC and monocytes in mice belongs to group II challenged with milk induction, whereas treatment with trial drug TTPC at both the dose level (250 and 500 mg/kg) has shown significant decrease in the Eosinophils, WBC and monocytes counts. Increase in the level of eosinophils triggers the event of bronchospasm drug like TTPC that subside this count shall cause broncho dilatation. It was concluded from the data's of the present study the formulation TTPC provides adequate protection against milk induced leukocytosis and also promotes bronchodilation as evident through histological finding. Hence this formulation may have considered as a drug of choice for clinical management of bronchial asthma.

KEY WORDS: ***Siddha preparation, Thoothula pazha chooranam, Asthma therapy, Milk-induced leukocytosis, Bronchodilation, Eosinophils.***

Corresponding Author: S. Arul Jothi
Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.
Email: jothimyloo@gmail.com

1. Introduction

Asthma, a chronic disease, is a major health care issue worldwide due to its increasing prevalence in many countries following trends of urbanization and industrialization. India alone has an estimated burden of more than 15 million patients [1]. Despite the existence of effective medicines, asthma control is poor globally and especially in developing countries [2]. Poorly controlled asthma is associated with significant morbidity, mortality and socio-economic problems [3]. In India, the estimated cost of asthma treatment per year for the year 2015 has been calculated at about 139.45 billion Indian rupees (approximately 2.3 billion US dollars). Interestingly, it has been deduced that this cost is likely to come down to about 48.5 billion Indian rupees if all asthmatics receive treatment according to evidence-based guidelines. It is noteworthy that this estimate does not include the indirect costs of asthma [4].

Globally, the employment of medicinal herbs and related preparation's as a substitute for orthodox drugs in the management of various diseases has been increasing due to the unavailability of modern health facilities, relative availability of medicinal herbs, poverty, and recent revelations that they possess active compounds that may be responsible for different biological and pharmacological actions [5]. Long acting beta 2 agonists are helpful in improving asthma control and airway functions when inhaled corticosteroids are insufficient. Long acting inhaled beta 2-agonist (formoterol and salmeterol) should not be used as a monotherapy in asthma as they do not appear to influence the airway inflammation in asthma. They are most effective when combined with inhaled glucocorticoids and this combination therapy is the preferred treatment when a medium dose of inhaled glucocorticoids alone fails to achieve control of asthma [6].

Requirement for daily inhalation with glucocorticoids is often a cause for patient discomfort, limiting the use of glucocorticoids in asthma therapy. In addition, the current therapy is not affordable for the patients in developing countries, who rely on the traditional medicine. Therefore, there is a significant need for new medications for the treatment of asthma that are highly efficacious, with low cost, easily managed and with few adverse effects [7].

In the search for new medications for asthma, plants through the traditional medicine are a credible alternative. Hence, plant have been used to treat several diseases for thousands of years. And nowadays following the traditional use of plants, some pharmacological screenings have led to new drugs discovery. Indeed, plants have been the leads of about 25% of drugs introduced into the market during the last 20 years [8,9]. Siddha preparation thoothula pazha chooranam majorly comprises of the herbal ingredients such as thoothulam pazham (solanum trilobatum), kandangkathiri pazham (solanum xanthocarpum), milagu (piper nigrum),thippili (piper longum), seeragam (cuminum cyminum), karunseeragam (nigella sativa),lavangam (syszgium aromaticum) and sugar. The main objective of the present research work is to evaluate the bronchodilator potential of the TTPC using milk-induced leukocytosis in albino mice model.

2.Materials and Methods

2.1. Experimental Animals

Healthy swiss albino mice were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air supported by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ¬± 2˚C and relative humidity 50–65%. They were provided with standard pelleted feed 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 with the IAEC approval number: SU/CLATR/IAEC/X/087/2018

2.2. Experimental Methodology

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 Thoothula Pazha Chooranam (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 Thoothula Pazha Chooranam (p.o) before milk injection for five days.

2.3. Induction of Leukocytosis [10,11]

Swiss albino 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. Biochemical Analysis [12]

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, eosinophile count.

2.5. Histopathological Analysis [13]

Sample obtained were immersed in 10% formalin for 24 h-48h for histopathological examination. After standard processing, the cut tissue was embedded in paraffin (Leica TP1020 tissue processor) and cut into 5 µm thick sections in a rotary microtome (Leica RM2255 - Fully Automated Rotary Microtome). The sections were stained with haematoxylin-eosin (Merck). Histological measurement and photographs were taken with Olympus CX31, Trinocular Biological Microscope (magnification 10x & 40 x).

2.6. Statistical Method

The statistical analysis was carried by one-way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). 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 TTPC on hematology profile of mice challenged against milk induced eosinophilia

It was observed that there was an abnormal increase in the level of Eosinophils, WBC and monocytes in mice belongs to group II challenged with milk induction, whereas treatment with trial drug TTPC at both the dose level has shown significant decrease in the Eosinophils, WBC and monocytes counts. Increase in the level of eosinophils triggers the event of bronchospasm drug that subside this count shall cause bronchodilatation. As shown in Table 1.

Table 1: Effect of TTPC on hematology profile of mice challenged against milk induced eosinophilia

GROUP	WBC count ($\times 10^3$ µl)	Eosinophil's (%)	Neutrophils $10^3/mm^3$	Lymph (%)	Mon (%)	PCT (%)
Control Group	2.917 ± 0.3	0.9117 ± 0.06	1.66 ± 0.08	68.75 ± 2.3	2.317 ± 0.2	0.6183 ± 0.07
Milk (4 mL/kg, s.c.)	13.15 ± 0.6*	3.533 ± 0.26*	4.483 ± 0.23*	97.6 ± 0.60*	7.75 ± 0.28*	4.083 ± 0.25*
Milk+ 250 mg/kg of TTPC,p.o	8.983 ± 0.4*	1.865 ± 0.16*	3.433 ± 0.22*	85.98 ± 2.15*	5.583 ± 0.30*	2.733 ± 0.17*
Milk+ 500 mg/kg of TTPC,p.o	6.167 ± 0.57 *	1.485 ± 0.06*	3.15 ± 0.16*	78.1 ± 1.7*	4.917 ± 0.18*	1.733 ± 0.17*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.2. Effect of TTPC on Lung weight of mice challenged against milk

It was observed that there was a marginal increase in the lung weight of the milk induced group when compare to that of the control group mice. TTPC at both the dose level has notable decrease in lung weight in treated mice belongs to group III and IV. As shown in Table 2.

Table 2: Effect of TTPC on Lung weight of mice challenged against milk

Group	Lung Weight in gms
Control Group	0.1967 ± 0.014
Milk (4 mL/kg, s.c.)	0.4317 ± 0.019
Milk+ 250 mg/kg of TTPC,p.o	0.2867 ± 0.01
Milk+ 500 mg/kg of TTPC,p.o	0.21 ±0.01

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.3. Effect of TTPC on Histopathological changes in Mice Lung (H&E) Staining under low and high power magnification

Histopathological analysis has revealed that the arrangement of epithelial and muscular appears normal in the lung of control group mice and further bronchial blood vessels and connective tissue appears normal. Severe bronchial obstruction with signs of hyper secretion and 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. Marginal dilatation with mild aggregation of inflammatory cells were observed in sample belongs to group III mice. Bronchial opening appears widen with no signs of infiltration observed in group IV samples when compare to that of the group II samples. As shown in Figure 1.

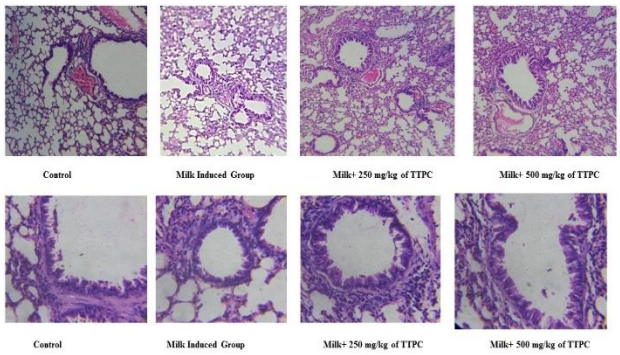

Figure 1: Histopathological changes in Mice Lung (H&E) Staining under low and high power magnification

4.Discussion

Bronchial asthma is the most common chronic respiratory disease, with an case burden of approximately 358.2 million in 2015. In 2015, about 0·40 million people died from asthma, a decrease of 26·7% from 1990, and the age-standardized death rate decreased by 58·8%. The prevalence of asthma increased by 12·6%, whereas the age-standardized prevalence decreased by 17·7% [14]. WHO estimates show that 300 million people currently suffer from asthma. Asthma deaths will increase in the next 10 years if urgent action is not taken [15]. Asthma cannot be cured, but proper diagnosis, treatment and patient education can result in good asthma control and management. Asthma occurs in all countries regardless of level of development. Over 80% of asthma deaths occur in low and lower-middle income countries. For effective control, it is essential to make medications affordable and available, especially for low-income families [16,17].

The current ‘gold standard' of asthma therapy is a combination inhaler containing a long-acting β2-agonist with a corticosteroid – an improved form of adrenal gland extract. Cromoglycate, derived from a plant product and theophylline, a dietary methyl xanthine, have also been extensively used in the therapy of asthma [18]. Herbs have always been a prototypical source of drugs and many of the formerly available drugs have been derived directly or indirectly from them. A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of several diseases [19].

According to the National Institute of Health (NIH), asthma is defined as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, mast cells, eosinophils, T-lymphocytes, neutrophils and epithelial cells [20].In the present study it was observed that an abnormal increase in the level of WBC and neutrophils count in the mice belongs to milk challenged group, whereas treatment with trial drug TTPC at both the dose level of 250 and 500mg/kg has shown significant decrease these in both WBC and neutrophil level.

Leukocyte recruited during asthmatic inflammation release the inflammatory mediators like cytokines, histamine, and major basic protein which promote ongoing inflammation. The eosinophil are the most characteristic inflammatory cells in bronchial biopsies taken from asthma patients and may be seen in the submucosal and epithelial layers. An abnormal increase in peripheral eosinophil count to more than 4% of total leukocyte is termed as eosinophilia [21]. In the present study it was observed that an abnormal increase in the level of eosinophils, lymphocytes and monocytes in mice belongs to group II challenged with milk induction, whereas treatment with trial drug TTPC at both the dose level has shown significant decrease these immune cells.

Release of mediators such as histamines, prostaglandins and leukotrienes, which subsequently lead to contraction of airway smooth muscle and bronchoconstriction [22-24]. Histopathological

analysis has revealed that the arrangement of epithelial and muscular appears normal in the lung of control group mice and further bronchial blood vessels and connective tissue appears normal. Severe bronchial obstruction with signs of hyper secretion and 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. Marginal dilatation with mild aggregation of inflammatory cells were observed in sample belongs to group III mice. Bronchial opening appears widen with no signs of infiltration observed in group IV samples when compare to that of the group II samples.

5.Conclusion

In search of alternate therapy from Indian system of traditional medicine the present investigation has been carried out for exploring the possible anti- asthmatic property of the siddha formulation thoothula pazha chooranam in milk induced mice model. Outcome of the study provided an evidence based data on revealing the control of the drug in limiting the milk induced eosinophils, WBC, neutrophils, lymphocytes and monocytes counts to the lower index. Further brocho dilatation were observed histologically in treatment group. By considering the merits and potential of the formulation it was concluded that this formulation may be suitable in treating bronchial asthma children's and adults with proper clinical validation.

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