ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Bronchodilator and Mast Cell Stabilizer Effect of Siddha Formulation Seenthil Chooranam

Vijayalakshmi. A*, Suganya M, Pankaj Kumar Giri, Jayakumari. S, Shanmugasundaram P.
Department of Pharmacognosy, School of Pharmaceutical Sciences, VISTAS, Vels University, Pallavaram, Chennai-117, Tamilnadu, India.
*Corresponding Author E-mail: **avijibaskaran@gmail.com**

ABSTRACT:
The present study aimed to evaluate anti-asthmatic activity of a classical Siddha formulation Seenthil chooranam (SC) by mast cell stabilizing effect and broncho dilator property by in-vivo method. Preliminary phytochemical and HPTLC analysis of SC were determined as per standard protocols. Phytochemical analysis of aqueous extract gave positive test for carbohydrates, phenols, glycosides, saponins, flavonoids, tannins and terpenoids. HPTLC finger print analysis of the aqueous extract showed the presence of possible number of components. The results of the in-vivo study demonstrate that SC has potent broncho dilator property with significant (***p<0.001) mast cell stabilizing activity and decrease in leukocytosis in dose dependent manner. These findings are clearly indicative of the role of SC as potent inhibitor of mast cell degranulation and ability to control the leukocytosis, have bronchodilation and hence can be used for the management of asthma supporting the traditional claims

KEYWORDS: Seenthil Chooranam, Siddha Formulation, Asthma, Mast Cell

INTRODUCTION:

Asthma is an inflammatory disease of the small airways characterized by episodic, reversible bronchial obstruction, polyphonic wheeze, dyspnoea, and cough which may be relieved spontaneously or as a result of therapy[1]. The prevalence of asthma has significantly increased in recent decades, that is, nearly 7% -10% of the world population was affected in each year. In urban areas, this problem is increasing due to increase in environmental smoke and air pollution resulting from urbanization.

Received on 23.08.2016 Modified on 11.10.2016
Accepted on 20.11.2016
Research J. Pharm. and Tech. 2017; 10(1): 252-256.
DOI: 10.5958/0974-360X.2017.00052.X

It can be triggered by various factors like allergens, drugs, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, chemicals, histamine and also hereditary[2]. Between 100 and 150 million people around the globe suffer from Asthma. In India 15-20 million people get affected. WHO recognize asthma as a disease of major public health importance and place a unique role in the co-ordination of international efforts against the disease. Asthma cannot be cured but it could be controlled[3].

Current synthetic drugs used in pharmacotherapy of asthma are unable to act at all the stages and targets of asthma. In traditional Ayurveda and Siddha systems of medicine, several drugs have been documented for the treatment of various respiratory disorders including asthma. Globally there is resurgence in usage of plant based medicines and WHO also recommends the same because of its holistic approach with maximum therapeutic value. However, well organized, pre-clinical

and clinical evidences are not adequately available in order to advocate their scientific merit and supremacy over the existing therapies. The majority of CAM (complementary and alternative medicine) therapy like Siddha medicine has not been rigorously tested in the way conventional western medicines have been. So in order to take the Siddha system of medicine in to the global lime light, we have come with one of the best traditional classical Siddha formulation known as Seenthil chooranam (Agathiyar vaidhya kaviyam 1500)[4]. consisting of *Widelia chinensis*, *Tinospora cordifolia* and *Poonagam*. Its traditional uses in folk medicine are multiple, and some of its therapeutic effects includes antiasthmatic, antidiabetic, antirheumatic, anti-inflammatory, antipyretic and antiallergic. The present study was undertaken to investigate the effect of the formulation of Siddha drug Seenthil chooranam quoted in the text Agasthiyar Vaithiya Kaviyam-1500 for its anti-asthmatic activity for mast cell stabilizing effect and broncho dilator property by in-vivo method.

MATERIALS AND METHODS:

Materials:

The Seenthil chooranam (SC) selected for the proposed study was procured from Indian Medical Practitioners Co-operative Pharmacy and Stores Ltd., Chennai. (IMCOPS). Dexamethazone and sodium chromoglycate were purchased from Sigma-Aldrich Chemical Co., USA. Egg albumin and other chemicals were purchased from Himedia Laboratories Pvt. Ltd., India. All the other chemicals were of analytical grade.

Extraction:

The SC was extracted with distilled water by cold maceration method. The extract was concentrated under reduced pressure at room temperature.

Preliminary Phytochemical Analysis:

The aqueous extract was dried and weighed. The extract was subjected to chemical analysis to detect the presence of different phytoconstituents[5].

High performance thin layer chromatography:

Chromatograph was performed on 10x10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel 60F 254 (E. Merck Ltd, Darmstadt, Germany) stored in a dessicator, application was done by Hamilton microsyringe (Switzerland), mounted on a Linomat V applicator. Spotting was done on the TLC plate, ascending development of the plate, migration distance 80 mm (distance to the lower edge was 10 mm) was performed at 25±20 °C in a camag chamber previously saturated for 30 min. After development the plate was dried at 60 °C in an oven for 5 minutes. Densitometric scanning was then performed with a Camag TLC Scanner 3 equipped with win CATS Software and the chromatograms were recorded[6].

Experimental animals:

Swiss Albino mice of either sex weighing 25-30 g were procured from Animal House. The entire process was approved by the Institutional Animal Ethical Committee which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. XVIII/VELS/PCOL/05/ 2000/CPCSEA/IAEC/04.02.2016. The animals were kept in clean and dry polycarbonate cages and maintained in a well ventilated animal house with 12 hrs light– 12 hrs dark cycles. The animals were fed with standard pellet diet and water ad libitum. For experimental purpose, the animals were kept fasting overnight but allowed for access to water.

Acute toxicity study:

Acute toxicity study was performed according to OECD guidelines 423 (Organization of Economic Co-Operation and Development). It is a stepwise procedure with three animals of single sex per step. Depending on the mortality and morbidity status of the animal, on average of 2-4 steps may be necessary to allow judgment on the test substance. The procedure is to fix a minimal number of animals, which allows acceptable database scientific conclusion. The method uses different defined doses (5, 50, 500, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the "Globally Harmonized System" (GHS) for the classification of extracts which cause acute toxicity[7].

Procedure:

Three healthy, Swiss Albino mice weighing 25-30 gm were selected for the study. The mice were fasted over-night and provided with water ad libitum. Following the period of fasting, the animals were treated with the seenthil chooranam at the dose of 2000 mg/kg body weight, orally. As most of the crude extracts possess LD50 value more than 2000 mg/kg body weight and this was used as starting dose. After oral administration, the mice were observed on hourly basis for 24 hrs to access mortality and to detect any changes in the autonomic or behavioral responses viz. alertness, aggressiveness, spontaneous activity, irritability, tremor, corneal reflex, salivation, urination, respiration and convulsion etc[8].

Bronchodilator Effect of Seenthil chooranam on Milk Induced Leucocytosis and Eosinophilia in Mice Model:

Induction of Leucocytosis and Eosinophilia:

The induction of Leucocytosis and Eosinophilia in all groups' mice was done by the administration of boiled and cooled milk subcutaneously at the dosage level of 4 ml/kg daily to all groups of mice.

Animal treatment:
Mice were randomly selected for milk administration and divided into five groups. In each group, six mice were selected for this procedure. Group 1 mice served as negative control (Normal control) and treated only with distilled water 1 ml orally. Administration of milk injection was done in group 2, 3 and 4. After 30 min of milk administration, group 2 and 3 were undergone treatment of test substance and standard drug was administered. Milk administered group 2 (Disease control) served as positive control. Milk administered Group 3 served as test group treated with the test Seenthil chooranam at the dose of 200mg/kg orally. Milk administered Group 4 served as test group treated with the test Seenthil chooranam at the dose of 400mg/kg orally. The absolute dose of test drug given to the mouse was calculated by the body surface area ratio between human intended dosages against mouse. Milk administered Group 5 served as test group treated with the standard drug Dexamethasone at the dose of 50mg/kg orally.

Haematological parameters analysis:
All experimental mice were fasted overnight of the study and 0.5 ml of blood was collected on next day by puncturing Retro orbital sinus using capillary tube. After 24 hour of milk administration, again the blood was collected from all groups of mice. A blood sample was sucked in WBC pipette up to mark and more diluted with eosin solution. Neubaur's chamber was used to counting eosinophil. The chamber was charged with this fluid and eosinophil count was completed. Total leukocyte count was done in each group before the drug administration and 24 hrs after boiled and cooled milk injection was calculated. Leucocytes counts were estimated by using Neubaur's chamber. The chamber was charged with above fluid and total leukocyte count was done[9].

Mast cell stabilizing effect of SC on ova albumin induced mast cell degranulation in mice model:
Mice were randomly selected from animal lab and it was divided into four groups. In each group six mice were selected to start the procedure. Group 1 animals served as control and treated with only 1 ml of distilled water orally for following three days. Group 2 mice served as standard and treated with standard drug sodium chromoglycate at the dose of 50 mg/kg orally for three days. Group 3 served as test group treated with the test drug SC at the dose of 200mg/kg orally for three days. Group 4 served as test group treated with the test drug SC at the dose of 400mg/kg orally for three days. The absolute dose of test drug given to the mouse was calculated by the body surface area ratio between human intended dosages against mouse. After three days of treatment procedure, all mice were injected with 0.9% Normal saline at the dose of 10 ml/kg into the peritoneal cavity as Intra Peritoneal injection. After this procedure abdomen of mice was gentle massaged, peritoneal fluid was collected by aspiration through 21 gauge needle and transferred into the test tube and mixed well. Test tube contains 10 ml of buffer medium (pH 7.2 - 7.4) made of L - Glutamine and 25mM Hepes buffer. The test solution was centrifuged at 400 - 500 rpm. Supernatant was discarded from that and mast cells were collected as pellet and washed with the same buffer medium two times by centrifugation. The cell suspensions were challenged with egg albumin (100µ g/ml) and incubated at 37°C for 10 min. Then the cell suspensions were stained with 1% toludine blue and observed under light microscope. Degranulated mast cells appeared as burst while normal mast cell appeared as intact was observed under light microscope. Total 100 mast cells of each cell suspension was collected from each group were counted and percent of protection against degranulation was calculated[9].

Statistical analysis
All the results for this study were expressed as mean ± SEM of six animals in each group. Analysis of variation was performed by one way ANOVA method followed by Tukey - Kramer multiple comparisons test. Probability values less than 0.05 ($P<0.05$) were considered as significant.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening:
The SC was extracted with distilled water and the yield was found to be 2.10%w/w. The aqueous extract of SC shows the presence of carbohydrates, phenols, glycosides, saponins, flavonoids, tannins and terpenoids.

High performance thin layer chromatography:
The aqueous extract was further subjected to HPTLC for the conformation of the active constituents. The aqueous extract showed 14 resolutions of spot and corresponding ascending order of Rf values start from 0.12 to 0.80 with the solvent system chloroform: ethyl acetate: benzene. The chromatogram and Rf values were correspondingly depicted in Fig. 1. Thus the developed chromatogram will serve the better tool for standardization of the formulation.

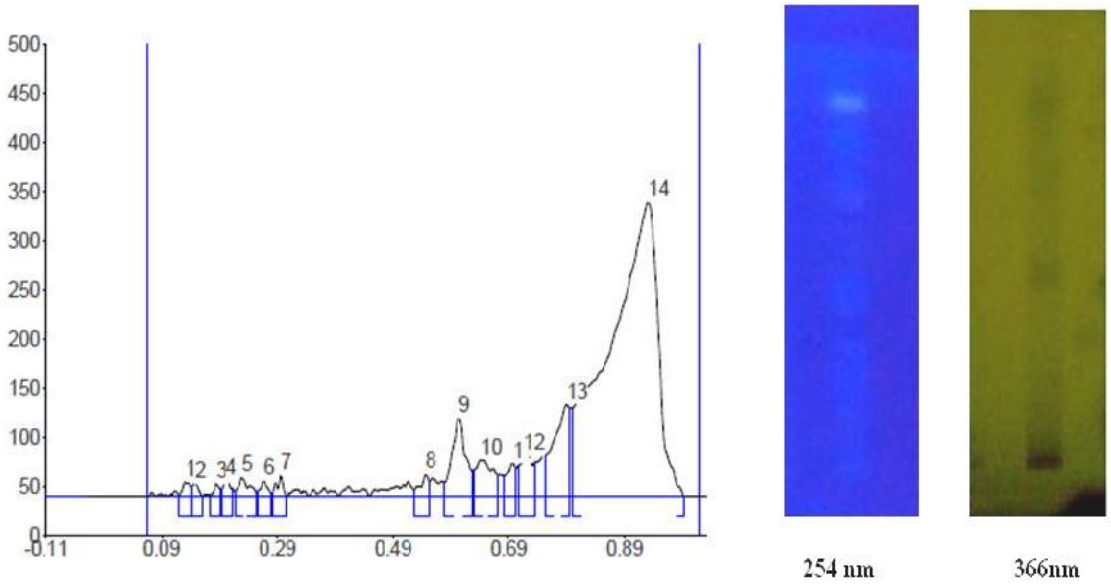

Figure 1: HPTLC chromatogram and finger print of Aqueous extract of SC

Antiasthmatic activity:

Bronchodilator Effect of SC on Milk Induced Leucocytosis and Eosinophilia in Mice Increased level of Leucocytes and eosinophils counts in our respiratory system play a vital role to induce bronchial hypersensitivity and produces airway inflammation in allergic and non-allergic asthma. The inflammatory reaction of bronchial walls in asthma is brought about increased level of bronchial eosinophils. It occupied in the later phase reaction of bronchial asthma. Subcutaneous administration of boiled and cooled milk into the Balb/c albino mice acts as antigen and reduced allergic response in mice increase the total leucocyte and eoisinophil count in 24 hour administration[10].

During asthmatic inflammation leukocytes release the following inflammatory mediators such as cytokines, histamine mainly and basic protein, which promote the endurance of inflammation[11]. Eosinophils infiltrating the airway also have an effect on mucus secretion by epithelial goblet cell[12]. Eosinophils part in bronchial asthma was quite an active in the development of allergic airway inflammation[13]. Eosinophil creates broncho constriction through the secretion of mediators such as eosinophil cationic protein, eosinophil-derived neurotoxin, and prostaglandin, which results in broncho constriction in respiratory tract. During bronchial asthma broncho constriction is developed by inflammatory changes of the airways. If a drug reduces or prevents bronchial inflammation of airways broncho dilation happens.

Administration of milk (4 ml/kg) by subcutaneous route exhibited significant ($^{***}P < 0.001$) increase in leukocyte count after 24 hours of its administration. In the test group, pretreated with SC at 200 mg/kg dose and 400 mg/kg dose, there was significant ($^{***}P < 0.01$) inhibition was found in leukocytosis. Subcutaneous injection of milk produced significant ($^{***}P < 0.001$) increase in total eosinophil count in milk-intoxicated group. SC at lower and higher dose of 200 mg/kg and 400 mg/kg showed significant reduction ($^{***}P < 0.001$) in leukocytosis and eosinophil count (Table 1). Finally the SC results represents reduce bronchial inflammation, helps airways to dilate and thus SC indirectly proves its broncho dilator activity in the management of asthma.

Table.1: Effect of SC on milk-induced Leukocytosis and Eosinophilia in mice

S.No	Group	Dose	Difference in no. of leucocytes (cu/mm)	Difference in no. of eosinophils (cu/mm)
1	Normal control	Distilled water, 1 ml	98±9. 06	97±6.36
2	Disease control	Milk, 4 ml/kg	4110±10.68	156.61±8.45
3	SC	200 mg/kg	2144±53.60***	81.72±6.3***
4	SC	400 mg/kg	1680±40.68***	63.80±4.32***
5	Dexamethasone	50 mg/kg	1560±46.20***	60.63±5.48***

Values are expressed in mean ± SEM. N = 6. Comparison was done by one way ANOVA followed by Tukey – Kramer Multiple comparisons test. ($^{***}P < 0.001$)

Mast cell stabilizing effect of SC on ova albumin induced mast cell degranulation in mice:

Mast cell derived mediators in respiratory system play a big role in allergic and non-allergic asthma. Mast cells are triggered by the inhalation of specific allergens leads to start degranulation. Degranulated mast cells in respiratory system release certain mediators of inflammation such as histamine, leukotrienes, platelet activating factors and eosinophils, neutrophils etc. that play important role in the development of airway inflammation and bronchoconstriction. Many of the pathologic features of bronchial asthma can attributed to the effects of mast cell-derived mediators. Mice mesentery mast cells following the exposure of egg albumin cause degranulation of mast cell release inflammatory mediators. Table 2 shows that SC showed maximum protection against mast cell degranulation by egg albumin. The results were significant and dose dependant compared with control group. Results of control group was compared with SC treated group represent reduce mast cell degranulation through significant protection against egg albumin induced mast cell degranulation by stabilizing it role in airway inflammatory pathway, which is responsible for the decreasing airway inflammation by preventing release of various inflammatory mediators.

Table.2: Effect of SC on mast cell degranulation in mice

S.No	Group	Dose	% Degranulation of mast cell
1	Normal control	Distilled water, 1 ml	82.62 ±4.12
3	SC	200 mg/kg	40.58±3.43***
4	SC	400 mg/kg	32.25±2.68***
5	Standard	50 mg/kg	29.36± 4.40***

Values are expressed in mean ± SEM. n = 6. Comparison was done by one way ANOVA followed by Tukey – Kramer Multiple comparisons test. ($^{***}P < 0.001$)

With the support of *in-vivo* pharmacological activity, it has been demonstrated that parental administration of milk produces a marked and significant increase in leukocyte and eosinophil count after 24 hours of administration. The milk-induced leukocytosis and eosinophilia in mice model helps to evaluate the stress-induced asthma. The results of present study revealed that SC caused reduction in the count of these inflammatory cells. Among the tested doses, 400 mg/kg has shown significant activity as compared with 200 mg/kg in a dose-dependent manner.Mast cell degranulation observed in control group was significantly prevented by standard drug sodium chromoglycate administered group. Results of control group was compared with SC treated groups represent reduce mast cell degranulation through significant protection against egg albumin induced mast cell degranulation by stabilizing its role in airway inflammatory pathway, which is responsible for the decreasing airway inflammation by preventing release of various inflammatory mediators.

CONCLUSION:

It can be concluded that SC may have the action like PGE, and may inhibits the tone of tracheal and bronchial muscles and thus has a broncho dilator action. It is possible that the broncho dilator activity of the SC may involve mainly, inhibition of prostaglandin synthesis. Mast cell stabilizing property of SC is possible to work by preventing mast cell degranulation. The mechanism is the blocking of IgE-regulated calcium channels in respiratory passages. Without intracellular calcium in cell, the histamine vesicles cannot fuse to the cell membrane and degranulate. From the above scientific evaluation, concludes that the drug SC is proficient with the new hope in the treatment of bronchial asthma which is cost effective and has fair preparation method.

CONFLICT OF INTEREST:

Conflict of interest declared none.

ACKNOWLEDGEMENT:

Authors acknowledge sincere thanks to Dr. Ishari. K. Ganesh, Chancellor, Vels University, Chennai, Tamil Nadu, India for providing all the facilities throughout the research work.

REFERENCES:

1 Buss WW, and Rosenwasser LJ. Mechanisms of asthma. J Allergy Clin Immunol. 2003; 111:s: 799-804.
2 Rakesh kumar, Himanshu Bhusan Sahoo, Biswakanth Kar, Nikunja Kishor Mishra, Rajaram Mohapatra, Sarada Prasad Sarangi. Pharmacological evaluation of calendula officinalis L. on bronchial asthma in various experimental animals. International journal of Nutrition, Pharmacology, Neurologial diseases. 2014; 4(2): 95-103.
3 Prashant K, Singh R Tushar Sahasrabudhe, Vinay and Dharmadhikar. Knowledge of Bronchial Asthma in Parents/Caregivers of Adolescent Asthma Patients. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2015; 6(3): 516-520.
4 Ramachandran SP. Yugimuni vaithiya chinthamani 800 (First Edition, Thamarai Noolagam, 1998) 269.
5 Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall, London, 1984.
6 Rich E and Schibli A, Thieme Medical Publisher, Inc, ISBN 13:978-1-58890-409-6(US), 2008.
7 Ecobichon DJ. The basis of toxicity testing. CRC Press, New York. pp. 3-86, Organization for Economic Co-operation and Development (OECD); Revised draft Guidelines 423. 2000. OECD Guidelines for the testing of chemicals No 423: Acute oral toxicity-Acute toxic classic method. Revised document, 1997, 1-26.
8 Organization for Economic Cooperation and Development (OECD) guidelines for acute toxicity of chemicals. No. 425, 2008.
9 Vasantha Kumar P, Pitchiah Kumar M, Velpandian V, Banumathi V. Bronchodilator and mast cell stabilizer effect of sIddha Herbo mineral formulation maha punnai ver kuligai international Journal of Research in Pharmaceutical and Nano Sciences, 2014; 3(5): 38 - 443.
10 Limbasiya KK, Modi VR, Tirgar PR, Desai TR, Bhalodia PN. : Evaluation of Anti asthmatic activity of dried whole plant extract of *Leucas aspera* using various experimental animal models.International Journal of Phytopharmacology. 2012; 3(3): 291-298.
11 Brekhman LI and Dardymov IV. New specific substance of plant origin, which increases nonspecific resistance. Annu Rev Pharmacol. 1969; 9: 419 - 428.
12 Shimizu T, Shimizu S, Hattori R, Y. Majima, A mechanism of antigen-induced goblet cell degranulation in the nasal epithelium of sensitized rats. J Allergy Clin Immunol. 2003; 112: 119-125.
13 Elizabeth R Walsh and Avery Auguest. Eosinophils and allergic airway disease: there is more to the story. Trends in Immunol. 2010; 31(1): 39-44.