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BRONCHODILATOR AND MAST CELL STABILIZER EFFECT OF SIDDHA HERBO MINERAL FORMULATION MAHA PUNNAI VER KULIGAI

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ABSTRACT

Asthma is one of the most important non communicable diseases in the world. It is also a chronic disease affecting our lungs commonly. More than 235 million people are currently affected in various countries and 100 million will be affected by 2025. To prove the anti-asthmatic property of a drug, need to evaluate mast cell stabilizing and bronchodilator property of the drug. Maha punnai ver kuligai (MPVK) is a herbo mineral compound having potent equal part of both herbal and mineral as ingredient. The present study aimed to evaluate the anti-asthmatic activity of a classical Siddha herbo mineral compound Maha Punnai Ver Kuligai through experimental models. The results demonstrate that drug has potent broncho dilator property with significant mast cell stabilizing activity in experimental animals.

KEYWORDS

Bronchodilator, Mast cell stabilizer, Herbo mineral and Non communicable disease.

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INTRODUCTION

Asthma is one of the most important non communicable diseases in the world[1]. It is also a chronic disease affecting our lungs commonly. "You Can Control Your Asthma" was the theme created on world asthma day at 6th may 2014 to create awareness about controlling asthmatic effects in worldwide[2]. More than 235 million people are currently affected in various countries and 100 million will be affected by 2025[3]. To control asthma, there are two ways one is medication and another is to improve surrounding environmental hygiene. Proper management of asthma enables people to enjoy a good quality of life.

Medication for asthmatic comes under two groups. They are reliever medications and controller medications. Reliever medications are usually bronchodilator that quickly relieves broncho spasm. Reliever group of medication includes ß2-agonist Bronchodilators, Anti cholinergic Bronchodilators and are majorly used for Bronchial asthma treatment. Controller group medications in bronchial asthma management act by providing anti-inflammatory action to prevent or diminish the inflammatory process and it helps to control the immune reactions that cause asthma attacks. A controller medication helps to reduce the incidence of attacks and sometimes preventing them from occurrence. Mast cell stabilizers, Leukotriene modifiers and corticosteroids come under this classification.

In present days Mast cell stabilizers provide side effects exhibiting dry cough and throat irritation while Leukotriene modifiers produce side effects like headache, nausea, stomach upset and diarrhoea[4]. Combined medications possess both reliever and controller properties are very good for asthmatic treatment. It contains both bronchodilating and mast cell stabilizing properties. Unfortunately this type of medication is very rare now.

Siddha herbo mineral preparations are bio-safe and have its own fast acting properties to control broncho constriction and relieves from asthmatic symptoms early. And they probably don't cause any adverse drug reactions. In classical siddha literature (Pathinen Siddhar Aruliya Rajavaithiya Bothini)[5], Maha Punnai Ver Kuligai (MPVK) was indicated for respiratory illness includes cough, sneezing, sinusitis and bronchial asthma. Thus the present study was focused to evaluate the bronchodilator and mast cell stabilizer properties of a herbo mineral drug on experimental animal models.

MATERIAL AND METHODS

Maha punnai ver kuligai was selected from the traditional siddha literature, Pathinen Siddhar Aruliya Rajavaithiya Bothini[5].

Ingredients and Preparation of the drug

Group I: (Herbals)

- Root bark of *Calophyllum innophyllum*(Punnai verpattai juice-steam boiled) – 50ml.
- Thrikadugu choornam (Dry ginger, Pepper and long pepper) – 35gram (1Palam).
- Purified Kayam(Asafoetita) – 35gram(1Palam).
- Purified Koraikilangu(*Cyprus rotandus*) – 35gram(1Palam).

Group II: (Minerals)

- Purified Rasam(Mercury) – 35gram(1Palam).
- Purified Kantham (Magnetic oxide)– 35gram(1Palam).
- Kalnar parpam (Calcinate form of Asbestos)– 35gram(1Palam).
- Vengara parpam (Calcinate form of Borax) – 35gram (1Palam).

Before starting to make a Maha Punnai Ver Kuligai all drugs were purified using classical methods to eliminate toxic contents of the ingredients and improve efficacy of the drug. Before starting grinding procedure Thrikaduku Chooranam (Powdered form of Dry ginger, Pepper and long pepper), Vengara parpam (Calcinate form of Borax) *and* Kalnar parpam (Calcinate form of Asbestos) were prepared. Then the mineral compounds was grinding one by one slowly followed by adding the herbal drugs one by one grind it gently in stone morter. After finished the above procedure decoction of *Calophyllum innophyllum* (Punnai) was added gradually and grinding together for 6 hours. After that the pill was rolled at the size of 130mg as per literature.

EXPERIMENTAL ANIMALS

Balb/c mice of either sex, weighed 20-30g was purchased from the animal house of King Institute of Preventive Medicine, Guindy, Chennai. All animals were maintained under standard laboratory condition with food and water *ad libitum*. Mice were allowed to acclimatize for 2 weeks before the commencement of experimental protocol. The animals were treated in line with the guide and care of laboratory animals as approved by the Institutional Animal Ethical Committee of Sairam Advanced Centre for Research, Chennai. (Registration no1545/po/a11/CPCSEA/1-18/2013).

Bronchodilator Effect of Mpvk 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 [6,7].

Animal treatment

Mice were randomly selected for milk administration and divided into four 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 drug Maha Punnai Ver Kuligai at the dose of 200mg/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 4 served as test group treated with the standard drug Dexamethasone at the dose of 50mg/kg orally was tabulated at Table No.1.

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[6,7].

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 were considered as significant.

Mast Cell Stabilizing Effect of Maha Punnai Ver Kuligai on Ova Albumin Induced Mast Cell Degranulation in mice Model

Mice were randomly selected from animal lab and it was divided into three 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 Maha Punnai Ver Kuligai at the dose of 200mg/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 (100µg/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[7,8].

Statistical analyses

All the results were expressed as mean ± SEM of six animals. Analysis of variance was performed by one way anova followed by Turkey – Kramer Multiple comparisons test. Probability values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Results of this study and statistical analysis results was tabulated given below Table No.2-6.

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 produced allergic response in mice increase the total leucocyte and eoisinophil count in 24 hour administration[9]. During asthmatic inflammation leukocytes release the following inflammatory mediators are cytokines, histamine mainly and basic protein, which promote the endurance of inflammation[10]. Eosinophils infiltrating the airway also have an effect on mucus secretion by epithelial goblet cell[11]. Eosinophils part in bronchial asthma was quite an active in the development of allergic airway inflammation[12]. 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[9].

In this study was observed that leukocytes count was decreased in mice treated with MPVK at doses of 200mg/ kg significantly as compared to disease control group. Result suggests that MPVK decreases milk induced leukocytes count in mice. And this study was observed that MPVK at doses of 200mg/kg significantly decreased milk induced eosinophils count in mice. Eosinophils counts of disease control group was compared with MPVK treated group results showed the drug reduces eosinophil counts in mice. Finally the test drug MPVK treated group mice leucocytes and eosinophils count was considerably reduced. During bronchial asthma broncho construction is developed by inflammatory changes of the airways.

If a drug reduces or prevents bronchial inflammation of airways broncho dilation happens. The effect of MPVK on reducing bronchial inflammation through reducing the increased leucocytes and eosinophils counts in mice. Finally the MPVK results represents reduce bronchial inflammation helps airways to dilate. MPVK indirectly proves its broncho dilator activity in the management of asthma.

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. their place was important 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. However, mast cell degranulation observed in control group was significantly prevented by standard drug sodium chromoglycate administered group.

Results of control group was compared with Maha Punnai Ver Kuligai treated group represent reduce mastcell 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. It strongly proves MPVK module in mast cell stabilizing activity.

Table No.1: Animal treatment groups and doses

S.No	Groups	Dose
1	Normal control	Distilled water, 1 ml
2	Disease control	Milk, 4 ml/kg
3	Maha punnai verkuligai	200mg/kg
4	Dexamethasone	50 mg/kg

Table No.2: Effect of Maha punnai ver kuligai 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	95±13.26	29.12±3.34
2	Disease control	Milk, 4 ml/kg	4214±111.13	145.31±9.33
3	MPVK	200 mg/kg	2334±67.45	105.42±5.6
4	Dexamethasone	50 mg/kg	1967±142.45	60.24±4.62

Note: Values are expressed in mean ± SEM. N = 6.

Table No.3: Statistical analyses table for the results - milk-induced Leukocytosis

S.No	Comparison of groups	Mean Difference	Q value	P value	Significance
1	1 vs 2	-4119.0	42.608	P<0.001	***
2	1 vs 3	-2239.0	23.161	P<0.001	***
3	1 vs 4	-1872.0	19.365	P<0.001	***
4	2 vs 3	1880.0	19.447	P<0.001	***
5	2 vs 4	2247.0	23.244	P<0.001	***
6	3 vs 4	367.00	3.796	P>0.05	Ns

Comparison was done by one way ANOVA followed by Tukey - Kramer Multiple comparisons test where q value is greater than 3.958 then the P value is less than 0.05.

Table No.4: Statistical analysis results of Milk induced Eosinophilia in mice

S.No	Comparison of groups	Mean Difference	Q value	P value	Significance
1	1 vs 2	-116.19	46.336	P<0.001	***
2	1 vs 3	-76.300	30.428	P<0.001	***
3	1 vs 4	-31.120	12.410	P<0.001	***
4	2 vs 3	39.890	15.908	P<0.001	***
5	2 vs 4	85.070	33.925	P<0.001	***
6	3 vs 4	45.180	18.017	P<0.001	***

Comparison was done by one way ANOVA followed by Tukey - Kramer Multiple comparisons test where q value is greater than 3.958 then the P value is less than 0.05.

Table No.5: Effect of Maha punnai ver kuligai on mast cell degranulation in mice

S.No	Group	Dose	% Degranulation of mast cell
1	Control – Distilled water	1 ml	77.62 ±3.46
2	Standard- sodium chromoglycate	50 mg/kg	24.16±2.12
3	Maha punnai ver kuligai	200mg/kg	48.69 ± 5.48

Note: Values are expressed in mean ± SEM. N = 6.

Table No.6: Statistical analyses of Maha punnai ver kuligai on mast cell degranulation in mice

S.No	Comparison of groups	Mean Difference	Q value	P value	Significance
1	1 vs 3	28.930	18.000	P<0.001	***
2	1 vs 2	53.460	33.263	P<0.001	***
3	3 vs 2	-24.530	15.262	P<0.001	***

Comparison was done by one way anova followed by Tukey - Kramer Multiple comparisons test where q value is greater than 3.958 then the P value is less than 0.05.

CONCLUSION

After evaluate the safety the drug, the Broncho dilator and Mast cell stabilizing and of Maha Punnai Ver Kuligai is elaborated. Hence it can be concluded that this drug 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 Maha Punnai Ver Kuligai may involve mainly, inhibition of prostaglandin synthesis. Mast cell stabilizing property of this drug 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 Maha Punnai Ver Kuligai is proficient with the new hope in the treatment of Bronchial asthma which is cost effective and has fair preparation method.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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