NVEO
Natural Volatiles &
Essential Oils

Systematic Study on Curcumin and Vasicine as A Novel Anti-Asthmatic Agent

Annie Susan S[1], Sengottuvelu S[1]*, Duraisami R[2], Prabha T[3], CN Marimuthu[4]

[1]Department of Pharmacology, Nandha College of Pharmacy, Erode – 638052, Tamilnadu, India
[2]Department of Pharmacognosy, Nandha College of Pharmacy, Erode – 638052, Tamilnadu, India
[3]Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode – 638052, Tamilnadu, India
[4]Nandha Engineering College, Erode – 638052, Tamilnadu, India

Abstract

Curcumin and Vasicine are a traditional Indian medicine both are used in the treatment of bronchial asthma and their activity were analyzed by different research workers. In this study we investigated the combination effect of Curcumin and Vasicine using*In-vitro* isolated guinea pig tracheal chain and also by *In-vivo* allergen induced airway inflammatory mouse model. In *In-vitro* method initially responses were recorded with histamine. After giving wash response were recorded in presence of single constituent and in combination. In *In-vivo* method after sensitized and challenged by Ovalbumin/ AloH, the BALB/c mice were administrated orally with the combination of Curcumin and Vasicine , total and differential count in BALF andIgE level in serum was counted using ELISA.Contraction produced by histamine was antagonized and produce relaxation by combination of Curcumin and Vasicine than single constituent. Treatment of mice with the Curcumin and Vasicine combination reduces the total and differential count of bronchoalveolar lavage fluid(BALF) and IgE serum level in the mice. The data indicate that the combination of drugs produce relaxation of smooth muscle, reduced the total and differential count and IgE, level in serum. From the result it was concluded that, Curcumin and Vasicinemay be an alternative for the conventional drug against asthma.

Key Words: Curcumin, Vasicine, Ovalbumin, Asthma and IgE

INTRODUCTION

The inflammatory response is complex and involves various mechanisms of protection against pathogens and tissue repair. In the lungs, inflammation is often caused by bacteria or exposure to toxins, dirt, irritants, and allergies. During inflammation, several types of inflammatory cells are activated and cytokines release individual and other mediators to alter the functions of other inflammatory cells. The formation of these cells and molecules leads to the evolution of inflammation. Clinically, severe inflammation is seen in pneumonia and severe respiratory depression, while chronic inflammation is seen with asthma and COPD. Because the lungs are an important organ in gas exchange, further inflammation is life-threatening. Whenever the lungs are exposed to harmful bacteria, a quick and decisive action is needed to eliminate the invaders. A soft balance between inflammation and anti-inflammatory is essential for lung homeostasis (1).

Curcumin is a polyphenolic compound also known as diferulayl methane, is an active compound from the golden spice turmeric (*Curcuma longa*). Curcumin has been shown to have several medicinal actions including anti-inflammatory, neuroprotective, anticancer, antioxidant and anti-bacterial effects. Curcumin has shown chemo-blocking properties, suppressing the tumorigenic activity of a variety of carcinogens in several types of cancer.It is a highly pleiotropic molecule that exhibit good anti-inflammatory activity, which is proved to treat allergic condition in asthma (2).

Vasicine is an alkaloid obtained from the plant *Adathodavasica.* It is an ancient drug which is used in the treatment of respiratory disorder. It is used as a mycolytic, bronchodilator and also contains anti-inflammatory activity. Both the constituents show great result in treating asthma through their anti-inflammatory activity (3). Plant origins are mainly used in inflammations and inflammatory mediators which responsible for various disorders (4).

The study mainly focuses on combining both the constituents together to find the synergetic effect. Anti-inflammatory effect of both the drugs is believed to be synergetic and increase the anti-inflammatory activity compared to the activity produced by single constituents (5). Based on above, the study was planned to evaluate the anti-asthmatic activity of Curcumin and Vasicine combination by *in-vitro* and *in-vivo* methods.

MATERIALS AND METHODS

Drugs and Chemicals

Curcumin, Vasicine, are natural constituent which is purchased from (Vital herbs) Maharastra. Dexamethasone was obtained from (Cipla) Maharastra. Ovalbumin and Aluminium Hydroxide was obtained from (NICE Chemical Pvt.Ltd) Cochin Sodium chloride, Potassium chloride, Calcium chloride, Magnesium chloride, Sodium dihydrogen phosphate, Sodium hydrogen carbonate, Glucose, Disodium hydrogen phosphate, Sodium chloride. All other chemicals and reagents used in the study were of analytical grade.

Animals and Ethical Considerations

Animals used for this experiment were Female BALB/c mice (20-25g) and Guinea pig (250-350g) which was procured from the Animal house, Kerala Veterinary and Animal Sciences Uniersity, Mannuthy, Kerala. The procured animals were placed in quarantine area for 1 week before experiment. The animals were housed under ambient temperature (21±2˚C) and relative humidity (55±5%) with fixed 12h light and 12h dark cycle. The animals were used after an acclimatization period of five days in propylene cage in the laboratory environment. During acclimatization period animal was provided with standard pellet diet (Hindustan Lever Pvt Ltd., Bangalore) and clean drinking water *ad libitum*. Experimental protocols (Proposal No: NCP/IAEC/2019-20/22) and procedures used in this study were in accordance of the guidelines of CPCSEA and was approved by Institutional Animal Ethics Committee of Nandha College of Pharmacy, Erode. (Reg.No:688/PO/Re/S/02/CPCSEA)

IN-VITRO STUDY

Effect of Curcumin and Vasicine on Histamine Induced Contraction in Isolated Guinea Pig Tracheal Chain Preparation (6)

The guinea pig fasting for the night was donated and the trachea was cut into individual rings and tied together in a series to form a chain. It was then hung in a washable Tyrode solution that was continuously ventilated and stored at 37 ± 0.5 ºC. One part of the trachea chain was attached to an S-shaped aerator tube and the other was connected to an isotonic frontal lever for writing on a drum. Tissues were allowed to simmer for 45 minutes, under a load of 400 mg. The dose-response curve of histamine is taken at a different molar concentration, keeping a time cycle of 5 minutes. After receiving a dose of histamine reaction curve in the tracheal chain, Curcumin (100µg / mL), Vasicine (30µg / mL) alone and combined were added to the water reservoir and responses were recorded with histamine doses. A percentage graph of the major contract response to the systematic logarithm and the negative molar concentration of histamine in abscissa was designed to record the curve to respond to the dose of histamine, if not present with the presence of Curcumin, Vasicine and a combination of both.

***IN-VIVO* STUDY**

Ovalbumin (OVA) Induced Allergic Airway Inflammation Mouse Model (7)

At days 0 and 14, the mouse was injected with an intraperitoneal injection of 20 µg of OVA emulsified with 2 mg aluminum hydroxide at a total volume of 200 µl PBS (pH 7.4) as an adjuvant. Mice were randomly divided into six groups of 6 mice in each group. Group I, acting as a general control, healthy mouse informed of aluminum hydroxide was then challenged with an equal volume of PBS from day 21 to day 23. GroupII acted as the OVA challenge group, was informed of 20 µg Ovalbumin and 2 mg of aluminum hydroxide in 200 µl of PBS (ip) on day 0 and day 14 and were challenged using 1% of OVA on PBS from day 21 to day 23. Group III acted as a reliable control group treated with Dexamethasone group (2mg / kg), mice sensitive to OVA and administered daily with inhaled Dexamethasone (2mg / kg) from day 0 to day 23 following OVA challenge from day 21 to day 23. Group IV functioned as a Curcumin treatment group, mice were OVA-induced and treated daily with Curcumin (100 mg / kg, po) from day zero to day 23 following OVA challenge from day 21 to day 23rd Group V operates as a Vasicine-treated group, mice are exposed to OVA and treated daily with Vasicine (3mg / kg, po) from day to day 23 following OVA challenge from day one -21 to day 23 Group VI acts as a group treated with Curumin and Vasicine, mice stimulated by OVA and treated daily. with Curcumin &Vasicine (100 mg / kg & 3mg / kg po) starting at zero day until day 23 following OVA challenge from day 21 to day 23.

Collection of Blood and Bronchoalveolar Lavage Fluid (BALF) (8)

Mice were anesthetized with an intraperitoneal injection of Ketamine + Xylazine (80mg / kg + 10mg / kg) 24 hours after the last challenge and blood was collected through retro orbital piercing and serum separated by centrifugation of samples in 4 ˚C (3000 rpm) 10 min, and serum was stored at − 80 ˚C to determine IgE in the form of an enzyme-linked immunosorbent assay antibody. After collection the animal blood was excreted with excess CO2 and BALF was collected by coughing and washing with two 0.8 mL aliquots of cold PBS. Samples were collected and immediately placed between 1000g at 4 ˚C for 10 minutes. The supernatants were to disassemble the cell pellets and reassembled into PBS and used for complete cell count and differentiation.

Statistical Analysis

All datas are presented as Mean ± SEM. Data were analyzed in a single variance analysis (ANOVA) followed by Dunnet ‘t’ testing using version 3 graph. P <0.05 was considered statistically significant.

RESULT

***IN-VITRO* STUDY**

Isolated Guinea Pig Tracheal Chain Preparation

Figure No 1. Effect of Curcumin and Vasicine on Dose Response Curve of Histamine in Guinea pig tracheal chain preparation

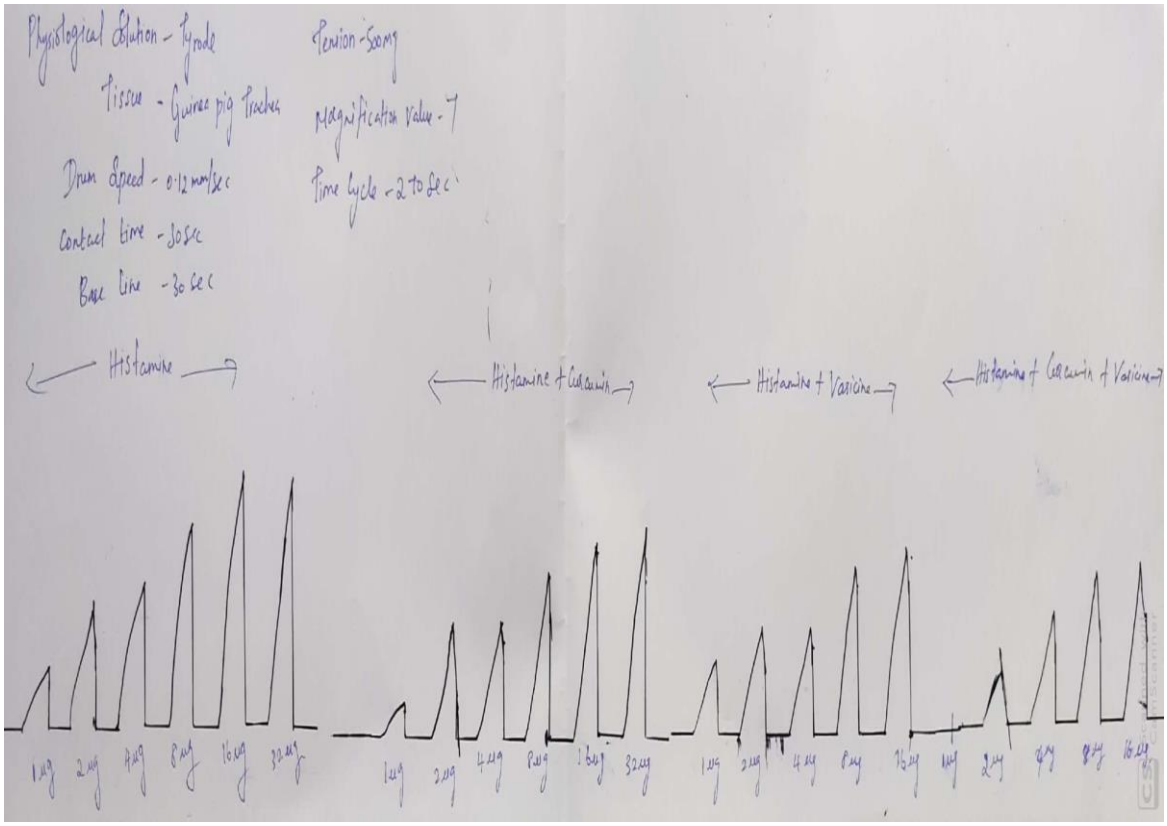
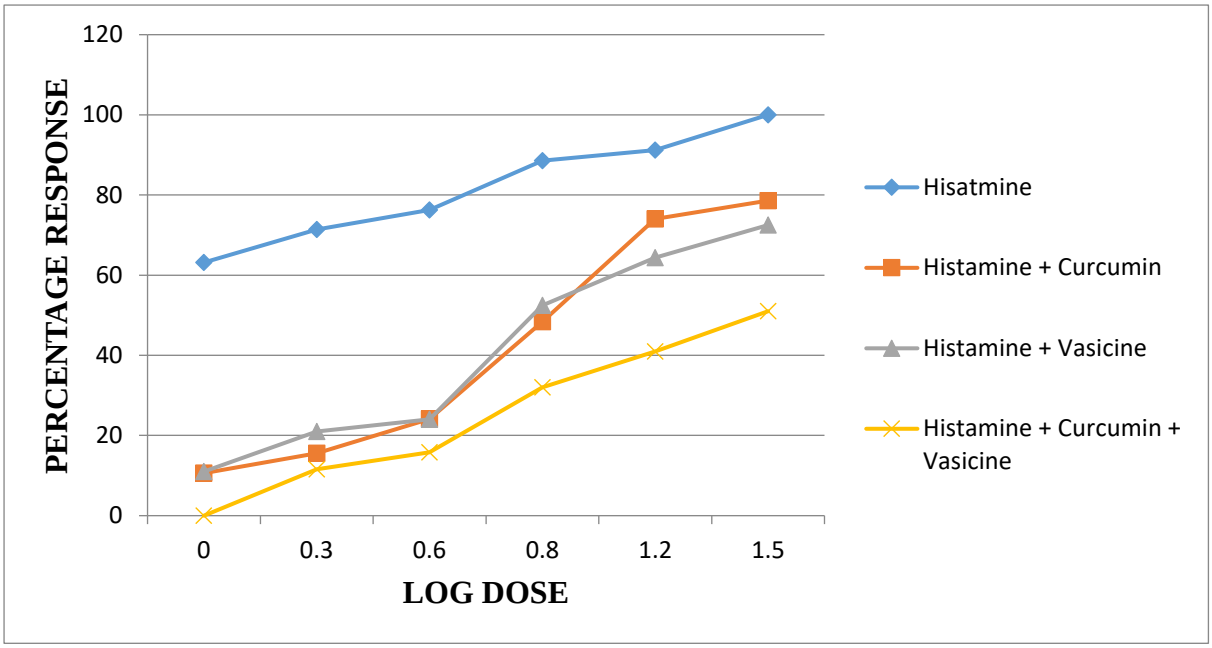


Table No 1. Dose Response Curve of Histamine and Percentage inhibition of Curcumin and Vasicine on Histamine induced contraction in isolated Guinea Pig TrachealChain preparation.

Dose (µg/ml)	Log dose of Histamine	% Responses			
		Histamine	Histamine + Curcumin	Histamine + Vasicine	Histamine + Curcumin + Vasicine
1	0	63.16±0.02	10.61±0.74**	11.32±0.86**	0
2	0.3	71.43±1.33	15.64±0.94**	21.44±0.41**	11.6±1.20***
4	0.6	76.31±1.37	24.10±0.65**	24.61±0.61**	15.88±1.43***
8	0.8	88.60±0.96	48.43±0.33**	52.56±0.45**	32.10±1.63***
16	1.2	91.26±0.77	74.21±0.81*	64.40±1.04*	41.42±0.51***
32	1.5	100.0±0.00	78.64±1.03*	72.51±0.41*	51.34±0.31***
% Inhibition of Histamine Induced Contractions			21.36%	27.49%	48.66%

Values are Mean ± SEM; ***P<0.001, **P<0.01, & *P<0.05 Vs Histamine

Chart No 1. Effect of Curcumin and Vasicine on Histamine induced contraction on isolated Guinea pig tracheal chain preparation



The antihistaminic activity of Curcumin and Vasicine against histamine induced contraction on guinea pig tracheal chain was shown in Figure No. 1, Table No. 1 and Chart No.1 In histamine induced contraction the sigmoid shaped graph falls near to the 'Y' axis which indicates histamine is a potent constrictor of smooth muscle. The response recorded in presence of Curcumin and Vasicinewith histamine, the sigmoid shaped graph falls away from the 'Y' axis, which indicates the test drug antagonizes the histamine induced contraction in guinea pig trachea. Further, the response recorded in presence of the combination of Curcumin and Vacisine with histamine, the sigmoid shaped graph further falls away from the 'Y' axis compared to the single constituent administration. From the readings, it was observed that, the combination of Curcumin and Vasicine produced more antagonistic action on histamine induced contraction on isolated guinea pig trachea compared to antagonistic action produced by single constituents. Percentage inhibition of histamine in isolated guinea pig trachea preparation showed significant inhibition of about 21.36% in presence of Curcumin, 27.49% in presence of Vasicine, 48.66% in presence of both Curcumin and Vasicine administration on comparison with the administration of histamine alone. From the above findings it was observed that the combination of Curcumin and Vasicine relaxes the smooth muscle than Curcumin and Vasicine alone by blocking the histamine receptors.

***IN-VIVO* STUDY**

Ovalbumin (OVA) Induced Allergic Airway Inflammation Mouse Model

Table No2. Effect of Curcumin and Vasicine on differential leucocyte count in OVA induced allergic airway inflammation in mice

Drug Treatment	Total WBC 10^3µl/ml	Eosinophil (%)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)

Group I Vehicle Control	7.03± 5.10	0.30± 0.02	54.00± 0.81	18.00± 1.94	2.70± 0.14
Group II Asthmatic Control(OVA)	10.60± 0.46	4.30± 0.47	73.00± 2.20	33.00± 2.54	6.30± 0.24
Group III (OVA+Dexamethasone)	8.43± 0.93**	2.30± 0.38**	64.30± 0.47**	24.60± 2.49**	3.00± 0.81***
Group IV (OVA+Curcumin)	7.60± 1.02**	2.90± 1.54**	62.40± 2.94**	23.60± 1.49***	2.30± 0.47***
Group V (OVA+Vasicine)	7.40± 1.02***	2.00± 0.10**	63.00± 2.15**	25.30± 4.10**	4.00± 0.81**
Group VI (OVA+Curcumin &Vasicine)	7.30± 0.32**	1.60± 0.08***	61.00± 0.89***	23.30± 2.05***	3.00± 0.37***

Values are mean ± SEM; ***P<0.001, **P<0.01, & *P<0.05 Vs Group II

Table 1, shows the effect of Curcumin and Vasicine on differential leucocyte count in OVA induced allergic airway inflammation in mice.Total WBC count, in the BALF fluid of asthma group was significantly higher than those of the control group (P<0.001). Significant decrease in total WBC count seen in asthmatic animal treated with a Curcumin, Vasicine and Curcumin &Vasicine combination compared to the untreated asthmatic control group (P<0.001). Dexamethasone treated group also significantly reduced total WBC count compared to untreated asthma group (P<0.01). The percentage of Eosinophil, Neutrophil, Monocyte and Lymphocyte in the BALF fluid of untreated asthmatic group was significantly more when compared to the control group (P<0.001).The percentage of differential count in the BALF fluid of Dexamethasone treated group was significantly decreased the count when compared to the untreated asthmatic group (P<0.01). The percentage of Eosinophil, Neutrophil, Monocyte and Lymphocyte in the BALF fluid of treated group Curcumin, Vasicinesignificantly reduced when compared to the untreated asthmatic group (P<0.01) and Curcumin and Vasicine combination treated group more significantly reduced the count compared to Curcumin ,Vasicine alone (P<0.001). From the above finding it shows that the effect produced by the combination was more when compared with the constituents alone and equal to the effect produced by standard drug treated group.

OVA-specific IgEin serum

Table No3. Effect of Curcumin and Vasicine on serum IgE concentrationin OVA induced allergic airway inflammation in mice

Drug Treatment	**Serum IgE (ng/ml)**
Group I Vehicle Control	17.39±1.14
Group II Asthmatic Control (OVA)	76.21±2.98
Group III (OVA+Dexamethasone)	34.74±0.11***
Group IV (OVA+Curcumin)	52.30±1.04**

Group V (OVA+Vasicine)	64.7±0.85*
Group VI (OVA+Curcumin &Vasicine)	36.71±1.02***

Values are mean ± SEM;
***P<0.001, **P<0.01, & *P<0.05 Vs Group II

Table 2, shows the effect of Curcumin and Vasicine on serum IgE concentration in OVA induced allergic airway inflammation in mice. In the control group, the IgE concentration was17.39±3.14 ng/mL, whereas sensitization and challenge with OVA promoted the IgEconcentration of about 76.21 ± 2.98 (P< 0.001). Dexamethasone treated group significantly reduced the IgE concentration (34.74±3.11) (P<0.001) compared with the untreated asthmatic control group.Treatment with Curcumin, Vasicine reduced the plasma IgE concentration (52.3±3.04, 64.7±4.85) (P< 0.01)When compared with the untreated asthmatic control.Treatment with combination of Curcumin and Vasicine shows significant reduction of IgE level (36.71±3.02) (P < 0.001)more when compared with negative control. From the above finding it shows that the effect produced by the combination is more when compared with the constituents alone and equal to the effect produced by standard drug treated group.

DISCUSSION

The present study was conducted to evaluate the synergetic effect of Curcumin and Vasicine on respiratory smooth muscles using different *In-vitro*&*In-vivo* animal models as a potent anti-asthmatic agent.In our Traditional system of medicine, Curcumin and Vasicine are well known medication for the treatment of respiratory problems. Curcumin showed anti- inflammatory andVasicine also shows anti-inflammatory effect along with bronchodilator effect. Hence, the combination of these constituents is believed to show good anti-asthmatic effect.

Histamine is released from mast cells and basophile by antigenic stimulants that cause smooth muscle contraction, increasing vascular penetration and mucous membranes. Histamine produces a volume based on the volume of the Guinea pig pig tracheal chain. In the present study, Curcumin (100µg / mL), Vasicine (30µg / mL) and a combination of Curcumin (100µg / mL) and Vasicine (30µg / mL) significantly inhibited histamine tracheal fixation of the tracheal chain -Ginean pig. The combination of Curcumin (100µg / mL) &Vasicine (30µg / mL) was found to be more effective than the ingredients alone.

Histamine produces dose dependent contraction of Guinea pig ileum preparation. In the present study, Curcumin (100µg/mL), Vasicine (30µg/mL)and combination of Curcumin (100µg/mL)&Vasicine (30µg/mL) significantly inhibited the histamine-inducedcontraction of isolated Guinea pig ileum preparation. Curcumin (100µg/mL)&Vasicine (30µg/mL) combination found to be more effective than the constituents alone.

Karaman *et al.,* (2011) studied the inflammatory activity of Curcumin using murine mouse model. The study demonstrated that Curcumin administration alleviates the pathological changes of chronic asthma (9). Curcumin might be a promising therapy for asthma in the future.Srinivasarao *et*

al., (2006) investigated the antioxidant and anti-inflammatory potential of vasicine isolated from leaves of the *Adhathodavasica* in murine model of asthma (10). After treatment with vasica showed good anti-oxidant anti-inflammatory effect.From the above studies Curcumin and Vasicine shows significant smooth muscle relaxant activity against histamine on isolated guinea pig tracheal chain preparation. So the combination of this constituent may produce better therapeutic effect for asthma.

Mice immunized with OVA/alum and then challenged with an OVA aerosol showeda significant increase in total white blood cell count inBALF when compared with the control group. Hence the increased level ofEosinophilic, Lymphocyte, Monocyte, Neutrophil count was observed in the asthma induction groups when compared with the control. But the combination of Curcumin &Vasicine treated group more significantly reduced the Total and differential WBC count when compared to the effect produced by the single constituent. And showing the equal effect to that of the standard reference control.

IgE levels in serum elevated in asthma groups, an asthmatic response after inhaling antigen in patients with allergic asthma effects from the IgE-dependent type-1 hyper-sensitivity reaction. IgE is associated with an early stage of allergic asthma. After the allergen binds to IgE, the complex binds to the IgE receptor and opens up mast cells to hide many mediators, making the symptoms worse (11, 12). This increased IgE level was reduced by the combination of the Curcumin &Vasicine treated group compared to the effect produced by a single component. And showing the same effect as that of standard reference control.

CONCLUSION

From the research findings it was concluded that, *In-vitro* guinea pig ileum and tracheal preparation on histamine induced contraction was reduced and produce relaxation by combination of Curcumin &Vasicine than constituents alone. Curcumin &Vasicine combination reduces the total and differential count of BALF, IgE serum level in the mice which is immunized with ova and aluminium hydroxide. Therefore this study revealed that Curcumin &Vasicine combination shows synergetic effect on the inhibition of inflammatory mediators which is useful to treat asthma.

REFERENCES

1. Moldoveanu B, Otmishi P, Jani P. Inflammatory Mechanism in the Lungs. Journal of Inflammation Research.2009; 2:1-11.
2. Divya S Nair, kirshnakumar K, Bibithakirshnan. Pharmacological Profile of Curcumin Review. Journal of Bio Innovation. 2017; 6(4):533-541.
3. Rachana R, Sujata Basu, Mamta Pant, SalujaSonam. Review and Future Perspectives of using Vasicine and Related Compounds. Indo Global Journal of Pharmaceutical Sciences. 2011; 1(1):85-98.
4. Pavithra K, SathibabuUddandrao VV, Chandrasekaran P, Brahmanaidu P, Sengottuvelu S, Vadivukkarasi S and Saravanan, G. Phenolic fraction extracted from *Kedrostisfoetidissima* leaves ameliorated isoproterenol-induced cardiotoxicity in rats through restoration of cardiac antioxidant status. Journal Food Biochemistry. 2020; 44: 13450.

5. Xian Zhou, Sai Wang, Dennis Chang, Hosen Kiat, Valentina. Synergistic Effect of Chinese Herbal Medicine. Frontiers in Pharmacology. 2016; 7:1-16.
6. Jawale N M, Shewale A B, Nerkar G S, Patil V R. Evaluation of Antiasthmatic Activity of Leaves of *Piper betal*linn. Pharmacologyonline. 2009; 3: 966-977.
7. Jingjing W*et al*. Anti-Asthmatic Activity of Osthole in an Ovalbumin Induced Asthma Murine Model.Respiratory Physiology and Neurobiology. 2017; (239): 64-69.
8. Tsukioka K, Matsuzaki M, Nakamata M, Kayahara H, Nakagawa T. Increased Plasma Level of Platelet Activating Factor (PAF) and Decreased Serum PAF Acetylhydrolase (PAFAH) Activity in Adults with Bronchial Asthma. Journal of Investigational Allergology and Clinical Immunology. 1996; 6:22-29.
9. Karaman M, Firinci F, Cilaker S, Uysal P, tugyan K, Yilmaz O, Uzuner N, Karaman O.Anti-inflammatory Effects of Curcumin in a Murine Model of Chronic Asthma. AllergologiaetImmunopathologia. 2011; 15: 988-996.
10. Srinivasarao D, Indisc A, Jayarraj, Jayara R, Lakshmi prabha M. A Study of Antioxidant and Anti-inflammatory Activity of VasicineAgoinst Lung Damage in Rats. IndianJournal of Allergy Asthma immunology. 2006;20(1):1-7.
11. Vasavdakrup, Hedge prakash L and Harini A. Pharmacological Activities of Turmeric. Journal of Homeopathy and Ayurvedic Medicine. 2013; 2 (4): 1-4.
12. Young Su Yang, *et al*,. Study of a BALB/c Mouse Model for Allergic Asthma. Official Journal of KoreanSociety of Toxicology. 2008; 24 (4) : 253-261.