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Effect of Jatiphala (*Myristica fragrans* houtt.) in experimentally induced allergic asthma in male wistar Rats

Sonali R Menthe, Vedantam Giridhar, Santosh F Patil and Ravichandra M

Abstract
Several drugs are mentioned in Ayurveda for anti-asthmatic properties under the term *shwasahara. Jatiphala* (*Myristica fragrans* Houtt.) is a unique speice explained by acharyas for the treatment of various disorders including *shwasa* (Asthma), *Kasa* (Cough), *Atisaara* (Diarrhoea) *etc.* In present study, *Jatiphala* was tried for its effect on experimentally induced allergic asthma in male wistar rats. *Jatiphala* was collected & subjected to fine powder. Rats were grouped in three groups & sensitized with intraperitoneal ovalbumin (OVA) & aluminium hydroxide & then challenged with OVA – PBS (phosphate buffer solution) aerosols. The blood parameters like TC, DC, AEC, Serum IgE & BALF cellular differential count was measured along with histological analysis of lung. *Jatiphala* group significantly reduced the eosinophil count in blood, IgE, AEC and number of inflammatory cells in BALF & lung tissues. Results revealed that *Jatiphala* may have an anti-inflammatory and anti-asthmatic effect on allergic bronchial asthma.

Keywords: Allergy, Asthma, *Jatiphala churna, IgE*

Introduction
Asthma is a chronic inflammatory lung disease that is characterized by airway hyper-responsiveness to allergens, airway oedema and increased mucus secretion. It affects over 100 million people worldwide and creates a burden in healthcare. This inflammation is associated with the infiltration of eosinophils, neutrophils and lymphocytes into the bronchial lumen and lung tissues [1].It affecting any age, race and socio-economic class globally and its prevalence is changing upwards worldwide. The increase in prevalence may be due to changes in life style, rapid industrialization, tobacco, smoke, viral infections, chemical irritants and increase in air pollution [2].

Worldwide approximately 300 million peoples currently have bronchial asthma, with estimates suggesting the prevalence of asthma in India is about 3%, whereas 57,000 deaths in India were tribute to asthma [3].

Allergic Asthma is characterized by symptoms that are triggered by an allergic reaction. Symptoms like coughing, wheezing, shortness of breath or rapid breathing, and chest tightness [4]. An allergic response occurs when immune system proteins (antibodies) mistakenly identify a harmless substance, such as tree pollen, as an invader. OVA albumin (OVA) is the main protein found in egg white, making up 60-65% of the total protein. In immunology it is commonly used to stimulate an allergic reaction in test subjects.

In an attempt to protect the body from the substance, antibodies bind to the allergen. The chemicals released by the immune system lead to allergy signs and symptoms, such as nasal congestion, runny nose, itchy eyes or skin reactions. For many people, this same reaction affects the lungs and airways, leading to asthma symptoms [5].

The aim of the WHO for CRDP (Chronic respiratory disease programme) is to reduce the charge of morbidity, disability and premature mortality related to chronic respiratory diseases [6] and it has been reported that many traditional herbal medicine have promising anti-asthmatic action [7].

Research on respiratory medicine drug is a developing area in modern biomedical science. Scientist who is trying to develop newer drugs from natural resources are looking towards the Ayurveda, the Indian traditional system of medicine. Several drugs of plant, mineral and animal origin are mentioned in Ayurveda for anti-asthmatic properties under the term *shwasahara.* Most of these medicines are plant origin only. Some of these plants have been screened for the evaluation of their anti-asthmatic activity in different pharmacological models and clinical trials, but the potential of most remains unexplored.

Jatiphala (*Myristica fragrans* Houtt.) is such a drug which is known to people since vedic period. Its well-known spices used in many food articles. In *Charaka samhita* (One of the most imp classical manuscript on Ayurveda) detail description about *shwasa vyadhi* is found in *chikitsa sthana*. *Shwasa* is also mentioned as the symptom as well as complication of many diseases. It is an illness of *pranavaha srotas*, caused due to predominant morbidity of *vata* and *kapha dosha* afflicting *rasa dhatu*. The causative factors of *shwasaroga* in general are also considered as the etiological factor of *tamaka shwasa* [8]. Environmental etiological factors include cold weather, cloudy weather; dust, smoke etc. can leads to *shwasaroga*.

Acharya Bhavamishra who is one among the *laghutrayi*, in his *Bhavaprakasha Nigantu*, mentioned some single drugs which are showing *shwasahara* properties. *Jatiphala* is such one herbal drug which acts as *kapha- vatahara, shotahara, shwasahara, vedana sthapana, vrsya, sthambhaka* and having specific action on *pranavaha srotas* [9], *Jatiphala* is frequently used in condition like diarrhoea, inflammation, aphrodisiac.

After assessing the available literature, it came into picture about unavailability of such study regarding respiratory disease on *Jatiphala* and also considering current need to evaluate an effective medicine by using influential plant to treat asthma, Hence the study was planned to know upto which extent the above said observations mentioned in the classics is appreciable pharmacologically in case of *Jatiphala* in allergic asthma, So an attempt was made to evaluate the effect of *Jatiphala* which may possibly work as anti-asthmatic in allergy induced asthma.

2. Materials and Methods

2.1 Plants Collection and Authentication

The fruits of *M. fragrans* (Nutmeg) were collected from Alapura region of Kerala, during *grishma ritu* as per classically mentioned [10]. The seeds were got identified and authenticated at AYUSH approved Central Research Facility at Shri BMK Ayurveda Mahavidyalaya and PG centre, Shahapur, Belagavi and voucher number (CRF/13/804) of the drugs given in Central Research Facility. For preparation of Churna, *Jatiphala* seeds were pounded little in khalwa to break its hard seed coat and then pulverized and sieved through 120 no sieve to get fine powder.

2.2 Animal Experimental Study

Male Wistar Rats, weighing 250 ± 20 gm were purchased from authorised Animal breeder (Animal House of KLEU's JNMC, Belgavi). Animals were housed in stainless steel cages. 7 days acclimatisation was given with ambient climatic conditions maintained throughout the experiment. The experimental procedures were approved by the institutional animal ethical committee (IAEC) (IAEC Reg no – 1017/C/06/CPCSEA) with the resolution No. BMK/IAEC/Res-04/2013. The acclimatized rats were divided into three groups: normal (n=2), OVA-control (n=6) and OVA- *Jatiphala group* (n=6).

- Total number of animals: 14
- Duration of study: 24 days.
- Dose of *Jatiphala* in human is 1 gm [11].
- Dose of *Jatiphala* for 200gm of wistar rats: 18 mg/kg/day

Animal Dose was calculated by dose conversion table of Paget and Barnes (1964) [12].

Table 1: Showing different groups of experiment

Groups		No of animals	Protocol used
Group 1	Normal Control	N=2	Normal
Group 2	Disease control	N=6	Ova-Control
Group 3	Experimental	N=6	Ova- *Jatiphala*

2.3 Experimental protocol

Rats were immunized with an intraperitoneal injection of a suspension containing 40 mg of OVA (Sigma life sciences Chemical Co USA 62-88% purity analytical grade) and 2 mg of aluminium hydroxide (SD Fine Chemicals, Mumbai) to all OVA group ie Group II, III, IV. Fifteen days after immunization, rats were challenged by exposure to a 1% OVA in phosphate-buffered saline (PBS) aerosol once daily for 30 min per day for 8 consecutive days (Day 15th – 22nd). The challenge was carried out in a histamine chamber by using an ultrasonic nebulizer (INCO company).The aerosol flow rate is approximately 0.4ml/min and the particle size is app. 5 µm. Control group rats were exposed to nebulised sterile saline using the same method. One day after the last challenge, that is on 24th day the blood and tissue samples were collected [13].

Table 2: Showing sensitization, dose, re-challenging drug and treatment adopted

Group		Sensitization	Dose and route	Rechallenge	Treated with
I	Normal control	Normal saline for induction	0.6 ml intra peritoneal	Normal saline aerosols	-
II	Disease control	Ovalbumin 40mg + $Al_2(OH_3)$	0.6 ml intra peritoneal	1% Ovalbumin + phosphate buffer (PBS)	Normal saline
III	Experimental drug	Ovalbumin 40mg + $Al_2(OH_3)$	0.6 ml intra peritoneal	1% Ovalbumin + phosphate buffer	*Jatiphala churna* from 1st day to 22ndday at dose of 18mg/kg/day

- Group I (Normal control group) rats were administered saline orally for 22 days after first sensitization.
- Group II (OVA-control) rats were administered saline orally for 22 days after first sensitization.
- Group III (OVA- *Jatiphala*) treatment group, administered with *Jatiphala churna* orally for 22 days after first sensitization at the dose of 18mg/kg/day. As *Jatiphala* is not fully dissolved in water for that purpose CMC (Carboxy Methyl Cellulose) suspension was prepared i.e. 10 mg of CMC powder mix in 100 ml of water and *Jatiphala churna* was mixed in CMC suspension which is freshly prepared daily.

Fig 1: Jatiphala oral dosing

Fig 2: Ova i.p Sensitization

3. Observations during experiment

On the first day of experiment, the rats from asthma group and *Jatiphala* group, were sensitized with OVA albumin and Aluminium hydroxide intra peritoneally. On 2nd day there is increased in rectal temperature were noticed in those groups. After 15 days of 1st sensitization that is from day 15th to the day 22nd rats were re-challenged by exposure to a 1% OVA in phosphate-buffered saline (PBS) aerosol once daily for 30 min/day for 8 consecutive days. During the experiment all possible subjective observations were noted and documented following as below Table no. (3)

Table 3: Subjective parameters observed during animal experiment

Si No.	Parameters
3.1.	Nasal Irritation and scratching
3.2.	Sneezing
3.3.	Lung auscultation: Crepts
3.4.	Lung auscultation: Wheezing
3.5.	Respiratory rate
3.6.	Sleeping pattern
3.7.	Movements: Activeness
3.8.	Food & water intake

3.1. Nasal irritation: It was stared to trace on from 1st day only and went on increasing in asthma group. Scratching reflex also seen to be increased further. On comparison with normal control group nasal irritation was observed less with delayed onset.

3.2. Sneezing: from 6rd day onwards sneezing reflex seen in asthma group whereas in *Jatiphala* group it started after aerosol rechallenge. Sneezing was present in all groups but comparatively less in the test drug and still lesser in saline control. Showing the typical development of allergy.

3.3. Crepts: Crepts were audible from 5thday onwards when ausculated with paediatric stethoscope and was more after the aerosol exposure.

3.4. Wheezing: usually seen immediately after aerosols exposure and approximately up till 2-3 hrs of post exposure from 2nd day onwards of aerosol exposure. Forced expiration was noted in asthma group. In normal saline group the crepts were heard after exposure to normal saline aerosols and disappeared after some time (approx. 1 hr) while wheezing was not heard.

3.5. Respiratory Rate: In asthma control group respiratory rate was 76-132/min in histamine chamber indicating of forced shallow respiration and probably dyspnoea while in normal saline group normal rate i.e. 60-100/min was observed.

3.6. Sleeping pattern: As seen in experimental images it was observed that rats were sleeping in sitting position instead of universal fetal position which normal. This substantiates the acharya's version in *tamaka shwasa* that is *Aasino labhatesukham* i.e. Sense of relief in prop-up position.

3.7. Movement & Activeness: Sense of fatigue was observed in all asthma control (OVA) rats while the observations were mixed in *Jatiphala* group. Same was not observed in Saline control group.

3.8. Food intake: It was more seen in both OVA treated group when compare to normal saline treated group. *Jatiphala* group rats appetite may be increased due to the deepana effect of drug. Following is an attempt to bring subjective parameters in close objectivity. Grading of irritation was done by numbering the scratching reflexes which was observed at nasal region. Other subjective parameters were also noted in table no. (5).

Table 4: Grading of nasal irritation

Scratches to nose and face	Grades
0 –10	1
11 – 20	2
21 – 30	3
More than 30	4

Table 5: Subjective Sign's of Asthma observed in rats with grading

Sl. No	Sneezing bouts	Nasal irritation	Crept	Sitting in one position	Sleeping in sitting position
Group I	15-20/min	1	+	Mixed	No
Group II	40-50/min	4	++++	Yes	Yes
Group III	20-25/min	3	++	Yes	Yes

Fig 3: Auscultation for wheezing & crypts

Fig 4: Aerosol exposure in Histamine chamber

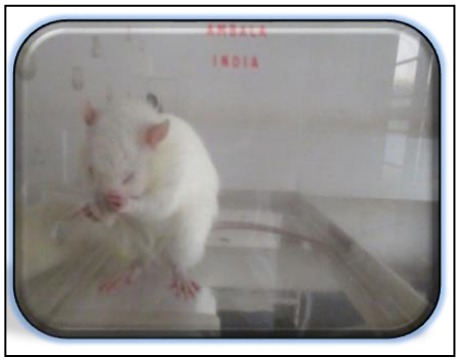


Fig 5: Nasal irritation

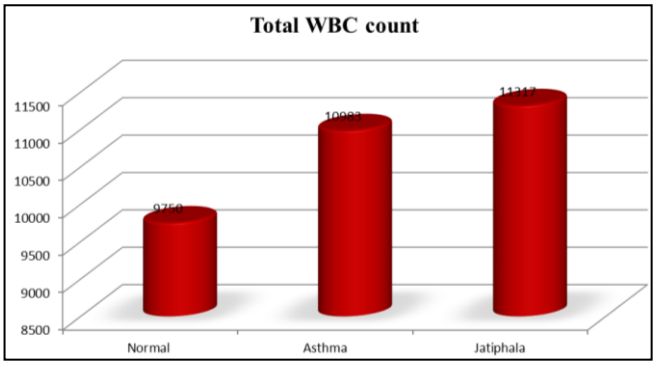
4. Experimental Results

The blood sample was collected after for sacrificing the animal to check the allergic markers and total count. Results obtained are tabulated below.

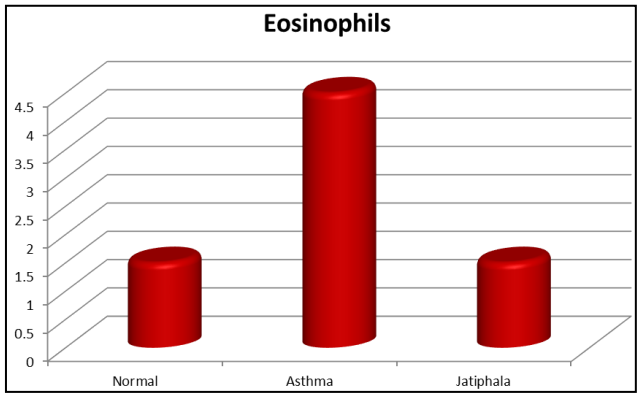
Table 6: Blood Total count, differential count, Absolute eosinophil count and IgE Mean ± SD.

Parameters	Normal saline group Mean ±SD	Asthma control group Mean ± SD	*Jatiphala* treated group Mean ± SD
Total count	9750± 6576	10983± 2338	11317 ± 2999
Neutrophil	18.5± 3.536	9.333± 3.445	9.833± 3.971[@]
Lymphocyte	77.5± 4.95	85± 5.099	85.33 ± 5.164
Eosinophil	1.5 ± 0.7071	4.5 ± 1.761	1.5 ± 0.5477[#]
Monocytes	2.5± 2.121	1.167± 0.4082	3.333± 1.211[*]
Absolute Eosinophil count	162.5 ±159.1	475± 213.9	166.7± 64.55[**]
IgE	9±1.02	15±1.09	5.7±0.97[***]

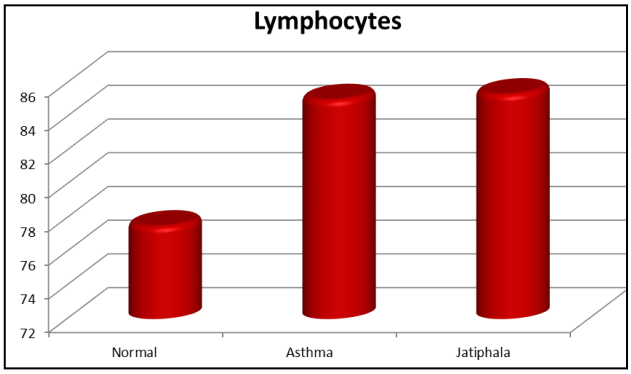
@ P < 0.05 when compared to Asthma Group
# p < 0.01 when compared to Asthma Control Group
* P < 0.05 when compare to Asthma Group
** P < 0.05 when compare to Asthma Group
*** P< 0.001 when compared to Asthma Control Group



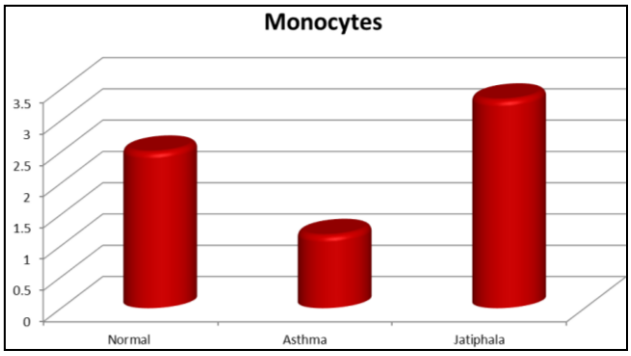
Graph 1: Showing Total WBC count comparison of all groups



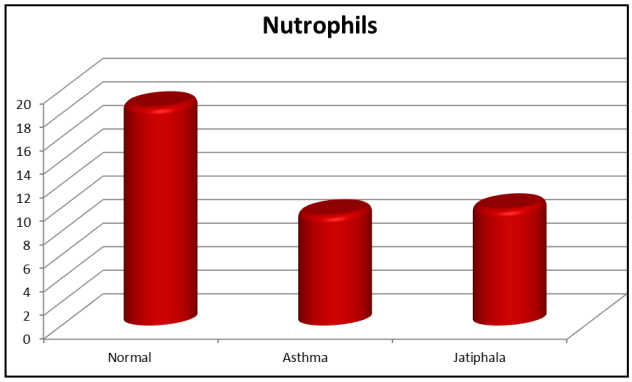
Graph 2: Showing Eosinophil levels comparison of all groups



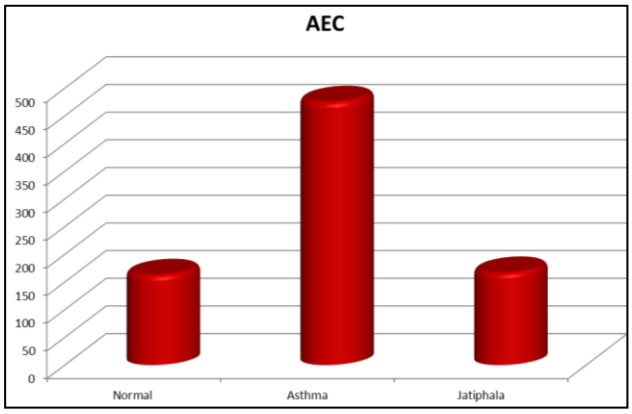
Graph 3: Showing Lymphocytes levels comparison of all groups



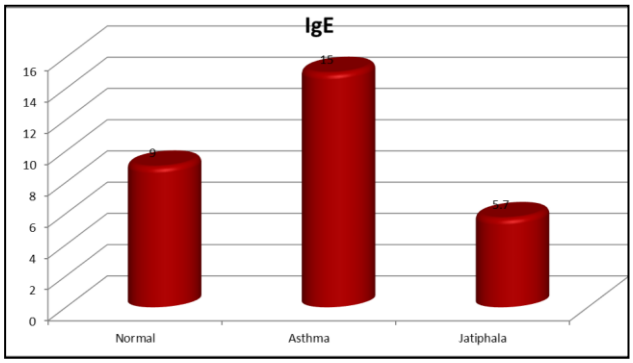
Graph 4: Showing Monocyte levels comparison of all groups



Graph 5: Showing Nutrophils levels comparison of all groups



Graph 6: Showing Absolute Eosinophils Count (AEC) levels comparison of all groups



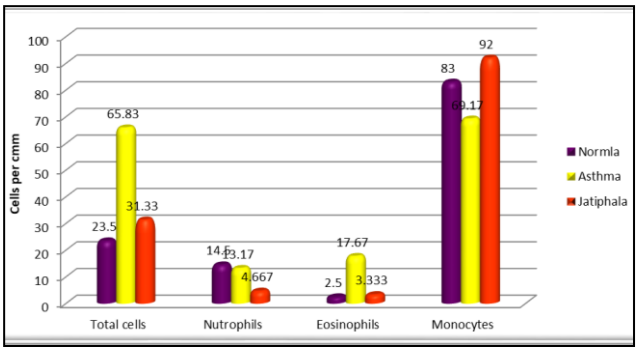
Graph 7: Showing Immunoglobulin E (IgE) levels comparison of all groups

4.1. Broncho-Alveolar Lavage Fluid

Lung was lavaged with Phosphate buffer Saline with pH 7.4 for Total count, differential count. Results obtained are tabulated in table No. (7).

Table 7: Broncho-Alveolar Lavage Fluid Total count, differential count.

Parameters	Normal saline group Mean ± SD	Asthma control group Mean ± SD	*Jatiphala* treated group Mean ± SD
Total count	23.5± 2.121	65.83± 5.492	31.33 ± 4.082*
Neutrophils	14.5 ± 0.7071	13.17 ± 2.317	4.667 ± 1.211*
Eosinophilss	2.5 ± 0.7071	17.67 ± 1.862	3.333± 1.211**
Monocytes	83 ± 0	69.17± 3.601	92± 1.265**
* *P* < 0.0001 When compare to Asthma Group ** *P* <0.0001 When compared to Normal and Asthma Group			

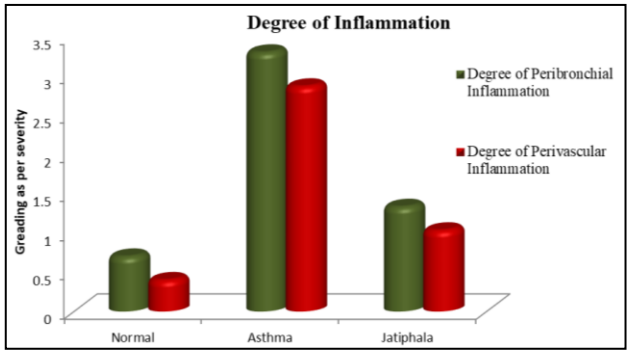


Graph 8: Showing Broncho-Alveolar Lavage Fluid Total count, differential count comparison between all the groups

4.2. Degree of Inflammation

Table 8: Showing degree of inflammation in BALF fluid

Parameters	Normal saline group Mean ± SD	Asthma control group Mean ± SD	*Jatiphala* treated group Mean ± SD
Peribronchial Inflammation	0.7 ± 0.1414	3.3 ± 0.7239	1.333 ± 0.4502
Perivascular Inflammation	0.4 ± 0	2.867 ± 0.6653	1.033 ± 0.4457
Test group showing significance at the level of P < 0.0001 with highly significant when compared with Asthma Group			



Graph 9: Showing degree of peribronchial and perivascular inflammation comparison between all groups

Fig 6: Retro orbital blood collection

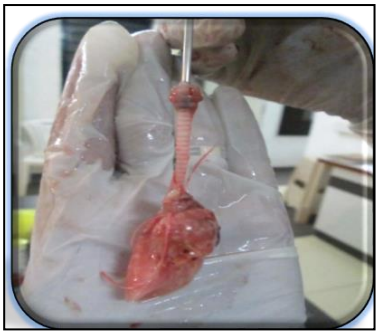


Fig 7: Lungs with Trachea

Fig 8: Collection of BALF

Fig 9: Spots observed on Lings

Figure 10: Redness seen on Lungs

5. Discussion

In the present study effect of *Jatiphala* in experimentally induced allergic asthma in rat was studied employing model of allergic asthma developed by Lu GP *et al.* [13]. This method was followed due to availability of all criteria needed for the study. Various parameters such as total count, differential count, IgE & absolute eosinophil count, as well as total count and differential count in BAL fluid were measured.

Sensitization was done with ova albumin and aluminium hydroxide injection by intra-peritoneal route (0.6 cc). After 15 days repeated exposure of 1% ova albumin PBS aerosols for 8 days resulted in induction of allergic asthma like condition as evidenced by raised serum IgE, Absolute eosinophils count, increased neutrophil and eosinophils count in BAL fluid and eosinophilslic infiltration into bronchial tissue when compared to normal saline group confirming the model executed by Sajida Abdureyim *et al.* [14].

Administration of *Jatiphala* reduced the allergic inflammatory markers such as serum IgE, AEC and infiltration of eosinophilsls at peribronchial and perivascular region in bronchial tissue (and BAL Fluid) as evidenced by histopathology results.

Clinical and experimental evidence suggesting that the imbalance between Th1 and Th2 leads to the manifestation of allergic disease including asthma [15] (Robinson DS *et al.*). The Th2 cytokines such as IL-4, IL-5, and TNF-*α* become overly abundant and this is seen to play a central role in the pathogenesis of allergic asthma. Antigen-induced IgE production, airway inflammation, and airway hyperresponsiveness have been well documented in patients with allergic asthma and in animal models [16] (Wills-Karp M *et al.*).

Th2 lymphocytes are the key orchestrators of this inflammation, initiating and propagating inflammation through the release of their cytokines. IL-4 induces IgE isotype switching in B lymphocytes and mucus production by goblet cells, as well as up-regulation of the expression of adhesion molecules required for inflammatory cell recruitment. The infiltration of eosinophils into the airways has been linked to the production of IL-5, which is important for eosinophil proliferation, activation and migration (Foster PS *et al.*). It has been suggested that eosinophils are key mediator of the pathology of bronchial asthma and contribute to tissue damage and airway inflammation. TNF-*α* is also an important chemo attractant for the recruitment of eosinophils into the lungs. This TNF-α is also a potent modulator of immune and inflammatory response.

Jatiphala has significantly reduced IgE (5.7± 0.97), a subtle marker of allergic inflammation and asthma when compared to Asthma control (15±1.09) and also it is to some extent comparable to historical data of standard group of dexamethasone (4.017± 0.68) (Patil S F *et al.* 2012). The Production of IgE is dependent on the Th2 cell activation and production of cytokine IL-4 finally activating B lymphocyte which then produces IgE. Hence we may speculate that *Jatiphala* may be acting by down regulating Th2 lymphocyte activation & its downstream signalling.

IgE is reduced significantly by *Jatiphala* (5.7± 0.97) when compared to normal saline group (9±1.02) hence one may suspect the diverse side effects could be possible by test drug hence further research on this line is undeniable.

Jatiphala significantly reduced Absolute eosinophils count (166.7 ± 64.55) when compared to Asthma control (475 ± 213.9) which is also marker of allergic inflammation & asthma.

Significant difference in results of BAL Fluid Eosinophils between *Jatiphala* group (3.333 ± 1.211) versus asthma control group (17.67 ± 1.862) was seen. IL-5, is important for eosinophils proliferation, activation, migration and infiltration of eosinophils into the airways. Hence we may assume that *Jatiphala* may be acting by inhibiting IL5 cytokine of Th2 lymphocyte activation & its downstream signaling.

Macelignan which is one of the phytoconstituent of *Jatiphala* has shown decreases activation of the T helper type 2 cell specific master transcription factor, also inhibits the secretion of histamine, prostaglandin E (2) and leukotriene C(4); decreased mRNA levels of cyclooxygenase2, IL4 IL13 and tumour necrosis factorα [17] (Han YS *et al.* 2012)

Increased in neutrophil is indication of severe persistent asthma and it is explained why severe asthma do not respond well to conventional asthma therapy, as neutrophilic airway inflammation may be resistant to corticosteroid treatment, where aseosinophilic inflammation is sensitive to steroids [18] (Keatings V. M. *et al.*)

Histological analysis of lung tissue for inflammation and cell infiltration into peribronchial and perivascular region showed highly significant difference ($P < 0.0001$) between asthma control (3.3 ± 0.7239) and Test drug *Jatiphala* group (1.333 ± 0.4502) Shown in Figure (11,12 & 13)

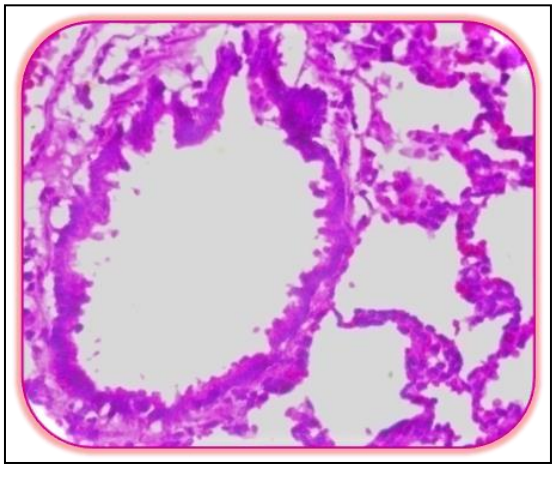


Fig 11: Normal Saline group

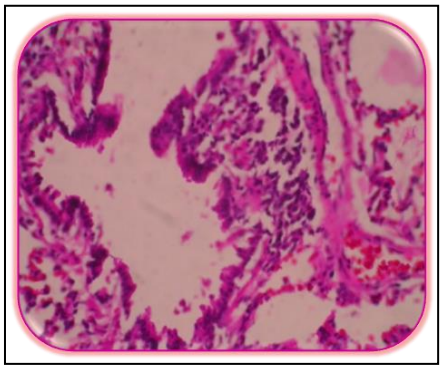


Fig 12: Asthma group

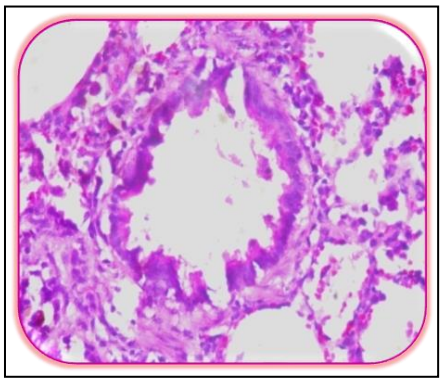


Fig 13: Jatiphala Group

The eosinophil count along with other parameters in BAL Fluid and circulation compared with histopathology they corroborate positive correlation confirming its anti-allergic activity.

Historical data of dexamethasone shows much decrease in weight on 23rd day than day one. The reason being glucocorticoids stimulate RNA and protein synthesis in the liver, they have catabolic and antianabolic effects in lymphoid and connective tissue, muscle, peripheral fat, and skin. Supraphysiologic amounts of glucocorticoids lead to decreased muscle mass and weakness and thinning of the skin (Bertram G. Katzung *et al*. 2009) whereas in test group there is no much variation found in food intake of all animals.

By streamlined results of IgE, AEC, BAL Fluid eosinophil and histopathology of lung tissue results given by *Jatiphala* group in comparison with asthma control group along with the recent researches of *Jatiphala* in regards to anti-inflammatory, anti-allergic and immune-modulatory, anti-microbial it may be assumed that the test drug is anti-allergic anti-inflammatory and immune-modulatory but at same time it is not suppressing the first line of defence which shows it may have advantage over the standard drug.

6. Conclusion

OVA challenge effectively produced allergic asthma symptoms in both groups. *Jatiphala* internal administration reduced the allergic inflammatory markers such as circulating serum IgE, AEC, and infiltration of eosinophills at peribronchial and perivascular region in bronchial tissue (and BAL Fluid) as evidenced by histopathological results, compared to asthma control group which was induced by OVA.

IgE is reduced significantly by *Jatiphala* hence one may suspect the varied side effects could be possible by test drug hence further research on this line is indisputable. Biochemical & Histological evidences from this study support the effectiveness of *Jatiphala* in allergy induced Asthma.

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