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# EVALUATION OF ANTI-HISTAMINIC ACTIVITY OF CHAUSATH PRAHARI PIPPALI IN GUINEA PIGS

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## ABSTRACT

Numerous formulations recommended in Rasashastra texts for allergic diseases including bronchial asthma. Rasaushadhis have a longer shelf life, a lower dose, a quicker action than herbal formulations.[1] *Chausath Prahari Pippali* is a formulation indicated in various conditions like *Shwasa* and *Kasa* as per the reference of *Ayurveda Sarasangraha.*[2] It is a potent herbal remedy for both respiratory and digestive disorders. It is prepared by *Mardana* of *Pippali churna* with *Gaja Pippali Phanta* (hot infusion) for 64 *Prahara* (192hr) to make it concentrated and efficient formulation. Physico-chemical, phytochemical, and chromatographic studies of the sample is carried out as per standard protocol. According to *Ayurveda Sarasangraha* this is a drug of choice for the Shwasa. Hence, an attempt is made to validate the anti – histaminic activity of *Chausath Prahari Pippali in Guinea Pigs.*

**KEYWORDS:** *Prahara*, Anti-Histamine, *Pippali*, *Gaja Pippali.*

## INTRODUCTION

Globally the incidence of allergic disorders is increasing alarmingly in recent decades. The allergic disorders especially asthma, allergic rhinitis affects the mankind with diversified clinical manifestation and threatening the quality of human life to a great extent. Bronchial asthma is a global health problem as per WHO which affects all age groups. About 250 – 300 million people suffer worldwide.[3] Deaths from this condition have reached over 3, 83,000

annually.[4] Unfortunately in spite of spending millions for effective anti-histamine drugs, safety and bio-medicine suffers major drawback in comprehensive allergic control. Hence, there is great scope for *Ayurveda* to offer safe and effective allergy management. *Ayurvedic* pharmaceutics are one of the fastest growing sectors in the world market. Globalization expects pharmaceutical standardization. Competition in pharma industry requires good quality products which have documentation regarding safety and efficacy issues. Ancient principles blended with current updated pharmaceutical technology helps in better appreciation. *Chausath Prahari Pippali Churna* is a classical *Ayurvedic* formulation mentioned in *Ayurveda Sarasamghraha*[2], a renowned text of Ayurveda, which is useful in *Vataja* and *Kaphaja* diseases, *kasa*, *shwasa* etc., It is a potent herbal formulation for both respiratory and digestive disorders. It is prepared by *Bhavana/Mardana* with *Phanta* (Hot infusion) of *Gaja pippali* to *pippali churna* for 64 *Prahara*$^2$(192hr) to make it more Potent and efficient formulation.

## METHODOLOGY

### Preparation of test drug

In khalva yantra fine powder of 1 part *Pippali Churna* was taken, 1 part of *Gaja Pippali Phanta* was added to it and triturated for 64 *Prahara*(192hrs) and stored in air tight container.[2] Every time fresh *Gaja Pippali Phanta* was added when *mardita churna* gets dried.

## EXPERIMENTAL STUDY

### Experimental animals

30 healthy adult guinea pigs of either sex, weighing between 350 and 400g, were obtained from the Bengaluru Biogen research animal facility. According to OECD norms, they were fed and housed. The animals were randomly selected and kept in a cage for 5 days before dosing to allow for acclimatization to the laboratory conditions. The Institutional Animal Ethics Committee (IAEC) provided the animal protocol with reference number PESCP/IAEC/136/2022. The experimental investigation was conducted at Bengaluru's PES College of Pharmacy.

### Dose of the Standard and Trial drugs[4]

- Based on various research publications available, the dose of standard drug Chlorpheniramine maleate was fixed as 2mg/kg body weight of guinea pigs.

- Human dose of the trial drugs were converted to animal dose based on standard dose converting formula.
- The human dose of CPP – 250mg/ day (45/ 60kg =5.55 mg/kg bw)
- Guinea pig (mg/kg bw) = Human dose (mg/kg bw) x $K_m$ ratio
- Guinea pig dose= 5.55 mg x 4.6 = 25.5mg/kg bw po is the medium dose.

**Study Design**

The effect was assessed by.

a. Histamine induced Broncho-constriction in guinea pigs.
b. Haematological evaluation.
c. Lung tissue parameters & Histo-pathological examination.

**Procedure**

**a. Histamine induced Broncho-constriction in guinea pigs**

Histamine was induced in II, III, IV, and V groups by histamine inhalation for 7 mins at the dose of 0.2% on day one in a modified histamine chamber. The flow rate of the nebulizer was set at 0.2ml/min. The animals were observed episodes of pre convulsive dyspnoea (PCD). The Guinea pig which exhibited respiratory symptoms were considered for the study. Treatment was provided according to study plan.[5]

30 Guinea pig are randomly grouped into 5 groups (n=6). Group I (NS) was treated with normal saline; Group II (S) was sensitized with Histamine; Group III was sensitized and treated with *Chlorpheniramine (CPM), Group* IV and V were sensitized by Histamine and treated with *CHAUSATH PRAHARI PIPPALI* using solvent as water and honey respectively.

**Group 1**- Normal Vehicle **Group 2**- Disease Control

**Group 3**- Standard (chloropheniramine maleate) **Group 4**- *Chausath Prahari Pippali*

**Group 5**- *Chausath Prahari Pippali* + honey.

**Table No. 1: Showing study design.**

| Group No | Group name | Treatment and dose | Frequency | Duration |
|---|---|---|---|---|
| I | Normal | Water *ad libitum* | Daily (7 days) | 21 days |
| II | Disease control (Histamine) | 0.2% w/v Histamine in D.w (Aerosol inhalation using nebulizer) | 1 day | 1 day |
| | | Vehicle (*anupana*)/ water | Daily | 21 days |
| III | Standard | 0.2% w/v Histamine in | 1 day | 1 day |

| | | | | |
|---|---|---|---|---|
| | Chlorpheniramine maleate | D.w (Aerosol inhalation using nebulizer) | | |
| | | Chlorpheniramine maleate 2mg/Kg.Oral | Daily | 21 |
| IV | *Chausath Prahari Pippali* | 0.2% w/v Histamine in D.w (Aerosol inhalation using nebulizer) | 1 day | 1 day |
| | | *Chausath Prahari Pippali* (25mg/kg body wt) Oral | Daily | 21 days |
| V | *Chausath Prahari Pippali* with *Anupana* | 0.2% w/v Histamine in D.w (Aerosol inhalation using nebulizer) | 1 day | 1 day |
| | | *Chausath Prahari Pippali* (25mg/kg body wt) Oral | Daily | 21 days |

**Measurement of allergic response-** On the 24$^{th}$ day, they were fasted overnight. The following day, the animals in each group were exposed to 1% w/v histamine aerosol using a nebulizer at a pressure of 300 mmHg in an air-tight Plexiglas chamber (24 × 14 × 24 cm). This caused progressive signs of difficulty in breathing leading to asphyxia. The time of onset of asphyxia was recorded. The time taken for each animal to fully recover was also measured as the recovery time (RT).The percentage protection offered by the treatment against asphyxia was calculated, using the formula.

$$\text{Percentage protection} = [(T_2 - T_1)/T_2] \times 100$$

Where $T_1$ is the time of onset of asphyxia before drug treatment

$T_2$ is the time of onset of asphyxia after drug treatment

Then the results were subjected to statistical analysis.

**b. Haematological Evaluation**

**Blood sampling-**At the end of the study (after 24h of the last day treatment) the blood was withdrawn from the lateral saphenous vein. Restrain the guinea pig and extend the hind leg downward while applying gentle pressure immediately above the knee joint to provide stasis. Puncture the vein using a 21G needle or lancet. The leg was gently manipulated to aid the blood flow, 0.5ml of blood was collected in an EDTA tube and used for the estimation of total WBC count and differential leucocyte count (DLC) using an automated hematological analyzer.

**c. Evaluation of Lung Tissue Parameters**

**Lung tissue isolation**

- The animals were euthanized by overdose anaesthesia using ketamine (120mg/kg bw ip).

- The lungs were isolated carefully and flushed with phosphate buffer solution.
- A known quantity of lung tissue was homogenized using homogenizer, then the content was transferred into eppendorff tubes and centrifuged at 10,000 rpm for 10 min at 4°C using Remi micro centrifuge.
- The clear supernatant was used for the estimation of total protein content, and for the oxidative stress markers – lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase.
- The bronchial tissues were collected from the euthanized animals and the portion of the lung tissue was placed in 10% formaldehyde-in saline solution.
- The tissues were subsequently processed and stained with haematoxylin and eosin dyes. Histological slides were examined using Olympus optical microscope.

## OBSERVATION AND RESULTS

**Table No. 2: Showing percentage protection against histamine induced allergy in normal and treated guinea pigs.**

| Group | | PCD in sec on 21st day(1hr) | PCD in sec on 21st day(24hr) | % of inhibition(1hr) | % of inhibition(24hr) |
|---|---|---|---|---|---|
| 1 | NC | - | - | - | - |
| 2 | DC | 69±6.444 | 81.33±4.84 | 25.11±5.79 | 4.49±2.94 |
| 3 | STD | 319.33±22.49 | 100.33±8.68 | 75.81±2.162 | 21.13±10.087 |
| 4 | CPPW | 82.83±6.554 | 69.83±4.86 | 5.028±8.256 | 11.76±9.749 |
| 5 | CPPH | 56.66±5.559 | 66.16±5.016 | 30.089±11.39 | 9.048±6.441 |

*NC – Normal control, DC – Diseased control, STD – Standard (Chlorpheniramine maleate) CPPW – *Chausath Prahari Pippali* with water, CPPH – *Chausath Prahari Pippali* with honey.

**Table No. 3: Showing effects on WBC count in normal & treated guinea pigs.**

| Group | | WBC |
|---|---|---|
| 1 | NC | 25257.83±1461.30 |
| 2 | DC | 26855.17±1553.71 |
| 3 | STD | 9633.91±557.37 |
| 4 | CPPW | 13078.17±756.64 |
| 5 | CPPH | 7038.25±407.20 |

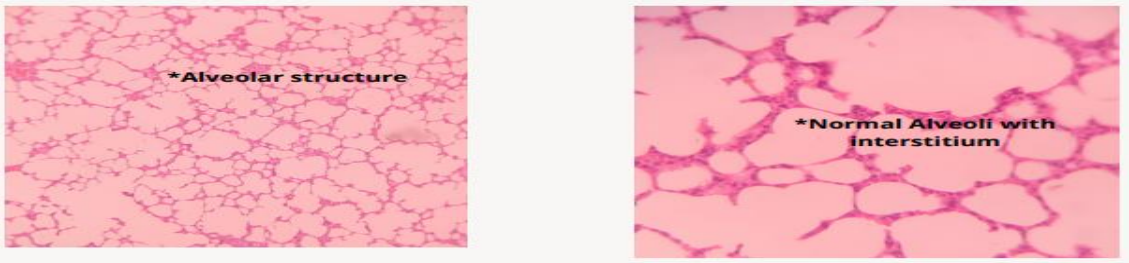
**Table No. 4: Showing effects on Differential Leucocyte count in normal and treated guinea pigs.**

| Group | | Neutrophils | Eosinophil's | Monocytes | Lymphocytes |
|---|---|---|---|---|---|
| 1 | NC | 61.56±3.90 | 3.90±0.248 | 4.886±0.310 | 27.36±1.7373 |
| 2 | DC | 55.70±3.536 | 6.840±0.434 | 3.90±0.248 | 31.27±1.985 |
| 3 | STD | 61.56±3.908 | 3.90±0.248 | 3.90±0.248 | 28.340±1.799 |
| 4 | CPPW | 61.56±3.908 | 3.90±0.248 | 4.886±0.310 | 27.36±1.7373 |
| 5 | CPPH | 61.568±3.908 | 4.886±0.310 | 3.90±0.248 | 27.36±1.7373 |

**Table No. 5: Showing effects on lung tissue parameters in normal and treated guinea pigs.**

| Group | | SOD(U/mg protein) | Catalase(U/mg protein) | LPO(U/mg protein) | GSH(U/mg protein) |
|---|---|---|---|---|---|
| 1 | NC | 257.20±21.66 | 0.068 ±0.089 | 58.45±10.60 | 0.265 ±0.0059 |
| 2 | DC | 174 ±20.64 | 0.017 ±0.059 | 108.00±10.033 | 0.129 ±0.011 |
| 3 | STD | 216.81 ±8.54 | 0.027 ±0.118 | 22.30±4.51 | 0.201 ±0.0075 |
| 4 | CPPW | 147.00 ±8.39 | 0.0121 ±0.086 | 43.17±16.83 | 0.128 ±0.0134 |
| 5 | CPPH | 103.37 ±9.44 | 0.029 ±0.073 | 17.86±2.855 | 0.161 ±0.006 |

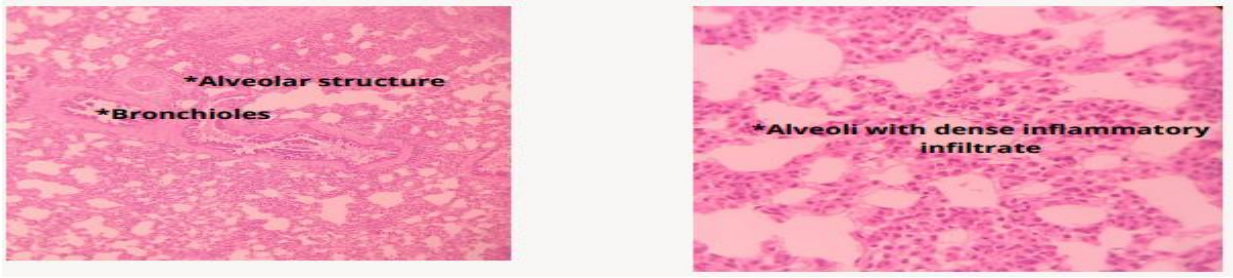
**HISTOPATHOLOGICAL EXAMINATION**




**H&E 50X** **H&E 400X**

**Fig No. 1: Group 1- Normal Control.**

- No significant change observed.




**H&E 50X** **H&E 400X**

**Fig No. 2: Group 2- Histamine induced 0.2%(disease control).**

- Samples show significant alveolar inflammation showing with dense interstitial

inflammatory infiltrate with alveolar wall thickening, exudation.

- Inflammatory infiltrate is composed of predominantly neutrophils as well as macrophages and few lymphocytes. Interstitium show edema and congested blood vessels.
- Peribronchial expansion with inflammatory infiltrate seen.
- Alveolar space show degenerated cells, proteinaceous debris and erythrocytes.

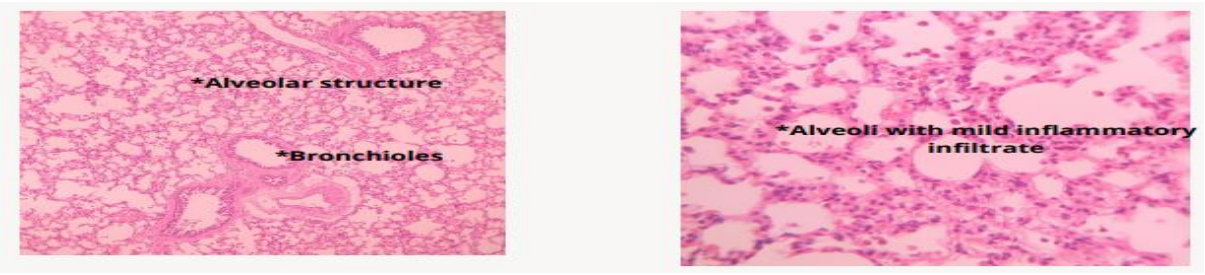



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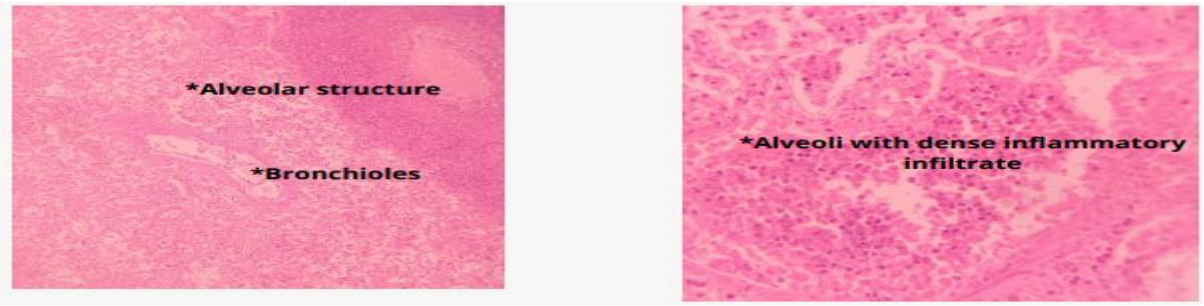
**Fig No. 3: Group 3- Standard control - Histamine induced & Chlorpheneramine treated. 2mg/kg body weight**

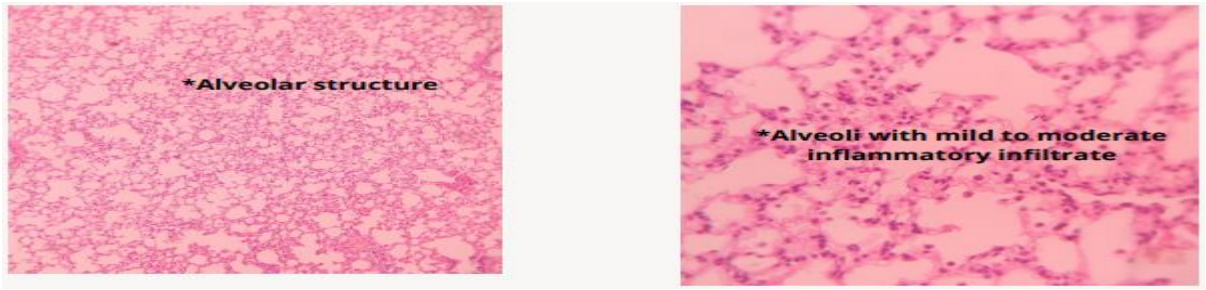
- Show alveolar structures with thickened septa and mild mixed inflammatory infiltrate composed of few neutrophils as well as macrophages and few lymphocytes
- Interstitium show edema and congested blood vessels




**H&E 50X** **H&E 400X**

**Fig No. 4: Group 4- Histamine induced & *Chaustath prahari pippali* treated oral dose 25mg/kg.**

- Show significant alveolar inflammation showing with dense interstitial inflammatory infiltrate with alveolar wall thickening, exudation
- Inflammatory infiltrate is composed of predominantly neutrophils as well as macrophages and few lymphocytes
- Interstitium show edema and congested blood vessels
- Alveolar space show degenerated cells, proteinaceous debris.




**H&E 50X** **H&E 400X**

**Fig No. 5: Group 5- Histamine induced & Chaustath prahari pippali treated oral dose 25mg/kg with honey.**

- Show mild alveolar inflammation showing with moderate interstitial inflammatory infiltrate with mild alveolar wall thickening.
- Inflammatory infiltrate is composed of few neutrophils, macrophages and few lymphocytes
- Interstitium show congested blood vessels.

## DISCUSSION

### Discussion on Experimental model

Guinea pigs were used in the present study because to their sensitivity and close anatomical and physiological relationship with the tracheal and bronchial musculature. Compared to other frequently utilised species, guinea pig receptor pharmacology is more similar to human receptor pharmacology.

### Discussion on Efficacy of test drugs on Histamine induced bronchospasm in guinea pigs

- There was not much significant response in trial groups IV, V compared to III (standard) i.e., delayed onset of pre-convulsive dyspnoea time (PCT) was seen.
- The group 3 standard control have a significant more inhibitory action with 75.81±2.162 percentage when compared with group 2 disease control 25.11±5.79. The group 4-test +water have a significantly less inhibitory action and group 5-test + honey have a significantly more inhibitory action with 5.028±8.25 and 30.089±11.39 respectively when compared with disease control.
- *Chausath Prahari Pippali* + honey showed better protection than *Chausath Prahari Pippali* + water and difference between them is significant, substantiating the claim of *acharyas* that *anupana/sahapana* potentiates the action of main drug and possibly counteracts possible ill effects.

**Discussion of Effects of test drugs on Haematological parameters**

**WBC count**

- Significant decrease in WBC count was observed in group IV and V when compared to group II and group III.
- Increased WBC count can indicate Infection, inflammation (possibly from allergies), tissue damage or stress.[6] Significantly *Chausath Prahari Pippali* (with and without vehicle) may have blocked the effect of histamine, hence the WBC count remained normal.

**Lymphocyte Count**

- Significant decrease in lymphocyte count was observed in group IV and group V.
- Increased numbers of lymphocytes are seen in Infections and inflammation, and *Chausath Prahari Pippali* (with and without vehicle) may have blocked the infection, hence the count is low in Group IV and V.

**MID Count**

- MID collectively refers to the number of Monocytes, Eosinophils and Basophils.
- Significant decrease is seen in Group III, IV and V animals (CPP) when compared to group II animals (diseased control).
- These cells respond to inflammation, infection and foreign bodies by ingesting and digesting the foreign material.

**Discussion on Effects of test drugs on Lung tissue parameters**

**Lipid peroxidation**

- The guinea pigs of group III, IV, and V have shown a significant decrease in malonaldehyde levels ($p<0.001$) when compared with group II animals.
- Lipid peroxidation is oxidative damage that affects cellular membranes, lipoproteins, and other molecules that contain lipids in conditions with oxidative stress, which may aid in monitoring the development and extent of pulmonary damage.
- Lipid peroxidation often precedes irreversible cell damage, being an early cause of cell death[7], the results showed there was damage of lung tissue in Group II(Control)

**Glutathione**

- The guinea pigs of group III, and V have shown significant increase in glutathione levels ($p<0.001$) when compared with group II and IV animals.

- GSH is a key intracellular reducing agent it is one of the fundamental anti-oxidant defense mechanisms in oxidant-induced lung injury and inflammation.[8] Decreased GSH count in Group II and IV was observed which shows there was lung tissue damage.

**Superoxide Dismutase**

- The guinea pigs of group IV, and V have shown a non-significant increase in SOD levels ($p<0.001$) when compared with group II and III animals.
- The superoxide dismutase (SOD) are a family of enzymes that play a pivotal role protecting tissues from damage by oxidant stress by scavenging superoxide anion, which prevents the formation of other more potent oxidants such as peroxynitrite and hydroxyl radical.
- In addition SOD prevents the accumulation of neutrophils in the focus of inflammation which secrete significant amount of lysosome enzymes that destroy nearby tissues.[9]
- The SOD count was decreased in group IV and V, which may indicate there was Oxidative stress

**Catalase**

- The guinea pigs of group III, and V have shown a significant increase in catalase levels ($p<0.001$) when compared with group II and IV animals.
- Catalase, plays a central role in the antioxidant screen of the lungs by virtue of its ability to convert hydrogen peroxide to oxygen and water. Hydrogen peroxide is a harmful byproduct of many normal metabolic processes; to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances.[10] Group II and III showed decreased catalase which showed there was damaged lung tissues.

**Discussion on Histopathology of Lung Tissue**

By comparing all the histo-pathological slides, significant regression of disease in group III, IV and group V is seen when compared to group II. But complete regression of the disease, both in trial and standard drug was not seen. Therefore it can be interpreted that it was necessary to continue the drugs for another few days and check for complete regression of disease through histopathology.

**Discussion on phytochemical analysis**

- All the ethanolic extracts were subjected to preliminary phytochemical screening,
- All the samples showed positive results for Phytosterols, and Phenols but negative for

Alkaloids, Tannins, Flavanoids.

- The change in array of Colours during Phytosterols Screening increases from *Chausath Prahari Pippali* at 16 *prahara* to 64 *prahara* which indicates increased in concentration of sterols.
- This validates that phytosterols and phenols both have anti-inflammatory, anti-oxidant in action which helps in proving *Chausath Prahari Pippali* has a significance role in anti-histamine action.

**PROBABLE MODE OF ACTION OF *CHAUSATH PRAHARI PIPPALI***

The barrier caused by *kapha* in *Tamaka Shwasa* causes the *Vayu* to become vitiated from its normal state. Therefore, to remove restriction in *strotas*, the therapeutic principles used should normalise *Vata* and *Kapha* and promote Agni. Except for the indications, there is no mention of the rasa, *guna, virya*, etc., of this formulation in the current research of *Chausath Prahari Pippali*. The *rasa, guna, virya*, etc. that were described for individual medicines as well as for groups of substances must therefore be taken into account. To understand this scientifically we should consider the related basic fundamental, that are described in the classics as.

i. The drugs are active due to their own inherent constituents (*Dravya Prabhava*), properties (*Guna prabhava*) together in particular time, on reaching particular site, with a particular mechanism and objective.

ii. The different properties of a drug are inferred by observing their effects on the body. Therefore keeping the above views in mind, we can establish the possible properties, actions and mode of action of our trail drugs by observing its pharmacological effects.

**By Virtue of its *Rasa***

***Katu Rasa***

- *Pippali and Gaja pippali which are main ingredients* present in *Chausath Prahari Pippali* has *Katu rasa*.
- *Katu rasa* is predominant in *vayu* and *agni mahabhoota* which have the tendency to act with upward direction.
- *Katu rasa* also acts as *Shothahara* (anti-inflammatory), *kandu vinashana* (anti-histamine), *krimin hinasthi* (anti-microbial), *margan vivrunoti* (broncho dialator), *kleda-kapha-malanupahanti* (eradicates excessive respiratory secretions, expectorant).
- It combats the *phena mala kaphautpatti* (excessive secretions) in the

*amashaya*(stomach).[11]

**By virtue of its *Guna***

***Teekshna Guna***

- *Pippali and Gaja pippali* has *teekshna guna* (penetrative quality),
- *Teekshna guna has agni mahabhoota* dominanace which is *kaphagna*[12](mucoltic-mucokinetic) and *Shodhaka* (expectorant effect) and are very rare essential pharmacological activities in treating *Kasa and shwasa roga*.

**Laghu & Snigdha guna**

- *Pippali and Gaja pippali* has *laghu & snigdha guna*
- *laghu guna* has tendency to increase *vata dosha* but due *to snigdha guna* it may not increase *vata,* so maintains normalcy *of dosha* and vice versa for *snighda guna &kapha dosha.*

***Yogavahi Guna***

- *Yogavahi guna of Pippali* accounts for quick absorption and easy transportation of the drug into cell membrane.

**By virtue of *Virya***

***Ushna virya***

- *Pippali and Gaja pippali* has *Ushna veerya* (hot potency)
- Helps in *pachana* (digestive), *swedana* (diaphoretic), *vilayana* (Iiquification) and as potent *vatakapha shamaka* property.[13] ie, it eradicates and reverses the pathophysiology of shwasa roga, by its mucolytic, expectorant, *kaphagna* (reduces phlegm), *lekhana* (scraping) and *ropana* (healing) property.
- *Ushna virya* liquefies *kapha* and remove *sroto avarodha*. Once *sroto avarodha* is removed, it makes *vatanulomana*, hence decreases *ati-pravritti* of *shwasavega* and normalises the function of *pranavaha srotas.*

**By Virtue of its *Karma***

***Deepana* and *Pachana***

- *Pippali and Gaja pippali* has *Deepana & Pachana guna.*
- Origin of *Shwasa* is from *Aamashaya* which is *pittasthana* and correction of *pitta* or *agni* is done by *Deepana guna.*

**Rasayana**

- *Pippali* is Immunological stabilizer of respiratory system hence is a respiratory *rasayana* most useful in all treatable cases of *Shwasa roga.*

**By Virtue of *Doshgnata***

***Vata-Kaphagna* properties**

- *Pippali and Gaja pippali* has *Vata-Kaphagna* property.
- *Samana vata dushti* in respiratory passage causes *kapha* vitiation and obstruction to *prana.*
- Hence treatment principle is removing the *malaroopi kapha* from *pranavaha srotas,* thereby clearing the passage of *prana* and normalising *samana vata* in *pranavaha srotas*
- *Vata-Kaphagna* property helps in removal of *kapha* and normalizing *vata*.

In total, all the ingredients of *Chausath Prahari Pippali* is synergistically a potent fast acting drug having *Vatakaphahara, Vatanulomaka, Marga Vivarana(Bronchodiaiator), Deepana, Pachana, Jwargna,* Krimigna,, *Kasa Shwasahara, Rasayana* effect. Hence *Chausath Prahari Pippali* is found to be promising drug in the treatment of *shwasa.*

## CONCLUSION

- In Histamine induced allergic response, *Chausath Prahari Pippali* showed significant response i.e, guinea pigs of group III, IV, and V have shown significant percentage protection when compared to group II.
- *Chausath Prahari Pippali* with *anupana (honey*) showed better response than *Chausath Prahari Pippali* plain, which is statistically significant

Lung tissue parameters showed

- Increased levels of glutathione and catalase in group III and V when compared to group II and IV which are fundamental anti-oxidant defence mechanisms in oxidant-induced lung injury and inflammation.
- The guinea pigs of group III, IV, and V have shown a significant decrease in lipid peroxidation level when compared with group II animals, which often precedes irreversible cell damage, being an early cause of cell death.

With the factual evidence obtained by experimental data, it has been concluded that *Chausath Prahari Pippali* is an effective Anti-Histaminic drug and *Anupana* augments Drug **action.**

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