AYURPUB.com
INTERNATIONAL AYURVEDA PUBLICATIONS

RESEARCH ARTICLE *ISSN 2456-0170*

COMPARATIVE EXPERIMENTAL STUDY OF MASTCELL STABILIZING ACTIVITY OF NAVA PIPPALI AND PURANA PIPPALI (*PIPER LONGUM* LINN.)

[1]Dr Rajendra H M [2]Dr Meenal D Lad

[1]Assistant Proffesor, Sri Jayendrasaraswathi Ayurveda College & Hospital, Chennai, Tamil Nadu, [2]Proffesor & HOD,Dept. of Dravyaguna Vigyan, College of Ayurved & Research Centre, Akurdi, Pune, Maharashtra, India.

ABSTRACT

Ayurveda, the ancient traditional system of medicine mentions various concepts, which is needed to explore and revalidate them through scientific parameters for better understanding and thereby extending its scope of utility. Among these, one of the concepts is mentioned in Sharangdhara Samhita which states that the drugs are to be used in Nava (fresh form) only except few drugs like Pippali (*Piper longum* Linn.) etc. should be used as Purana (old form). Hence there is necessary to revalidate the concept through scientific parameters by evaluating the Qualities of Nava Pippali (Fresh form) and Purana Pippali (Old form). Many inflammatory cells are known to be involved in asthma with no key cell that is predominant. Among them mast cells are important in initiating the acute broncho-constrictor responses to allergens and several other indirectly acting stimuli such as exercise and hyperventilation, as well as fog. Hence Antiasthmatic activity is chosen on Animal models in which mastcell stabilizing activity is selected in order to evaluate the effect of Nava and Purana Pippali on Bronchial Asthma. Studies showed that Purana Pippali significantly protects compound 48/80 induced degranulation of mast cell when compared to Nava Pippali, which is responsible for decreasing airway inflammation by preventing release of various inflammatory mediators.

KEYWORDS: Nava pippali, Purana Pippali, Bronchial asthma, Mastcell degranulation.

INTRODUCTION

Ayurveda, the ancient traditional system of medicine mentions various concepts, which is needed to explore and revalidate them through scientific parameters for better understanding and thereby extending its scope of utility. Among these, one of the concepts is mentioned in *Sharngadhara Samhita* which states that all the plant drugs are to be used in *Nava* (fresh form) only except few drugs like *Vidanga* (*Embelia ribes*), *Krishna* (*Piper longum Linn.*), *Guda* (Jaggery), *Dhanya* (Cereals), *Ajya* (Ghee), *Makshika* (Honey) should be used as *Purana* (old form). *Pippali* which is the synonym of *krishna* is one among these drugs which should be used as *Purana* (old form) only[1]. *Adhamalla's Dipika,* commentary on *Sharngadhara Samhita* mentions that *Vidanga (Embelia ribes), Pippali* etc. drugs

if used in old form will be of good Quality/Potent and these drugs must be used one year old only[1]. Quality of a drug is given much importance in order to achieve its therapeutic efficacy[2].

So there is necessary to revalidate the concept through scientific parameters by evaluating the Qualities of *Nava Pippali* (Fresh form) and *Purana Pippali* (Old form).

As per the classical literatures, *Pippali* is widely used in *Shwasa*[3](Bronchial asthma) and maximum formulations used in this disease contain *Pippali* as one of the ingredient, which indicates its importance in alleviating the disease. *Shwasa* can be considered as Bronchial Asthma as one of the entity under *Shwasa***.** Among several respiratory diseases affecting human, bronchial asthma is the most common disabling syndrome. The morbidity and mortality of the disease is increasing and making it a global concern. Asthma is one of the most common chronic diseases globally and currently affects approximately 300 million people worldwide. In developing countries where the prevalence of asthma had been much lower, there is a rising prevalence, which is associated with increased urbanization[4].

Many inflammatory cells are known to be involved in asthma with no key cell that is predominant. Among them mast cells are important in initiating the acute broncho-constrictor responses to allergens and several other indirectly acting stimuli such as exercise and hyperventilation, as well as fog. Activated mast cells are found at the airway surface in asthma patients and also in the airway smooth muscle layer. Mast cells release several broncho-constrictor mediators, including histamine, prostaglandin D_2 and cysteinyl leukotrienes, but also several cytokines, chemokines, growth factors and neutrophins.

Hence Antiasthmatic activity is chosen on Animal models in which mastcell stabilizing activity is selected in order to evaluate the effect of *Nava* and *Purana Pippali* on Bronchial Asthma. As Biological evaluation of plant drugs is useful to determine pharmacological activity, potency and toxicity and moreover this is an important evaluation for drugs because by their biological effects, this evaluation will conclude the effect[5].

MATERIALS

Plant material:

Nava Pippali: Freshly collected fruits of *Pippali* (*Piper longum Linn*.)

The samples were dried in shade.

Purana Pippali: Freshly collected fruits of *Pippali* preserved for one year at room temperature.

All the samples were identified and authenticated by Agharkar Research Institute, Pune.

All these samples were standardized by Physicochemical and Phytochemical analysis.

Invivo Study:

Institutional Animal Ethics Committee approved the experimental protocol of *Piper longum* Linn. fresh fruit (*Nava Pippali*) with reference no. IAEC/XXXI/SRU/232/2012 and of one year old fruit (*Purana Pippali*) with reference no. IAEC/ XXXVI / SRU / 328/2013. The pharmacological work was carried out as per norms of CPCSEA

(Committee for the Purpose of Control and Supervision of Experiments on Animals).

METHODOLOGY

Test Drug: *Nava Pippali/Purana Pippali*

The overnight fasted male Wistar albino rats (180-220 g b. wt) were sacrificed with excess dose of anesthetic ether and the abdomen was cut open to expose the intestine. Pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out and cut into small pieces and placed in a beaker containing Ringer Locke solution for 30 min. Different dilutions of *Nava Pippali/Purana Pippali* samples (10, 30, 100, 300, 1000 µg/ml) were prepared in Ringer Locke solution. Then the tissues were incubated with Compound 48/80 (0.8 µg/ml) for a period of 30 min. The pieces of mesentery were then placed on a clean slide. Excess fatty layers and adhering tissues were carefully removed. The trimmed tissue was placed in 4% formaldehyde solution containing 0.1% O-Toludine blue for 20–30 min and the tissue was then de-stained with acetone and xylene (two changes each) for 5 min[6]. Three pieces of mesentery were used for each concentration of the test sample.

The stained mesentery pieces were examined under a digital light microscope at 100 x magnification and 100 mast cells were counted, starting from the left hand side of the field and then proceeding clockwise. The number of intact, fragmented or disrupted mast cells was counted. A mast cell was considered disrupted if four or five granules were observed around the mast cells. The percentage of fragmented or disrupted and intact mast cells was calculated[7]

Statistical analysis:

The results were reported as mean ± SEM and analysed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnett's '*t*'-test, for individual comparison of test samples with that of control. The analysis was carried out using Graph Pad Prism 4.0 Version.

OBSERVATIONS AND RESULTS

Nava Pippali

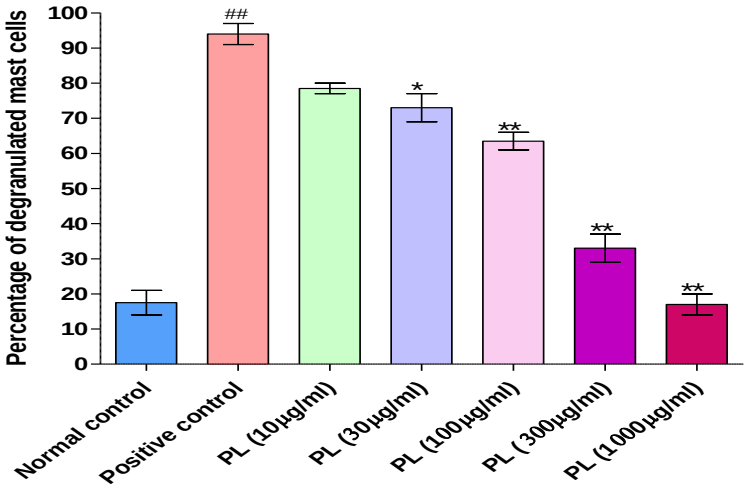
Table 1: Effect of *Nava Pippali* on Compound 48/80 induced Mast Cell Degranulation

S.No	Concentration (µg/ml)	% Degranulation
1	Normal control	17.50±3.50
2	Positive control (0)	94.00±3.00
3	PL-10	78.50±1.50
4	PL-30	73.00±4.00
5	PL-100	63.50±2.50
6	PL-300	37.00±4.00
7	PL-1000	17.00±3.00

n=3/group; i.e. experiments were performed in triplicates.

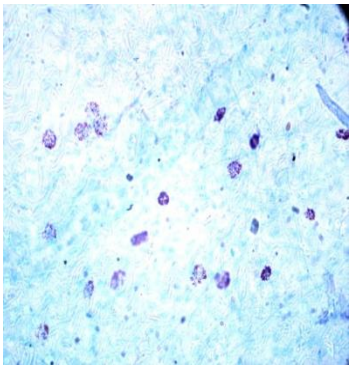
PL - *Piper longum Linn.* fruit (*Nava Pippali*).

Fig.1: Effect of *Nava Pippali* on Compound 48/80 induced Mast Cell Degranulation

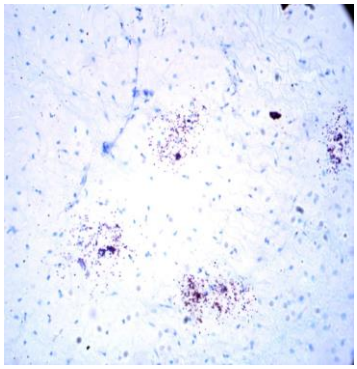


Each bar represents mean ± SEM (n=3). **p < 0.001as compared to positive control group.

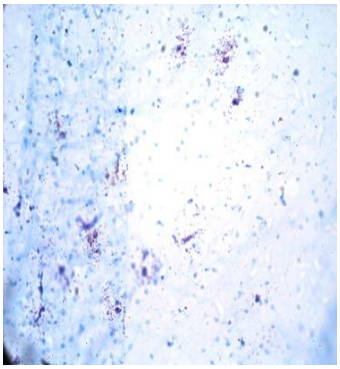
Fig.2: Mast Cell stabilization potential of *Nava Pippali*



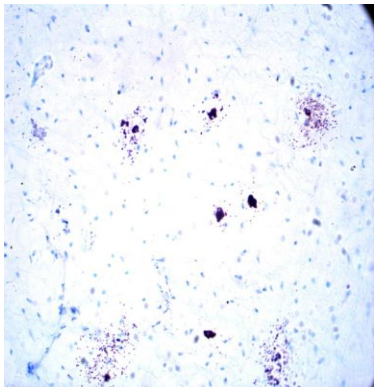
Normal Cells



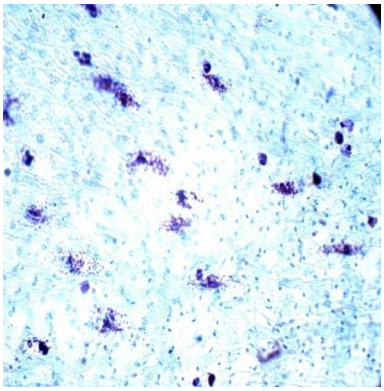
C 48/80 + Vehicle



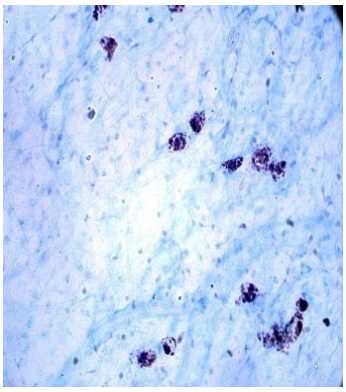
C 48/80 + PL 10µg/ml



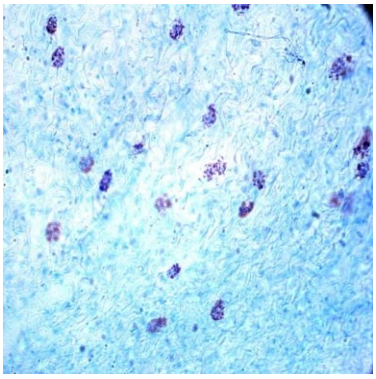
C 48/80 + PL 30 µg/ml



C 48/80 + PL 100 µg/ml



C 48/80 + PL 300 µg/ml



C 48/80 + PL 1000 µg/ml

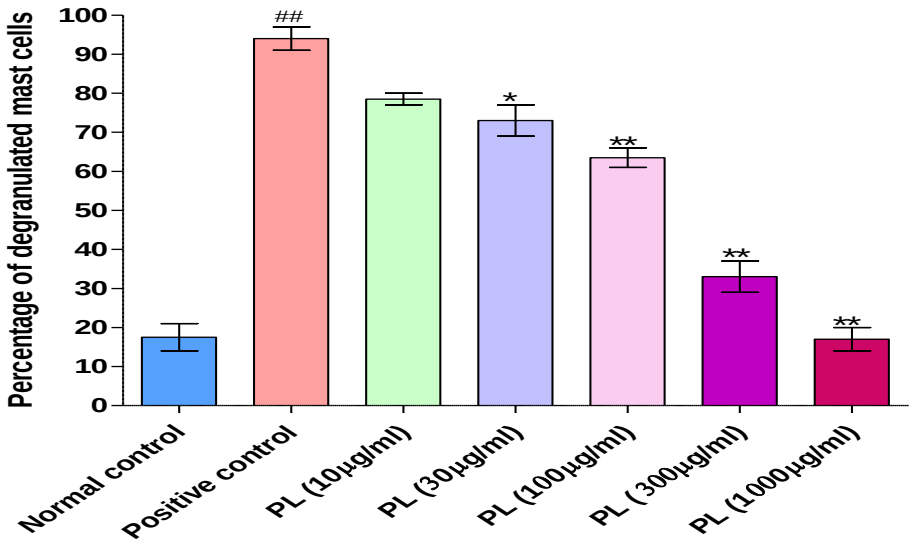
PL - *Piper longum Linn.* fruit (*Nava Pippali*).

4.2 *Purana Pippali*

Table 2: Effect of *Purana Pippali* on Mast Cell Degranulation

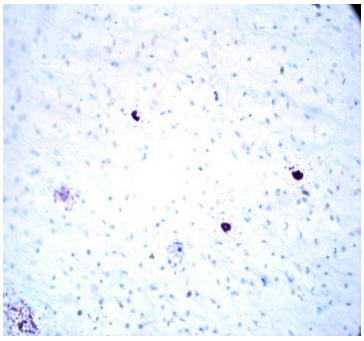
S No	Concentration (µg/ml)	% Degranulation
1	Normal control	12.33±1.20
2	Positive control (0)	96.67±0.88
3	PL-10	76.33±0.88
4	PL-30	70.33±2.73
5	PL-100	49.67±3.76
6	PL-300	33.67±1.86
7	PL-1000	14.67±0.33

Fig.3: Effect of *Purana Pippali* on Mast Cell Degranulation

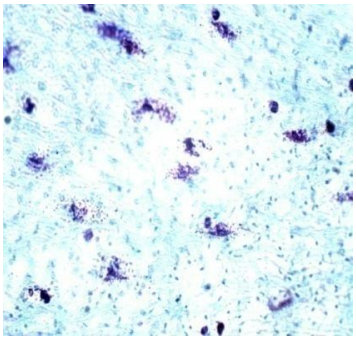


Each bar reprents mean ± SEM (n=3). **p < 0.001as compared to positive control group.

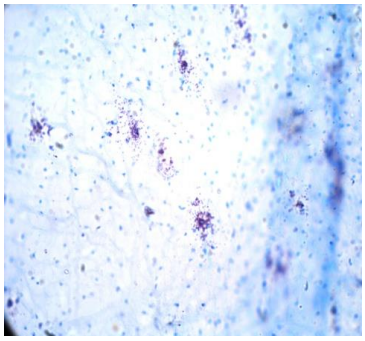
Fig.4: Mast Cell stabilization potential of *Purana Pippali*



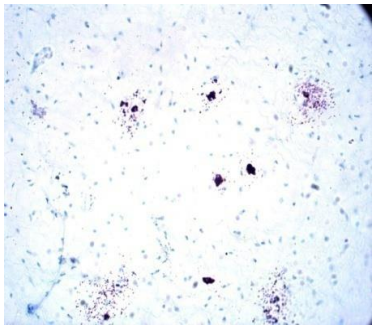
Normal Cells



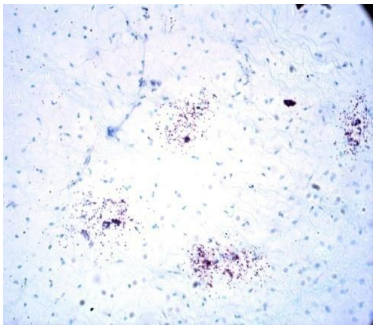
C 48/80 + Vehicle



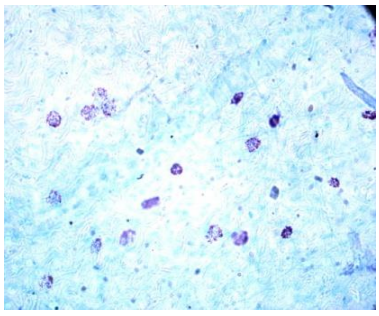
C 48/80 + PL 10µg/ml



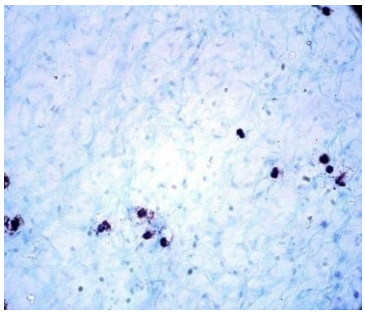
C 48/80 + PL 30 µg/ml



C 48/80 + PL 100 µg/ml



C 48/80 + PL 300 µg/ml



C 48/80 + PL 1000 µg/ml

PL - *Piper longum Linn.* fruit (*Purana Pippali*).

DISCUSSION

In Normal control, mast cell degranulation was found to be 17%.

In Positive control, Compound 48/80, a known mast cell degranulating agent produced significant rat mesenteric mast cell degranulation which was 94%.

Nava Pippali produced significant reduction in the Compound 48/80 induced mast cell degranulation in a dose-dependent manner. The % inhibition of mast cell degranulation of *Nava Pippali* at concentration of 10µg/ml, was found to be 78%, at concentration of 30µg/ml, mast cell

degranulation was found to be 73%, at 100µg/ml, mast cell degranulation was found to be 63%, at 300µg/ml, mast cell degranulation was found to be 37% and at concentration of 1000µg/ml, mast cell degranulation was found to be 17% respectively.

In Positive control, Compound 48/80, a known mast cell degranulating agent produced significant rat mesenteric mast cell degranulation which was 97%.

Purana Pippali produced significant reduction in the Compound 48/80 induced mast cell degranulation in a dose-dependent manner. The % inhibition of mast cell degranulation of *Purana Pippali* at concentration of 10µg/ml, was found to be 76%, at concentration of 30µg/ml, mast cell degranulation was found to be 70%, at 100µg/ml, mast cell degranulation was found to be 49%, at 300µg/ml, mast cell degranulation was found to be 33% and at concentration of 1000µg/ml, mast cell degranulation was found to be 14% respectively.

In this study, *Purana Pippali* significantly protects compound 48/80 induced degranulation of mast cell when compared to *Nava Pippali*, which is responsible for decreasing airway inflammation by preventing release of various inflammatory mediators.

SCOPE FOR FURTHER RESEARCH

Comparative study of *Nava Pippali* and *Purana Pippali* on Bronchial Asthma can be performed through Clinical trials.

Comparative study of *Nava Pippali* and *Purana Pippali* on other Pharmacological activities like Anti-inflammatory etc. can be done.

Evaluation of Quality and Potency of *Purana Pippali* in each successive year is to be assessed.

CONCLUSION

Purana Pippali is found to be more effective than *Nava Pippali* in antiasthmatic activity (Invitro mast cell degranulation by compound 48/80) as it showed significant protection against compound 48/80 induced mast cell degranulation by stabilizing it.

REFERENCES

1.Pandit Parasurama Sastri, Vidyasagar, Sharngadhara Samhita with the commentary of Adhamalla's Dipika & Kasirama's Gudhartha Dipika, Prathama Khanda, Chp 1, Sixth Edition 2005, Chaukhambha Orientalia, Varanasi, Page no.11.

2. Dr.Ramkaran Sharma and Vaidya Bhagwan Dash (editors) - Caraka Samhita, Vol I, Reprint 2012, Chaukhamba Sanskrit Series Office, Varanasi, page no.183 & 186.

3. Dr.G.S.Pandey, Bhavaprakasha Nighantu of Sri Bhavamisra, Reprint 2006, Chaukhamba Bharati Academy, Varanasi, page no.15.

4. Yash Pal Munjal (editor) - API Textbook of Medicine, Volume II, Ninth Edition 2012, The Association of Physicians of India, Mumbai, page no.1704-1710.

5.Biren.N.Shah and A.K.Seth - Textbook of Pharmacognosy and Phytochemistry, Second Edition 2014, Elsevier, a division of Reed Elsevier India Private Limited, New Delhi, page no.119.

6. Dnyaneshwar J Taur et al. - Mast Cell Stabilizing, Antianaphylactic and Natural Medicines 2011, 9(5): 359-362.

7. Gupta, et al. – Peritoneal mast cell stabilization potential of Pothos scandens L.,

Indian Journal of Pharmacology Feb 2013; 45: 83-6.

8. Vadnere GP, Rahul SS et al. - Studies on antiasthmatic activity of aqueous extract of Clerodendron Phlomidis. Pharmacology online 2007; 1, 487-494.

9.Priyashree S, Jha S – Bronchodilatory and mast cell stabilizing activity of Cressa cretica L:evaluation through invivo and Invitro experimental models, Asian Pac J Trop Med.2012 March;5(3):180-6.

10.Divya Kajaria, Tripathi JS – Antihistaminic, mast cell stabilizing and bronchodilator effect of hydroalcoholic extract of polyherbal compound-Bharangyadi, Ancient Science of Life 2012 Jan-Mar;31(3):95-100.

CORRESPONDING AUTHOR

Dr Rajendra H M,
Assistant Proffesor, Sri Jayendrasaraswathi Ayurveda College & Hospital, Nazarathpet, Chennai, Tamil Nadu,-India.
E-mail: ayurrajhm@gmail.com

Source of support: Nil,
Conflict of interest: None Declared

Cite this article as
Rajendra H M: Comparative Experimental Study of Mastcell Stabilizing Activity of Nava Pippali and Purana Pippali (*Piper Longum* Linn.) ayurpub 2017;II(2):406-413