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In-vivo Antiasthmatic Activity of *Nava Pippali* and *Purana Pippali* (Piper longum Linn.)

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Abstract

Ayurveda, the ancient traditional system of medicine mentions various concepts, that needs to be explored and revalidated 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).

So it is important to revalidate the concept through scientific parameters by evaluating the Qualities of *Nava Pippali* (Fresh form) and *Purana Pippali* (Old form). Evaluation of a drug ensures the identity of a drug and determines the quality and purity of drug.

Hence antiasthmatic activity is chosen on Animal models i.e., In vivo study in which histamine induced bronchospasm is selected in order to evaluate the effect of *Nava* and *Purana Pippali* on Bronchial Asthma. Studies showed that *Purana Pippali* is found to be more effective than *Nava Pippali* in antiasthmatic activity (Histamine induced bronchospasm) as it showed increased preconvulsion dyspnea time and maximum protection against histamine aerosol exposure.

Keywords

Nava pippali, Purana Pippali, Bronchial asthma, Histamine induced bronchospasm

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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* as:

नवान्येव हि योज्यानि द्रव्याण्यखिलकर्मसु।

विना विडङ्गकृष्णाभ्यां गुडधान्याज्यमाक्षिकै:॥[1]

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].

विडङ्गादि द्रव्यं विना तेन विडङ्ग प्रभृतिकं पुरातनं गुणकरमिति तात्पर्यार्थ:॥(आढमल्ल टीका)

Adhamalla's Dipika, commentary on *Sharngadhara Samhita* mentions that *Vidanga, Pippali* etc. drugs if used in old form will be of good Quality/Potent.

पुरातनत्वं संवत्सरादुपरि भवति। (आढमल्ल टीका)

The time of these drugs which are to be used in old form is mentioned by *Adhamalla* as: *Pippali* and other drugs must be used one year old.

The quality of a drug is given much importance in order to achieve its therapeutic efficacy.

So *Dravya*(drug) is considered as second important factor next to *Vaidya*(Physician) in *Chikitsa Chatushpada*(four aspects of treatment), which are responsible for the cure of diseases, provided they have requisite qualities like its abundance, suitability, available in various forms and should possess all the properties[2]. Hence it emphasizes that drug should be selected of good quality in order to achieve maximum therapeutic efficiency.

Therefore, there is necessary to revalidate the concept through scientific parameters by evaluating the Qualities of *Nava Pippali* (Fresh form) and *Purana Pippali* (Old form). Evaluation of a drug ensures the identity of a drug and determines the quality and purity of drug.

पिप्पली रेचनी हन्ति कासश्वासोदर ज्वरान्।

कुष्ठ प्रमेह गुल्मार्श: प्लीहशूलाममारूतान्॥[3]

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***.** Hence antiasthmatic activity is chosen on Animal models i.e., in vivo study in which histamine induced bronchospasm is selected in order to evaluate the effect of *Nava* and *Purana Pippali* on Bronchial Asthma.

Biological evaluation of plant drugs is useful to determine pharmacological activity, potency and toxicity. Moreover this is an important evaluation for drugs as it 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.

In-vivo 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

Invivo study of *Nava* and *Purana Pippali:*

Acute oral toxicity of *Nava Pippali/Purana Pippali*:

Acute oral toxicity study of *Nava Pippali/Purana Pippali* was performed according to the OECD test guideline 423-Acute Toxic Class Method. Young healthy adult Sprague Dawley female rats weighing between 160-180g body weights were divided into two groups of 3 animals /group[6]. Animals were housed individually in a well ventilated polypropylene cage. A 12-h light/12-h dark artificial photoperiod was maintained. Room temperature 22°C (±3°) and relative humidity 50–70% were maintained in the room. Animals had free access to pelleted feed (Nutrilab rodent,

Tetragon Chemie Pvt Ltd., India) and Reverse osmosis (Rios, USA) purified water *ad libitum*. Animals kept in their cages for 5 days prior to dosing for acclimatization to the laboratory conditions. Prior to *Nava Pippali/Purana Pippali* sample administration animals were fasted for overnight and then 3- 4hrs post administration of test sample. This experiment was conducted with step wise procedure. The test sample was administered once orally via gastric intubation at a dose level of 2000 mg/kg body weight. Lethality and abnormal clinical signs were observed on the day of dosing of test sample and thereafter for 14 days. Body weights were recorded just prior to dosing and thereafter once in a week till completion of the experiment. Gross pathological changes were also observed at the end of experiment[7].

Antiasthamatic activity of *Nava Pippali/Purana Pippali* studied by:

Histamine induced bronchospasm.

Research Design: Informal Experimental Research Design.

Study type: Before and after with Control design.

Histamine induced bronchospasm of *Nava Pippali/Purana Pippali*:

Animal husbandry

Young adult male Dunkin Hartley Guinea pigs (400-600 g b. wt) were used for the study. Animals were housed individually in polypropylene cages in a well-ventilated room (air cycles: 15/min; recycle ratio: 70:30) under an ambient temperature of 22±3°C and 40–65% relative humidity, with a 12-h light/dark artificial light cycle. They were provided with fresh lucerene and purified water *ad libitum*. Animals were acclimatized at least for 7 days to the laboratory conditions prior to initiation of the experiment[8].

Experiment Design and Treatment

Following acclimatization, animals were grouped into four (4 animals / group) and fasted overnight.

Group I : Positive control

Group II : Standard control (Salbutamol)

Group III : *Nava Pippali/Purana Pippali* (Low dose)

Group IV : *Nava Pippali/Purana Pippali* (High dose)

Prior to drug administration, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The preconvulsion dyspnoea time (PCD) was noted for each animal. PCD is the time of

aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as preconvulsive dyspnoea commenced, animals were removed from the chamber and placed in fresh air to recover from dyspnoea for 24 h. The time for preconvulsive dyspnoea was recorded as basal value. After 24 h, animals of Group I received Normal saline and Group II received Salbutamol per oral. Animals of Group III and Group IV were treated with aqueous extract of *Nava Pippali/Purana Pippali* at low dose and high dose per oral. The experimental animals were again subjected to histamine aerosol later at an interval of 1, 4 and 24 h to determine PCD[9]. The protection offered by the treatment was calculated using the formula:

Percentage Protection = (1-T1/T2) x 100

Where,

T1 = Mean of PCD before administration of test drugs.

T2 = Mean of PCD after administration of test drugs at1h, 4h and 24 hrs.

Parameter for assessment: The time taken from aerosol exposure to the onset of dyspnoea.

End Point: Preconvulsion dyspnoea (PCD)[10].

Statistical analysis:

The results were reported as mean ± SEM and analyzed 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.

RESULTS

Nava Pippali

- There were no treatment related deaths, abnormal clinical signs, remarkable body weight changes or gross pathological changes were observed in all the experimental animals fed by Control vehicle & *Nava Pippali as seen from Table and Fig 1*.

- **Acute Oral Toxicity:**

Table 1 Body weight of experimental animals fed by Control vehicle & *Nava Pippali*

Values are expressed in mean ± SEM; n=3

Group	Treatment	Body weight (g)

		1 day	1 hr before *Nava Pippali* administration Day 1	6h on Day 1	Day 2	Day 7	Day 14
I	**Control (vehicle)**	162.33±0.88	157.33±2.96	165.67±2.19	172.00±0.58	187.67±2.73	203.33±2.03
II	***Nava Pippali* (2000mg/kg b.wt.)**	162.33±1.86	154.00±1.86	166.67±2.19	176.67±2.67	187.00±2.31	198.67±2.96

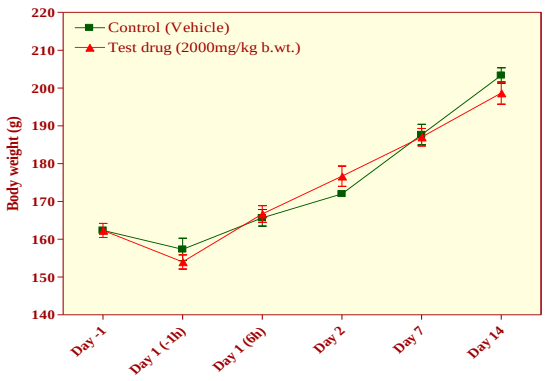

Fig.1 Body weight of the experimental animals fed by Control vehicle & *Nava Pippali*

- From the above results, LD_{50} of *Nava Pippali* was found to be greater than 2000mg/kg b.wt. Hence, the test drug falls in the "category-5" or "unclassified" in accordance to the Globally Harmonised System of classification of chemicals.

- **Antiasthmatic activity**

Histamine induced bronchospasm as shown in Table 2 and Fig 2.

Table 2 Effect of *Nava Pippali* on histamine induced bronchospasm

Group	Treatment	Preconvulsive dyspnea (sec)			
		Before treatment	After treatment		
			1h	4h	24h
I	Normal control	43.75±0.48	44.25±1.11	51.75±1.25	54.75±0.48
II	Standard control (Salbutamol)	45.00±0.82	175.00±9.08**	201.75±6.29**	157.25±10.13**
III	*Nava Pippali* (200 mg/kg)	45. 00±1.08	82.00±2.04**	109.50±3.12**	81.50±3.12*
IV	*Nava Pippali* (400 mg/kg)	42.75±0.85	126.25±5.11**	181.75±6.46**	125.00±2.04**

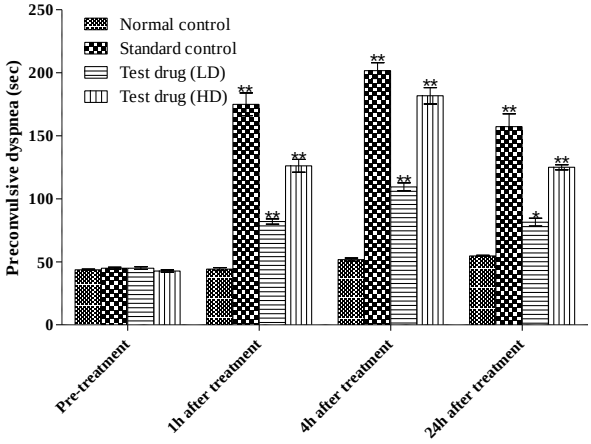

Fig 2 Effect of Nava Pippali on histamine induced bronchospasm

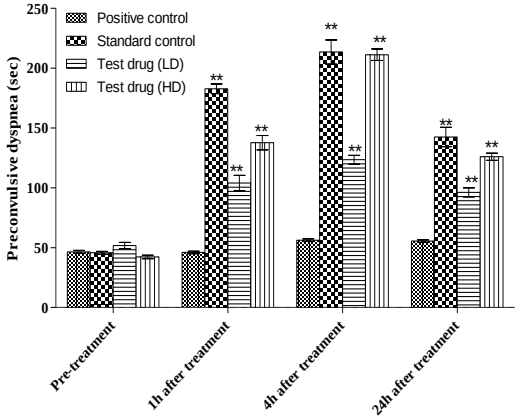

Fig.3 Effect of *Purana Pippali* on histamine induced bronchospasm

The results are expressed in mean ± SEM (n =4); Statistical analysis was done using prism 4.0 Version, Unpaired t test, and p values positive control Vs test drug ** (0.01); Using graph pad prism.

Purana Pippali

There were no treatment related mortality, abnormal clinical signs or remarkable body weight changes were observed in experimental animals fed by *Purana Pippali as seen in Table 4.*

Table 3 Percentage Protection of *Nava Pippali* against histamine induced bronchospasm

Group	Treatment	% Protection		
		1h	4h	24h
II	Standard control (Salbutamol)	74.12±1.10	77.67±0.30	71.03±1.92
III	*Nava Pippali* (200 mg/kg)	45.01±2.06	58.85±1.09	44.44±3.24
IV	*Nava Pippali* (400 mg/kg)	66.03±1.00	76.37±1.11	65.74±1.25

- **Acute Oral toxicity:**

Table 4 Individual animal body weight fed by *Purana Pippali*

Step/Dose	Animal number	Body weight (g)		
		Day 0	Day 7	Day 14
I *Purana Pippali* 2000 mg/kg b.wt.	1	165.2	175.0	188.6
	2	157.0	174.2	183.0
	3	158.6	180.4	192.2
II*Purana Pippali* 2000 mg/kg b.wt.	4	168.2	172.6	190.2
	5	174.6	186.2	197.8
	6	168.0	177.2	189.4

- No gross pathological observation was recorded in all the experimental animals (Table 5).
- From the above tested conditions, LD_{50} of *Purana Pippali* was greater than 2000mg/kg body weight classified under GHS hazard category 5.

Table 5 Individual animals gross pathological observation after fed with *Purana Pippali*

Step/Dose	Animal Number	Organs	Observations
I *Purana Pippali* 2000mg/kg b.wt.	1	Skin, eyes, brain, lungs, heart, liver, kidney, adrenals, spleen and sex glands	No abnormality detected
	2		No abnormality detected
	3		No abnormality detected
II *Purana Pippali* 2000mg/kg b.wt.	4		No abnormality detected
	5		No abnormality detected
	6		No abnormality detected

- **Antiasthamatic activity**

Histamine induced bronchospasm (Table 6)

Table 6 Effect of *Purana Pippali* on histamine induced bronchospasm

Group	Treatment	Preconvulsive dyspnea (sec)		
		Before treatment	After treatment	
			1h	4h	24h
I	Positive control	46.50±1.19	46.00±1.08	56.25±1.11	55.50±1.04
II	Standard control (Salbutamol)	45.75±1.18	182.75±4.01	213.50±10.14	142.50±8.11
III	*Purana Pippali* (200 mg/kg)	51.75±2.66	104.00±6.49	123.50±3.71	96.25±3.73
IV	*Purana Pippali* (400 mg/kg)	42.25±1.44	137.75±5.98	211.75±64.59	126.00±3.03

Table 7 Percentage Protection of *Purana Pippali* against histamine induced bronchospasm

Group	Treatment		% Protection		
			1h	4h	24h
II	Standard control	(Salbutamol)	74.97±0.30	78.41±2.44	67.71±2.42
III	*Purana Pippali*	(200 mg/kg)	49.29±5.11	57.87±3.03	46.24±1.87
IV	*Purana Pippali*	(400 mg/kg)	69.16±1.60	80.20±1.02	66.42±1.33

DISCUSSION

Acute oral toxicity: Acute oral toxicity study of *Nava* and *Purana Pippali* was performed according to the OECD test guideline 423-Acute Toxic Class Method. There were no treatment related deaths, abnormal clinical signs, remarkable body weight changes or gross pathological changes were observed in all the experimental animals. From the results, LD_{50} of *Nava Pippali* and *Purana Pippali* were found to be greater than 2000mg/kg b.wt. Hence, the test drugs falls in the "category-5" or "unclassified" in accordance to the Globally Harmonized System of classification of chemicals (Table 1 and Fig 1).

Antiasthmatic activity

To evaluate the efficacy of antiasthmatic property of a drug, evaluation of bronchodilator activity is used as pharmacodynamic parameter (Table 2 and Fig 2).

Histamine induced bronchospasm:

- In Normal control, Preconvulsion dyspnea (PCD) was observed in 43sec before treatment and after treatment PCD was observed in 44sec, 51sec and 54sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.
- In Standard control, Preconvulsion dyspnea (PCD) was observed in 45 sec before treatment and after treatment PCD was observed in 175sec, 201sec and 157sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.
- In *Nava Pippali* at low dose of 200 mg/kg, Preconvulsion dyspnea (PCD) was observed in 45 sec before treatment and after treatment PCD was observed in 82sec, 109sec and 81sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.
- In *Nava Pippali* at high dose of 400 mg/kg, Preconvulsion dyspnea (PCD) was observed in 42 sec before treatment and after treatment PCD was observed in 126sec, 181sec and 125sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.
- In *Purana Pippali* at low dose of 200 mg/kg, Preconvulsion dyspnea (PCD) was observed in 51 sec before treatment and after treatment PCD was observed in 104sec, 123sec and 96sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.
- In *Purana Pippali* at high dose of 400 mg/kg, Preconvulsion dyspnea (PCD) was observed in 42 sec before treatment and after treatment PCD was observed in 137sec, 211sec and 126sec at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.
- Hence Preconvulsion dyspnea (PCD) time was found to be significantly increased in *Purana Pippali* when compared to *Nava Pippali* at an interval of 4h, which confirmed the bronchodilator activity.
- In Standard control, the protection offered was 74%, 77% and 71% at an interval of 1h, 4h and 24h of histamine aerosol exposure, respectively (Table 3).
- In *Nava Pippali* at low dose of 200 mg/kg, the protection offered was 45%, 58% and 44% at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.

- In *Nava Pippali* at high dose of 400 mg/kg, the protection offered was 66%, 76% and 65% at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.
- In *Purana Pippali* at low dose of 200 mg/kg, the protection offered was 49%, 57% and 46% at an interval of 1h, 4h and 24h of histamine aerosol exposure, respectively (Table 7).
- In *Purana Pippali* at high dose of 400 mg/kg, the protection offered was 69%, 80% and 66% at an interval of 1h, 4h and 24h of histamine aerosol exposure respectively.
- In this study, *Purana Pippali* showed maximum protection when compared to *Nava Pippali* at an interval of 4h, thereby protecting the animals to a significant extent from the development of asphyxia produced by histamine aerosol confirming that it has bronchodilator activity.

CONCLUSION

Purana Pippali is found to be more effective than *Nava Pippali* in antiasthmatic activity (Histamine induced bronchospasm) as it showed increased Preconvulsion dyspnea time and maximum protection against histamine aerosol exposure.

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