Pharmacological Activity of *Poonaga parpam*

**Keywords:** *Poonaga parpam* (PNP), Bronchodilator, Anti-inflammatory, Antipyretic, Siddha medicine

**ABSTRACT**

*Poonaga parpam* (PNP) a novel Siddha formulation administered for the management of bronchial asthma by traditional healers and siddha physicians. It is a fine ash obtained through incineration. *Poonagam* (earthworm) and *Aaduthinnapaalai* (*Aristolochia bracteata*) are used as an ingredient for the preparation of PNP. The aim of the study was to screen the bronchodilator, anti-inflammatory and antipyretic pharmacological activities of PNP on animal model. The Bronchodilator activity of the *Poonaga Parpam* (PNP) has been estimated in the Histamine induced bronchoconstriction in guinea pig. PNP at the dose of 100 mg/kg, 200 mg/kg significantly and dose-dependently increased the time of PCT compared with Chlorpheniramine maleate – 2mg/kg treated Group. The anti-inflammatory activity was evaluated using carrageenan-induced paw edema models in Wistar albino rats. PNP has shown significant (*P* < 0.001) inhibition of paw oedema on 3rd hour at the doses of 12 and 24 mg/kg, respectively. The Antipyretic activity has been estimated rats by Brewer’s yeast induced pyrexia in Wistar albino rats. PNP at doses of 12 mg/kg and 24 mg/kg caused significant lowering of body temperature when compared to the control group animals.
INTRODUCTION

Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. It is well known that all the eyes of the world are turning to natural medicine, especially indigenous system of medicine to find out a more acceptable drug for incurable diseases.

Bronchial asthma is an important allergic disorder and it is defined as ‘a chronic inflammatory disorder of the airways associated with increased airway hyper-responsiveness, recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night/early morning. It can be triggered by various factors like allergens, drugs, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, chemicals, histamine and hereditary. The incidence of bronchial asthma is increasing nowadays. Prevalence of asthma is between 100 and 150 million people around the globe and India has an estimated 15-20 million asthmatics. The prevalence of current asthma was 11.9% in children. Allergies in Childhood (ISAAC) have provided data on asthma prevalence in 6-7 and 13-14 year old Indian children.

The drugs like Bronchodilators, Anti-inflammatory agents, Mast cell stabilizers, LT receptor antagonists are used for bronchial asthma all over the world. Both preventions of inflammatory response and bronchial hyperactivity are important for the long term control of asthma. Despite the availability of a wide range of Anti-asthmatic drugs, the relief offered by them is mainly symptomatic and short lived with more or less side effects.

In Siddha system, the symptoms of Bronchial asthma can be correlated with the symptoms of Swasakasam as quoted by Yugi muni. In other system of medicine, three different drugs such as bronchodilators, anti-inflammatory and sometimes anti pyretic were used for the management of bronchial asthma. But in siddha system, single formulation can be used for the treatment of bronchial asthma which has all these properties. Many herbal, herbo-mineral and animal origin formulations which have bronchodilator, mast cell stabilizer, and anti inflammatory activity are used for the treatment of bronchial asthma (Swasa Kasam).

Siddha system which has got hoary of antiquity is based on five elemental Theory (Pancha Pootha Theory). The therapeutic potency of any drug was designed depending on the following parameters namely Suvai, Gunam, Veeriyan, Vibhaham. In siddha system of medicine there is an interrelation between veeriyan and treatment.
Poonaga parpam (PNP) is an animal origin siddha formulation that is indicated for bronchial asthma\(^4\). All the raw drugs that are Poonagam (earthworm) and Aaduthinnapaalai (Aristolochia bracteata) and the finished medicine PNP, a nano-sized formulation possesses veppa veeriyam which is mainly related to the treatment of Bronchial asthma.

**MATERIALS AND METHODS**

*Poonaga parpam* Preparation\(^4\):

It is prepared through the special oxidation procedure involving purified form of earthworm processed under herbal juice. The preparation of PNP was based on the Siddha classical literature “Sikicha Rathina Deepam”.

**Collection and authentication of the test drug:**

The ingredients of PNP are purified Poonagam (Earthworm), juice of Aduthinna paalai (Aristolochia bracteata) and buttermilk. All these raw materials were collected from in and around Thanjavur district, Tamilnadu and authenticated by Botanist, competent authority of Gunapadam Department, National Institute of Siddha. All the ingredients were purified (detoxification) by the suitable method specified in the Siddha literature.

**Method of Preparation\(^4,5,6\):**

Purified Poonagam was ground well and juice of Aduthinna Paalai (Aristolochia bracteolata) was added little by little to it for one day and made into pellet and dried. The pellet was then placed in between two earthen saucers and it was covered by mud sealed cloth. Then it was subjected into pudam by using 100 cow dung cakes. The above mentioned procedure was repeated for 9 times and finally, the parpam was obtained and powdered well. Then it was stored in an airtight container.

**Assessment of Bronchodilator Activity\(^7,8\)**

Bronchodilator activity of PNP was evaluated using histamine induced bronchoconstriction in guinea pig.
Selection of Experimental animals:

Healthy albino guinea pig weighing 700 gms of male sex was used in this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory IAEC approved no: IAEC/ LI/23/CLBMCP/2017.

The animal was kept in plastic cage and maintained under controlled environment (temperature 25±2°C and 12hrs dark and light cycle) with standard diet, water ad libitum during experiment. The animal was allowed an acclimatization period of 14 days before actual experiment. The animal experiment was performed with accordance to legislation on welfare.

Experimental design

Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Overnight fasted Guinea pigs with free access to water were divided into four groups each containing 6 animals.

- Group-I was treated as Vehicle Control- Honey (p.o)
- Group-II received standard drug Chlorpheniramine maleate (2 mg/kg, i.p)
- Group-III PNP (100mg/kg, p.o)
- Group-IV PNP (200mg/kg, p.o)

Experimental procedure

All the doses were given orally once a day for 5 days. Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol using an ultrasound nebulizer in an aerosol chamber under constant pressure of 40mm/Hg. The required time for appearance of pre convulsive dyspnoea produced by the histamine was noted for each animal. The pre convulsive time (PCT) was determined from the time of exposure to onset of dyspnoea leading to the appearance of convulsions. As soon as the PCT were noted, the animal were removed from the chamber and placed in fresh air for recovery. This time for pre convulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 2 days. In order to observe the Bronchodilator effect of the test substance on induced broncho contractions, the test material PNP was added in a cumulative
fashion (100 and 200 mg) to obtain the concentration-dependent inhibitory responses. These animals were again subjected to histamine aerosol after 1hr of drug administration and PCT was determined. The protection offered by treatment was calculated by using the formula:

\[
\text{Percentage Protection} = (1 - \frac{T1}{T2}) \times 100
\]

Where,

T1 = the mean of PCT before administration of test drugs.

T2 = the mean of PCT after administration of test drugs \(^{10}\).

Assessment of Anti-Inflammatory Activity \(^{11}\)

Anti-inflammatory activity of PNP was evaluated by carrageenan-induced hind paw edema in wistar albino rats.

Selection of Experimental animals:

Healthy Wistar albino rats (140-160g) of both sexes were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no: NIS/IAEC-III/03/29092016.

The animals kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water \textit{ad libitum}. They were fed with standard diet and kept in well ventilated animal house and they were also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show any signs of infection they were excluded from the study. The animal experiment was performed with accordance to legislation on welfare.

Experimental design \(^{12,13}\)

Both sex of Adult wistar Albino rats weighing (140-160g) were used in this study. Rats were divided into 4 groups, consisting six animals for each group.

Group I : Vehicle control - received only honey orally

Group II : Received Standard drug Indomethacin (10mg/kg orally)

Group III : Received PNP (12 mg/kg orally)
Group IV : Received PNP (24 mg/kg orally)

Experimental procedure

Carrageenan was administrated by sub-plantar injection of 0.1 ml freshly prepared 1% suspension in right hind paw in rats. Group II, III, and IV of animals were pretreated with 10 mg/kg body weight standard drug Indomethacin, PNP 12 mg/kg and 24 mg/kg at 1hr before eliciting paw edema. Rat’s paw volume was measured initially and then 1, 2, 3 hours after the carrageenan injection by using plethysmographic method.

The edema inhibitory activity was calculated according to the following formula-

\[
\text{Edema (\% inhibition)} = (1 - \frac{D}{C}) \times 100
\]

Where, D-represents the percentage difference in increased paw volume after the administration of test drugs to the rats, C-represents the percentage difference of increased volume in the control groups.

Statistical analysis

All the results were reported as mean ± SD. They were further analyzed using one way analysis of variables (ANOVA) followed by Dunnet’s multiple comparison test.

Assessment of Anti-Pyretic activity

Anti-pyretic activity of PNP was evaluated by Brewer’s yeast induced hyperpyrexia method in wistar albino rats.

Selection of Experimental animals:

Healthy Wistar albino rats (140-160g) of both sexes were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no: NIS/IAEC-III/03/29092016.

The animals kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water ad libitum. They were fed with standard diet and kept in well ventilated animal house and they were also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show...
any signs of infection they were excluded from the study. The animal experiment was performed with accordance to legislation on welfare.

**Experimental design**

Both sex of Adult wistar Albino rats weighing (140-160g) were used in this study. Rats were divided into 4 groups, consisting six animals for each group.

- **Group I**: Control - Honey plus yeast injection
- **Group II**: Received Standard drug Paracetamol (150 mg/kg orally plus yeast injection)
- **Group III**: Received PNP (12 mg/kg orally plus yeast injection)
- **Group IV**: Received PNP (24 mg/kg orally plus yeast injection)

**Experimental procedure**

The animals were fasted overnight with free access to water prior to the experimental procedure. The normal temperature of each rat in four groups was measured rectally at one hour interval on a clinical thermometer. Before yeast injection the basal rectal temperature of rats was recorded by inserting the clinical thermometer to a depth of 2 cm into the rectum and after recording animals were given subcutaneous injection of 10ml/kg of 20% w/v yeast suspended in normal saline below the nape of the neck for elevation of body temperature of rats.

After 8 hours of yeast injection, rats which show a rise in temperature of at least 1°C were taken for the study. The honey was administered orally to the control groups of animals and paracetamol at the dose of 150mg/ml was administered orally to the standard groups of animals. PNP was administered orally at a dose of 12 mg/kg and 24 mg/kg of body weight to Group –III and Group –IV respectively. Rectal temperature was recorded by clinical thermometer at 0, 1, 2, 3hrs after drug administration and tabulated.

**Evaluation of parameters**

Antipyretic activity was evaluated by comparing initial rectal temperature (°C) before yeast injection, with rectal temperature (°C) after 8 hours of yeast injection at different time intervals.
Statistical analysis

All the results were reported as mean ± SD. They were further analyzed using one way analysis of variables (ANOVA) followed by Dunnet’s multiple comparison test.

RESULTS AND DISCUSSION

Evaluation of bronchodilator activity of PNP using histamine induced bronchoconstriction in guinea pig

Table.no.1: Effect of PNP against Histamine induced bronchoconstriction in guinea pig

<table>
<thead>
<tr>
<th>Groups</th>
<th>Onset of Convulsion in sec.</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I Vehicle Control- Honey (p.o)</td>
<td>93.75±0.39</td>
<td>--</td>
</tr>
<tr>
<td>Group-II Standard (Chlorpheniramine maleate–2mg/kg, i.p.)</td>
<td>994.51±0.44**</td>
<td>100</td>
</tr>
<tr>
<td>Group-III PNP (100mg/kg, p.o)</td>
<td>280.66±0.22 **</td>
<td>29</td>
</tr>
<tr>
<td>Group-IV PNP (200mg/kg, p.o)</td>
<td>632.10±0.22**</td>
<td>64</td>
</tr>
</tbody>
</table>

The results were expressed as mean ± SD and was analyzed statistically using one way ANOVA followed by Dunnett’s multiple comparisons test.

Chart.no.1: Bronchodilator effect of PNP in Guinea pig
PNP significantly (p<0.01) and dose dependently increased the time of PCT following exposure to histamine aerosols induced bronchospasm in guinea pigs. The percentage protection was found to be 64% in 200 mg/kg of PNP treated animals, when compared with the untreated control group. The standard group also significantly (p<0.01) delayed the onset of pre convulsive dyspnoea time and the percentage protection was found to be 100% when compared with untreated control group.

The study resulted in deep-rooted the bronchodilator properties of the trial drug PNP justifying it's claiming in the treatment of asthma.

**Evaluation of anti-inflammatory activity of PNP in wistar albino rats**

**Table.no. 2: Inhibitory effect of PNP against carrageenan induced paw oedema in Wistar albino rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage of inflammation after carrageenan injection at different hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Vehicle Control- Honey (p.o)</td>
<td>0.89±0.05</td>
</tr>
<tr>
<td>Standard Indomethacin 10 mg/kg(p.o)</td>
<td>0.91±0.22</td>
</tr>
<tr>
<td>PNP 12 mg/kg(p.o)</td>
<td>0.90±0.06</td>
</tr>
<tr>
<td>PNP 24 mg/kg(p.o)</td>
<td>0.93±0.05</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; n = 6 animals in each group: * P<0.05, ** P< 0.01, ***P<0.001 is considered significant when compared with control rats and followed by one way ANOVA.
Chart.no. 2. Anti-inflammatory effect of PNP in Wistar albino rats

The effect of PNP on carrageenan-induced rat paw edema at different hours of study was compared to that of control for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume. The Group I is carrageenan induced along with oral administration of vehicle honey which showed an elevated level of paw volume in each hour. At the end of the 3rd hour, the paw volume is higher than the Initial Paw Volume. In Group II the Standard Indomethacin is orally received which gives low paw volume in each hour (1st to 3rd hr). Finally at the end of 3rd hour paw volume shows least value. In Group III & IV the Carrageenan is subcutaneously induced along with the oral administration of PNP of 12 mg/kg and 24 mg/ kg respectively. The anti-inflammatory activity was found to be dose dependent in carrageenan-induced paw edema model. PNP has shown significant ($P < 0.001$) inhibition of paw oedema on 3rd hour at the doses of 12 and 24 mg/kg, respectively.
Evaluation of antipyretic activity of PNP in wistar albino rats

Table.no. 3: Antipyretic effect of PNP in Wistar albino rats by Brewer’s yeast induced pyrexia.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Rectal temp.in °c</th>
<th>Rectal temp.in °c after 8 hrs of yeast injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0hr</td>
</tr>
<tr>
<td>Group-I Control</td>
<td>Control Honey(p.o)</td>
<td>36.97±0.27</td>
</tr>
<tr>
<td>Group-II Standard</td>
<td>Paracetamol 150mg/kg p.o</td>
<td>36.50±0.21</td>
</tr>
<tr>
<td>Group-III PNP</td>
<td>12mg/kg p.o</td>
<td>36.90±0.28</td>
</tr>
<tr>
<td>Group-IV PNP</td>
<td>24mg/kg p.o</td>
<td>36.73±0.41</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; n = 6 animals in each group: * P<0.05, ** P< 0.01, ***P<0.001 is considered significant when compared with control rats and followed by one way ANOVA.

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature at the 8th hour after administration. The results obtained from the study showed that there was significant increase in the body temperature of rats injected with Brewer’s yeast.

Rats treated with the standard drug Paracetamol (150 mg/kg) has shown maximum reduction in rectal temperature during 3rd hour after injection of Brewer’s yeast. It was found that PNP...
at doses of 12 mg/kg and 24 mg/kg caused significant lowering of body temperature when compared to the control group animals. Inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol. Also, there are several mediators underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyretic activity.

CONCLUSION

The pharmacological activities like Bronchodilator, Anti-inflammatory and Antipyretic activity of *Poonaga parpam* (PNP) shows significant effect in dose dependent manner. It may be used for the management of bronchial asthma.

REFERENCES