

**EVALUTION OF ANTIHISTAMINIC ACTIVITY OF  
LEAVES OF *PIPER BETEL* LINN.**

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**Summary**

Antihistaminic activity of Ethanolic extract and Hydrodistilled extract of leaves of *Piper betel* Linn. was pharmacologically evaluated by using various models like isolated guinea pig tracheal chain, isolated guinea pig ileum and histamine induced bronchoconstriction in guinea pig.

In the present study the histamine induced dose dependent contraction of guinea pig tracheal chain and isolated guinea pig ileum preparation was significantly inhibited ( $p < 0.05$ ) by both the extracts ( $100\mu\text{g/ml}$ ). Ethanolic extract ( $61.02 \pm 1.03$  and  $61.93 \pm 0.56$ ) was less effective as compared to Hydrodistilled extract ( $56.06 \pm 1.27$  and  $55.42 \pm 1.43$ ) of *Piper betel* Linn. in both the cases.

In histamine induced bronchoconstriction, the Ethanolic extract ( $100\mu\text{g/ml}$  and  $200\mu\text{g/ml}$ ) and Hydrodistilled extract ( $100\mu\text{g/ml}$  and  $200\mu\text{g/ml}$ ) of *Piper betel* Linn. were found to protect the guinea pigs against histamine aerosol induced bronchoconstriction ( $p < 0.001$ ). Hydrodistilled extract ( $200\mu\text{g/ml}$ ) was more effective as compared to Ethanolic extract of *Piper betel* Linn.

**Key words** – Piper betel,  $H_1$ -antagonist, Ethanolic extract, Hydrodistilled extract, Antihistaminics.

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## **Introduction**

The current approaches to the treatment of asthma are mainly focused on anti-inflammatory drugs such as steroids, bronchodilators and blocking of inflammatory mediator<sup>1</sup>.

Histamine plays an important role in the symptomatology of allergic reactions. Drugs which have the capacity to control the histamine release and its further effects can be called as antihistaminic or antiallergic drugs<sup>2</sup>.

Intensive research during the last several decades has highlighted the role of lymphocytes, mast cells and histamine in the etiopathogenesis of allergic conditions. In spite of the voluminous literature on the subject, treatment of asthma and allergic diseases continues to be far from satisfactory. The available treatment for respiratory tract diseases has major limitations owing to low efficacy and associated adverse effects. Ayurveda, an Indian system of medicine, has described several drugs from indigenous plant sources for the treatment of bronchial asthma and allergic disorders<sup>3</sup>.

The selected plant of *Piper betel* have multiple therapeutic activities like antibacterial, treating eczema, lymphangitis, to cure cough, cold, pruritis, asthma, treating rheumatism. Among these diseases some diseases are related to histaminic activity of the body. Even though the selected plant *Piper betel* is effective in histamine activity related diseases, antihistaminic activity of *Piper betel* is still not scientifically investigated<sup>4,5</sup>.

Thus, taking into the consideration the traditional claims and reported activities of *Piper betel* Linn., the present study was planned to evaluate the antihistaminic activity of *Piper betel* Linn. Using, a) Isolated guinea pig tracheal chain preparation, b) Isolated guinea pig ileum preparation and c) Histamine induced Bronchoconstriction in Guinea pig.

## **Experimental**

### **Material and Methods –**

#### **Drug sample –**

Leaves of *Piper betel* Linn. were collected from local habitat Faizpur, Dist-Jalgaon, (Maharashtra) and authenticated at Department of Botany, Rashtrasant Tukadoji Maharaj, Nagpur University, Amravati Road, Nagpur-33 and authentication no. is 9147.

**Preparation of Extracts –**

Leaves dried under the shade were powdered and then charged into soxhlet apparatus and exhaustive extraction was carried out using Ethanol as solvent. Extract was concentrated by distilling the excess of solvent to obtain the crude extractive. *Piper betel* leaves were cut into small pieces. The pieces of leaves were placed in a round bottom flask with distilled water and hydrodistillation was carried out for 4 hours<sup>9,10</sup>.

**Animals –**

Dunkin-Hartley Guinea pigs weighing 350-400 gm were used for the present study. Animals were housed under a standard 12 hour light/dark cycle and were provided with food and water *ad libitum*. The animal protocol was approved by the Institutional Animal Ethical Committee and the study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

**Dose Selection –**

Acute toxicity studies on the leaf extract of *Piper betel* has been reported. From this doses are selected as 100mg and 200mg in histamine induced bronchoconstriction. Calcium channel antagonist activity of the constituents of *Piper betel* has been reported; from this the dose for isolated guinea pig tracheal chain preparation and isolated guinea pig ileum preparation is selected as 100µg.

**Preparation of drug solution –**

*Piper betel* Ethanolic extract and Hydrodistilled extract were dissolved in distilled water. Chlorpheniramine maleate was dissolved in distilled water. Histamine was dissolved in physiological saline solution.

**Isolated guinea pig tracheal chain preparation –**

Overnight fasted guinea pig was sacrificed and Trachea was cut into individual rings and tied together in series to form a chain. It was then suspended in bath of Kreb's solution which was continuously aerated and maintained at  $37 \pm 0.5$  °C. One end of the tracheal chain was attached to an S-shaped aerator tube and other attached to an isotonic frontal writing lever to smoked drum. Tissue was allowed to equilibrate for 45 min. under a load of 400 mg. A dose response curve for histamine was taken in variant molar concentrations, by maintaining 15 min time cycle. After obtaining a dose response curve of histamine on trachea, the *Piper betel* Ethanolic extract and Hydrodistillated extract (100µg/ml) were added to the reservoir and same doses of histamine were repeated. Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of extracts<sup>12,13,14</sup>.

**Isolated guinea pig ileum preparation –**

Overnight fasted guinea pig was sacrificed and ileum was mounted in an organ bath containing Tyrode solution which was continuously aerated and maintained at  $37 \pm 0.5^\circ\text{C}$ . One end of ileum was attached to an S-shaped aerator tube and other attached to isotonic frontal writing lever to smoked drum. The tissue was allowed to equilibrate for 30 min. under a load of 500-mg. Contact time of 30 sec. and 15 min time cycle was followed for recording the response of Histamine. After obtaining a dose response curve of histamine on ileum, the *Piper betel* Ethanolic extract and Hydrodistilled extract (100 $\mu\text{g/ml}$ ) were added to the reservoir and same doses of histamine were repeated in presences of extracts. Graph of percentage of maximum contractile response on ordinate and negative Logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of extracts<sup>11</sup>.

**Histamine induced bronchoconstriction in guinea pig –**

Overnight fasted guinea pigs were divided into six groups each containing 6 animals. Group 1 was treated as control, Group 2 received standard drug Chlorpheniramine maleate (2 mg/kg). Animals belonging to groups 3, 4 received Hydrodistilled extract in dose (100,200 mg/kg) and groups 5, 6 were administered Ethanolic extract in dose (100,200mg/kg). All the doses were given orally. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The preconvulsive time (PCT) was determined from the time of exposure to onset of convulsions. As soon as the PCT were noted, the animal were removed from the chamber and placed in fresh air. 24 hours later the animals of group 3, 4 received Hydrodistilled extract and group 5, 6 received Ethanolic extract. Group 2 received Chlorpheniramine maleate. 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 following formula<sup>14</sup>.

$$\text{Percentage Protection} = (1 - T_1/T_2) \times 100 \quad \text{Where,}$$

$T_1$  = The mean of PCT before administration of test drugs.

$T_2$  = The mean of PCT after administration of test drugs.

**Statistical Analysis –**

The drug was compared with control and results were analysed by one way ANOVA followed by Dunnet's multiple comparison test for isolated guinea pig tracheal chain and ilium preparation and Neuman Keul's test for histamine induced bronchoconstriction.  $P < 0.05$  and  $P < 0.001$  were considered to be statistically significant respectively.

### Results and Discussion

Allergy is a complex, multifactorial process that involves formation and release of different mediators including histamine. Histamine is released from mast cells and basophiles by antigenic stimulation causing smooth muscle contraction, increased vascular permeability and mucus formation<sup>6</sup>. Mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate. The pharmacological agent that increase intracellular level of cAMP relaxes smooth muscles and inhibit the release of Autacoids and tissue and basophils<sup>15</sup>. Histamine can provoke bronchoconstriction, it may also be responsible for bronchial hypersensitivity which is a common feature of asthma<sup>6</sup>. Targeting histamine, either prevention of its release from mast cells or use of histaminergic receptor antagonists becomes part of antihistaminic therapy in allergic diseases<sup>16</sup>.

The qualitative phytochemical studies revealed the presence of tannins, alkaloids, carbohydrates and amino acids in the plant extract. The desired medicinal properties of the plant may be attributed to the synergistic or individual effect of these phytoconstituents.

Histamine produces dose dependent contraction of Guinea pig tracheal chain preparation. In the present study, Ethanolic extract and Hydrodistilled extract of *Piper betel* Linn. (100µg/ml) significantly inhibited ( $p < 0.05$ ) the histamine-induced contraction of isolated Guinea pig tracheal chain preparation. Hydrodistilled extract found to be more effective. (Table 1, Figure 1)

The tracheal muscle has histamine H<sub>1</sub>, M<sub>3</sub>, B<sub>2</sub> receptors . The stimulation of histamine H<sub>1</sub> receptors cause contraction of bronchiole smooth muscles<sup>17</sup>. In present study, both the extracts (100µg/ml) significantly inhibited histamine induced contraction of Guinea pig tracheal chain preparation, indicating histamine H<sub>1</sub> receptor antagonist activity.

Guinea pig ileum is used for screening of antihistaminic activity. The stimulation of H<sub>1</sub> receptors produces graded dose related contraction of isolated guinea pig ileum<sup>17</sup>.

In present study, Ethanolic extract and Hydrodistilled extract of *Piper betel* Linn. (100µg/ml) significantly inhibited ( $p < 0.05$ ) the histamine-induced contraction of isolated guinea-pig ileum preparation indicating H<sub>1</sub> receptor antagonistic activity. Hydodistilled extract was more effective. (Table 2, Figure 2)

Histamine when inhaled has been shown to induce bronchoconstriction by direct H<sub>1</sub>-receptor activation and also by a naturally mediated bronchoconstrictor effect via vagal reflexes<sup>7</sup>.

In the present study, ethanolic extract and hydrodistilled extract of *Piper betel* Linn. (100,200mg/kg) significantly protected ( $p<0.001$ ) the Guinea pigs against histamine-induced bronchospasm. The percentage protection with hydrodistilled extract was found to be 81.5% at 200mg/kg dose. The guinea pigs exposed to histamine aerosol showed signs of progressive dyspnoea leading to convulsions. The Ethanolic extract and Hydrodistilled extract of *Piper betel* Linn. significantly prolonged the latent period of convulsions (PCT) as compared to control following the exposure of histamine aerosol. The action started after 1 hr of drug administration.

The antihistaminic drug Chlorpheniramine maleate used in the study produced a significant increase in the latent period of convulsion after 1 hour. Therefore, the result of present study indicates the utility of the Ethanolic extract and Hydrodistilled extract of *Piper betel* Linn. in antihistaminic activity, more prominently of Hydrodistilled extract due to its more effectiveness. (Table 3, Figure 3)

Thus, it can be concluded from the results obtained in the present investigation that ethanolic extract and hydrodistilled extract, especially hydrodistilled extract of *Piper betel* Linn. possess significant antihistaminic activity which closely mimicked to the standard, Chlorpheniramine maleate. Hence it is worthwhile to study further by isolating and identifying the active constituents of *Piper betel* Linn.

Table 1: Results Obtained From Guinea pig tracheal chain preparation

Sr. No .	Dose of Histamine (30 ug/ml) (ml)	-Log molar concentration of Histamine	Control % maximum response	Standard % maximum response	Hydrodistilled extract % maximum response	Ethanollic extract % maximum response
1	0.1	6.61	22.430±1.60	9.89± 1.32*	11.20±1.24**	20.02± 2.26**
2	0.2	6.31	44.610±1.56	24.03±1.56*	26.02±1.65**	34.08± 3.72**
3	0.4	6.01	60.450±2.10	29.56±2.02*	32.02±2.78**	39.03± 3.24**
4	0.8	5.71	75.120±1.56	37.12±1.03*	39.16±2.03**	44.20± 2.12**
5	1.6	5.40	87.320±2.46	46.08±1.89*	50.08±1.03**	58.05± 2.53**
6	3.2	5.10	99.230±1.46	52.06±2.45*	56.06±1.27**	61.02± 1.03**

(Values are mean±SEM (n=6) \*p<0.05 when compared to control group, \*\*p<0.05 when compared to standard group).

Table 2 Guinea pig ileum preparation

Sr. No .	Dose of Histamine (10 ug/ml) (ml)	-Log molar concentration of Histamine	Control % maximum response	Standard % maximum response	Hydrodistilled extract % maximum response	Ethanollic extract % maximum response
1	0.1	7.08	32.56±1.023	12.55±1.560*	17.67±1.93**	24.63±1.05**
2	0.2	6.79	51.91±1.450	23.10±2.065*	26.98±2.01**	33.03±1.33**
3	0.4	6.48	78.26±2.030	35.23±1.020*	39.31±1.31**	46.00±2.53**
4	0.8	6.18	92.90±2.560	42.65±1.670*	47.86±1.20**	53.89±2.48**
5	1.6	5.88	99.58±1.051	51.32±1.250*	55.42±1.43**	61.93±0.56**

(Values are mean±SEM (n=6) \*p<0.05 when compared to control group, \*\*p<0.05 when compared to standard group).



Table 3 Histamine induced Bronchoconstriction in Guinea pigs

Sr.No.	Group	Onset Convulsion sec.	of in	% protection
1	Control	91.45±0.093	-	
2	Standard	1028.0±4.553*		91.10
3	Hydrodistilled extract %100	406.2.±0.357**		77.48
4	Hydrodistilled extract %200	495.1±0.253**		81.5
5	Ethanolic extract 100	254.0±0.396**		64.0
6	Ethanolic extract 200	257.6±0.400**		64.5

(Values are mean±SEM (n=6)\*p<0.001 when compared with control group, \*\*p< 0.001 when compared with standard group).

Figure 1 Guinea pig tracheal chain preparation

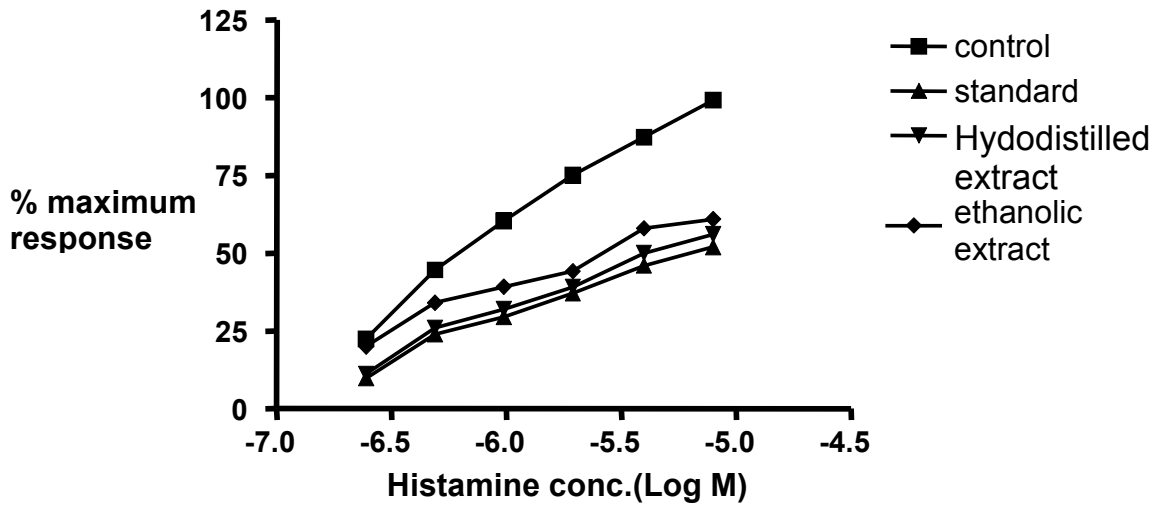


Figure 2 Guinea pig ileum preparation

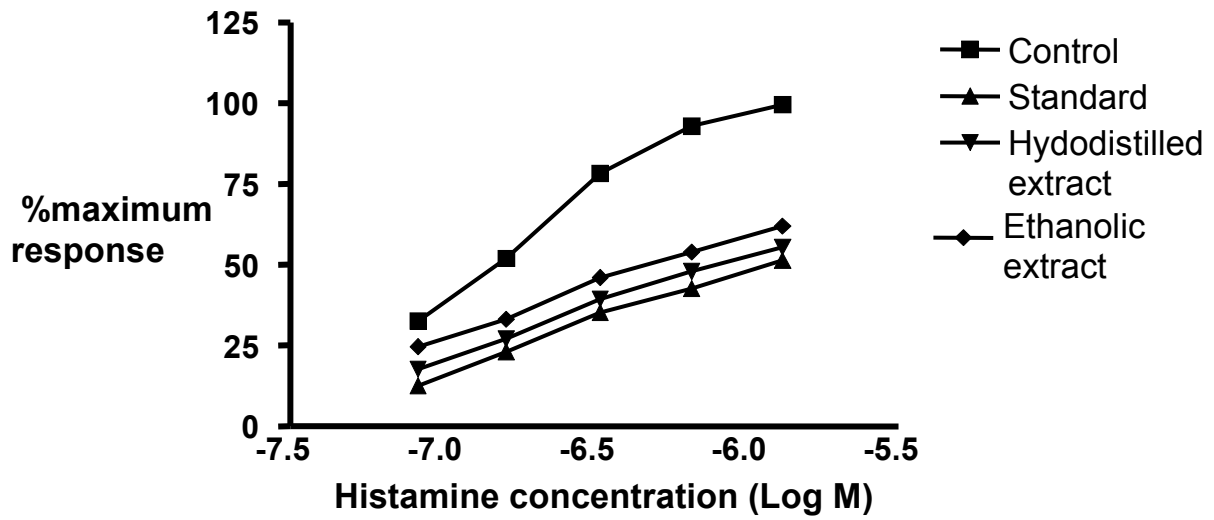
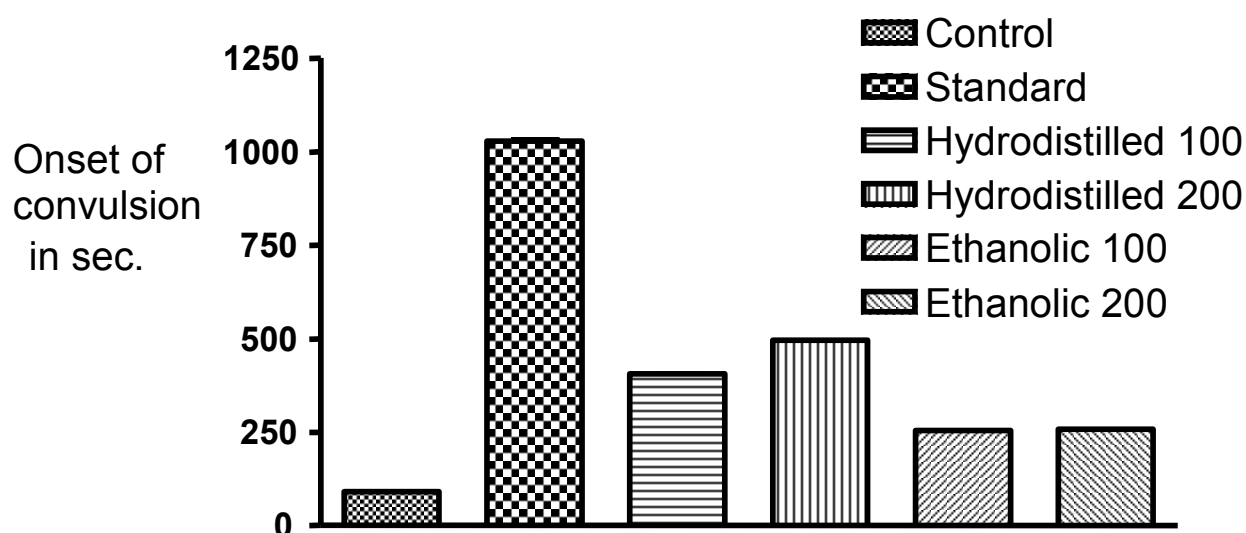


Figure 3 Histamine induced Bronchoconstriction in Guinea pig



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