

EVALUATION OF ANTI-ASTHMATIC EFFECT OF ETHANOL EXTRACT OF *PIPER BETLE* LINN. AGAINST HISTAMINE INDUCED BRONCHOSPASM IN GUINEA PIGS

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ABSTRACT

Piper betel Linn. Is commonly known as Pan or Tamboli. This has a long history of use in India being applied in multiple therapeutic activities like antibacterial, treating eczema, lymphangitis and treating rheumatism. So this plant is effective in histaminic activity related diseases. In asthma histamine also plays an important role. So here ethanolic extract of *Piper betel* Linn. at a dose of 100mg/kg body weight and 200mg/kg body weight is studied in Guinea Pigs for its effect in asthma. It is compared with a standard drug that is Chlorpheniramine, which is an antihistaminic drug. The Preconvulsive time of all the drugs were compared. Here asthma is induced by 0.2% histamine aerosol. The results obtained from this study shows that *Piper betel* Linn. at a dose of 100mg/kg body weight and 200mg/kg body weight has significant antiasthmatic property.

Keywords: *Piper betel* Linn., Ethanolic Extract, Antihistaminic, Antiasthmatic, Guinea pig

INTRODUCTION

Asthma is defined as Hyperresponsiveness of tracheobronchial smooth muscle to a variety of stimuli. Asthma is now recognized to be a primarily inflammatory condition, chronic inflammation underlying hyperreactivity. The hyperresponsiveness leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing particularly at night or in the early morning. These episodes are usually associated with widespread, but variable airflow obstruction within the lung that is often reversible either spontaneously or with treatment" (Global Strategy for Asthma Management and Prevention, 2010).

Although asthma is a chronic obstructive condition, it is not considered as a part of chronic obstructive pulmonary disease as this term refers specifically to combinations of disease that are irreversible such as bronchiectasis, chronic bronchitis, and emphysema (Self *et al.*, 2009). Unlike these diseases, the airway obstruction in asthma is usually reversible however, if left untreated the chronic inflammation from asthma can lead the lungs to become irreversibly obstructed due to airway remodeling (Delacourt, 2004).

Drugs that inhibit inflammation and bronchoconstriction are used as pharmacologic agents to treat asthma. As the increasing incidence of asthma entails a significant burden of disability, economic cost and death new targets for therapeutic intervention like improving existing therapies by altering the ratio of benefit to adverse effect, devising new targeted therapies and attempting to prevent or reverse permanent airway remodeling in long-standing asthma is necessary. Anti-inflammatory medications, particularly corticosteroids, are mainstays in the pharmacologic treatment of asthma. As the complex pathophysiology of asthma is further elucidated, more targeted therapies will be developed (Lango *et al.*, 2008).

The anti-inflammatory and anti-oxidant properties of an ethanol extract of the leaves of *P. betle* Linn was evaluated in rat model of chronic inflammation (Ganguly *et al.*, 2007). Mechanism of action was also investigated (Sarkar *et al.*, 2008) as asthma is an inflammatory condition, *P. betle* Linn. is studied here as an anti-inflammatory agent in bronchial asthma. PB is a plant of antiquity with its global spread in terms of distribution, its acceptance by diverse cultural groups and known for ethno medicinal properties – is bestowed with a unique position in the list of medicinal plants. Due to the higher phenol content in the leaf, the plant possesses high antioxidant activity and other pharmacological activities. A number of pharmacological activities such as antidiabetic, anti-ulcer, hepato-protective, anti-infective, immunomodulatory, cardiovascular and anticancer were demonstrated in the last two decades.

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Aims and Objectives

Aim

- To evaluate therapeutic efficacy of *Piper betel* Linn in asthma

Objectives

- To evaluate Anti-Histaminic effect of ethanol extract of *Piper betel* Linn. in guinea pig model.
- To compare the Anti-Histaminic effect of ethanol extract *Piper betel* Linn. with Diphenhydramine in guinea pig model.

MATERIALS AND METHODS

Materials

▪ *Chemicals & Solutions*

- Histamine
- Ethanol extract of *Piper betel* Linn.
- Diphenhydramine hydrochloride
- Propylene glycol
- Normal saline

▪ *Animals*

Guinea male pigs weighing 450gm on average.

▪ *Equipment*

- Histamine chamber
- Aerosol
- Infant feeding tube
- Hypodermic syringe
- Measuring jar
- Glass beakers
- Animal weighing balance
- Cotton
- Animal cages
- Stopwatch



Figure 1: Guinea pig

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Figure 2: Histamine chamber

Methods

Before starting the study permission from animal ethical committee of Maharajah's Institute of Medical Science, Vizianagram was taken.

1. Weight of the animals was measured before experiment.
2. All guinea male pigs weighing 450gm on average are selected for the study.
3. Guinea pigs were randomized into 4 groups (Control, Standard, Test1 and Test 2 groups). Each group contains 6 animals.
4. All animals are overnight fasted.
5. Prior to the experiment preconvulsive time for all the animals was noted by exposing to 0.2% histamine aerosol and was tabulated.
6. To the control group guinea pigs 1ml of normal saline is administered orally 1 hour before exposing to 0.2% histamine aerosol and the preconvulsive (PCT) time was noted. After 1 hour the second reading was noted.
7. To the standard group guinea pigs diphenhydramine 25 mg/kg BW is administered orally 1 hour before exposing to 0.2% histamine aerosol and the preconvulsive (PCT) time was noted, second reading was noted after 1 hour
8. To the test-1 group guinea pigs *Piper betel* Linn. 100 mg/kg BW is administered orally 1 hour before exposing to 0.2% histamine aerosol and the preconvulsive time was noted. Again preconvulsive time was noted after 1 hour.
9. To the test-2 group guinea pigs *Piper betel* Linn. 200 mg/kg BW is administered orally 1 hour before exposing to 0.2% histamine aerosol and the preconvulsive time was noted. Again after 1 hour the preconvulsive time was noted.

Percentage of protection of asthma by different agents are calculated by following formula

$$\text{Percentage of protection} = (1 - T1/T2) \times 100$$

Where, T1 = The mean of PCT before administration of the drug.

T2 = The mean of PCT after administration of the drug.

Data are analysed by ANOVA by using Microsoft Excel and SPSS software.

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RESULTS

For this evaluation study of anti-asthmatic effect of ethanol extract of *Piper betel* Linn. in guinea pigs by using histamine chamber 24 guinea pigs of weight 450gms were selected and are divided into four groups each containing 6 guinea pigs (i.e., group I, II, III and IV respectively). The pre-convulsive time of each guinea pig is recorded by exposing to 0.2% histamine aerosol before injecting the drug.

- In the I group (control) of guinea pigs before administration of drug the mean of preconvulsive time is 98.3 sec (Table 1) and after administration of 1 ml of normal saline showed mean preconvulsive time of 100 ± 0.83 sec with SD of 2.040 and SE of 0.8328 at 1st hour (Table 2) and preconvulsive time of 99.80 ± 0.33 sec with SD of 0.812 and SE of 0.3315 at 2nd hour (Table 4).
- In the II group (standard) of guinea pigs before administration of drug the mean of preconvulsive time is 117.5 sec (Table 1) and after administration of 25 mg/kg BW diphenhydramine showed mean preconvulsive time of 322.50 ± 0.95 sec with SD of 2.340 and SE of 0.9553 at 1st hour (Table 4) and mean preconvulsive time of 405 ± 16.55 sec with SD of 40.54 and SE of 16.55 at 2nd hour (Table 4).
- In the III group (test – 1) of guinea pigs before administration of drug the mean of preconvulsive time is 113.3 sec (Table 1) and after administration of 100 mg/kg BW *Piper betel* showed mean preconvulsive time of 262.50 ± 4.41 sec with SD of 10.807 and SE of 4.412 at 1st hour (Table 2) and mean preconvulsive time of 295 ± 2.14 sec with SD of 5.24 and SE of 2.141 at 2nd hour (Table 4).
- In the IV group (test – 2) of guinea pigs before administration of drug the mean of preconvulsive time is 112.5 sec (Table 1) and after administration of 200 mg/kg BW *Piper betel* showed mean preconvulsive time of 281 ± 5.87 sec with SD of 14.400 and SE of 5.879 at 1st hour (Table 2) and mean preconvulsive time of 315 ± 4.08 sec with SD of 10.00 and SE of 4.082 at 2nd hour (Table 4).
- In comparison of control group with standard group the mean difference of preconvulsive time at 1st hour is -222.50 with 95% confidence interval from -237.26 to -207.74 with a p value of <0.001 (Table 3).
- In comparison of control group with test - 1 group the mean difference of preconvulsive time at 1st hour is -162.50 with 95% confidence interval from -177.26 to -147.74 with a p value of <0.001 (Table 3).
- In comparison of control group with test - 2 group the mean difference of preconvulsive time at 1st hour is -181.00 with 95% confidence interval from -195.76 to -166.24 with a p value of <0.001 (Table 3).
- In comparison of standard group with test - 1 group the mean difference of preconvulsive time at 1st hour is 60.00 with 95% confidence interval from 45.2 to 74.76 with a p value of <0.001 (Table 5).
- In comparison of standard group with test - 2 group the mean difference of preconvulsive time at 1st hour is 41.500 with 95% confidence interval from 26.73 to 56.261 with a p value of <0.001 (Table 3).
- In comparison of test - 1 group with test - 2 group the mean difference of preconvulsive time at 1st hour is -18.500 with 95% confidence interval from -33.26 to -3.739 with a p value of <0.001 (Table 3).
- In comparison of control group with standard group the mean difference of preconvulsive time at 2nd hour is -305.20 with 95% confidence interval from -339.21 to -271.19 with a p value of <0.001 (Table 5).
- In comparison of control group with test - 1 group the mean difference of preconvulsive time at 2nd hour is -195.20 with 95% confidence interval from -229.21 to -161.19 with a p value of <0.001 (Table 5).
- In comparison of control group with test - 2 group the mean difference of preconvulsive time at 2nd hour is -215.20 with 95% confidence interval from -249.21 to -181.19 with a p value of <0.001 (Table 5).
- In comparison of standard group with test - 1 group the mean difference of preconvulsive time at 2nd hour is 110.00 with 95% confidence interval from 75.99 to 144.01 with a p value of <0.001 (Table 5).
- In comparison of standard group with test - 2 group the mean difference of preconvulsive time at 2nd hour is 90.00 with 95% confidence interval from 55.99 to 124.01 with a p value of <0.001 (Table 5).

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- In comparison of test - 1 group with test - 2 group the mean difference of preconvulsive time at 2nd hour is -20.00 with 95% confidence interval from -54.00 to 14.00 with a p value of >0.05 (Table 5). Comparison between mean preconvulsive time of control, standard, test - 1 and test - 2 groups showed statistically significant p value of < 0.001 at both 1st hour and 2nd hour except in comparison between test - 1 and test - 2 groups at 2nd hour showed statistically nonsignificant p value of >0.05. Percentage of protection offered by diphenhydramine 25 mg/kg BW (standard drug) at 1st hour is 63.5% and at 2nd hour is 70.98%, *Piper betel* Linn. 100 mg/kg BW (test drug) showed 56% at 1st hour and 61.4% at 2nd hour and *Piper betel* Linn. 200 mg/kg BW (test drug) showed 60% at 1st hour and 64.28% at 2nd hour. This indicates that diphenhydramine showed a better percentage of protection when compared to *Piper betel* Linn (Table 6). Comparison of preconvulsive time at 1st and 2nd hour is shown in the bar diagram (Figure 3).

Table 1: Baseline Preconvulsive Time

S.No.	Group	Number of animals	Mean PCT (Sec)
1.	Control	6	98.3
2.	Standard	6	117.5
3.	Test - 1	6	113.3
4.	Test - 2	6	112.5

Table 2: Results after 1st hour

S.No	Group	Number of animals	Mean PCT (Sec)	SD	SE
1.	Control	6	100.00 ± 0.83	2.040	0.8328
2.	Standard	6	322.50 ± 0.95	2.340	0.9553
3.	Test - 1	6	262.50 ± 4.41	10.807	4.412
4.	Test - 2	6	281.00 ± 5.87	14.400	5.879

Table 3: Comparison of mean PCT 1st Hour

Comparison	Mean difference	p Value
Control Vs Standard	-222.50	< 0.001
Control Vs Test - 1	-162.50	< 0.001
Control Vs Test - 2	-181.00	< 0.001
Standard Vs Test - 1	60.00	< 0.001
Standard Vs Test - 2	41.500	< 0.001
Test - 1 Vs Test - 2	-18.500	< 0.05

Table 4: Results after 2nd Hour

S.No.	Group	Number of animals	Mean PCT (Sec)	SD	SE
1.	Control	6	99.80 ± 0.33	0.812	0.3315
2.	Standard	6	405.00 ± 16.55	40.54	16.55
3.	Test - 1	6	295.00 ± 2.14	5.24	2.141
4.	Test - 2	6	315.00 ± 4.08	10.00	4.082

Table 5: Comparison of mean PCT 2nd Hour

Comparison	Mean difference	p Value
Control Vs Standard	-305.20	< 0.001
Control Vs Test - 1	-195.20	< 0.001
Control Vs Test - 2	-215.20	< 0.001
Standard Vs Test - 1	110.00	< 0.001
Standard Vs Test - 2	90.00	< 0.001
Test - 1 Vs Test - 2	-20.00	> 0.05*

* Statistically Not Significant

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Table 6: Percentage of protection

S.No	Group	% of Protection	
		1 st Hour	2 nd Hour
1.	Control	–	–
2.	Standard	63.5	70.98
3.	Test – 1	56	61.4
4.	Test – 2	60	64.28

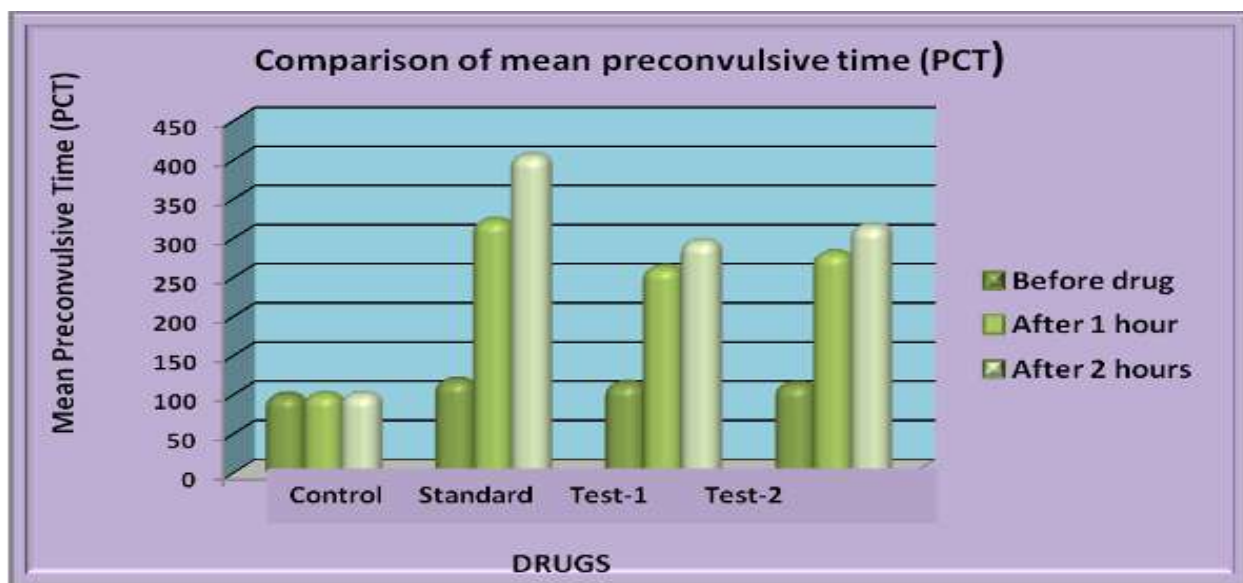


Figure 3: Comparison of mean preconvulsive time (PCT)

DISCUSSION

So in this study it is observed that *Piper betel* Linn. at doses of 100mg/kg BW and 200 mg/kg BW produced statistical significant prolongation of PCT ($p < 0.001$) in comparison to control.(Table 3) Percentage of protection offered by diphenhydramine 25 mg/kg BW at 1st hour is 63.5% and at 2nd hour is 70.98%, *Piper betel* Linn. 100 mg/kg BW showed 56% at 1st hour and 61.4% at 2nd hour and *Piper betel* Linn. 200 mg/kg BW showed 60% at 1st hour and 64.28% at 2nd hour. This indicates that *Piper betel* Linn has antiasmatic property, though its action is less than that of diphenhydramine .The results are comparable to study done by Hazare *et al.*, (2011).

The anti-inflammatory activity of *Piper betel* linn. was studied by Ganguly *et al.*, (2007). As Bronchial asthma is an inflammatory condition, anti-inflammatory activity of *Piper betel* linn may be the reason for reducing bronchial asthma.

Free radical and superoxide may be responsible for bronchial asthma, so antioxidant property of *Piper betel* linn., studied by Srimani *et al.*,(2009) and Rathee *et al.*, (2006), may be responsible for reducing bronchial asthma.

Histamine may cause bronchoconstriction so the antihistaminic activity of piperbetel linn. which was Studied by Hajare *et al.*, (2011), may be causative agent in reducing some bronchial asthma cases.

Conclusion

The ethanolic extract of *Piper betel* Linn. has significantly prolonged the latent period of convulsions (PCT) as compared to control following the exposure of histamine aerosol. Bronchial asthma is symptom complex arising as a result of hypersensitivity of bronchial tree arising as a result of inflammation, superoxide formation and histamine and other mediator’s release. The present study shows protection against histamine induced experimental bronchial asthma in guinea pigs which may be due to

- 1) Anti-inflammatory activity

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- 2) Antioxidant action
- 3) Antihistaminic action

But in humans for asthma other mediators like Leukotriene plays an important role. So the effect of *Piper betel* Linn. in human asthma is to be studied in future.

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