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Study of therapeutic efficacy of Piper betel Linn with asthma in guinea pig model

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Abstract

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. Male guinea male pigs were selected for the experiment. Animals were weighed with the help of weighing machine. The guinea pigs weighing 450gm on average were selected for the experiment. The study was conducted in MIMS (Maharajah's Institute of Medical Sciences). In this study evaluated the anti-asthmatic effect of ethanol extract of piper betel Linn in guinea pigs by using histamine chamber 24 guinea pigs were selected and are divided into four groups each containing 6 guinea pigs (i.e., group I, II, III and IV respectively). Comparison of control group with standard group the mean difference of preconvulsive time at 1st hour was -222.50 with 95% confidence interval from -237.26 to -207.74 with a p value of <0.001. Comparision of control group with test - 1 group the mean difference of preconvulsive time at 2nd hour was -195.20 with 95% confidence interval from -229.21 to -161.19 with a p value of <0.001. Bronchial asthma is an inflammatory condition so anti-inflammatory activity of piper betel linn, may be the reason for reducing bronchial asthma. Present study shows protection against histamine induced experimental bronchial asthma in guinea pigs which may be due to anti-inflammatory activity, antioxidant action and antihistaminic action.

Keywords: Anti-Histaminic, Piper betel Linn, Diphenhydramine, asthma and guinea pig model.

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Introduction

Asthma is a chronic condition. These symptoms may be due to liberation of endogenous and intrinsic mediators like histamine, leukotrienes (LTs), bradykinin, prostaglandins (PGs), nitric oxide, platelet activating factors (PAF), chemokines and endothelin from mast cells during the allergic reactions and inflammation of the air passages in the lungs. Nearly 7-10% of world population suffers from bronchial asthma. [1] Asthma is defined by the Global Initiative for Asthma (GIA) as "a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. [2] The chronic inflammation is associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing particularly at night or in the early morning.[3] These episodes are usually associated with widespread, but variable airflow obstruction within the lung that is often reversible either spontaneously or with treatment". [4]

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. [5] 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. In contrast to emphysema, asthma affects the bronchi, not the alveoli [6].

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. [7] 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. [8]

The anti-inflammatory and anti-oxidant properties of an ethanol extract of the leaves of Piper betle Linn was evaluated in rat model of chronic inflammation. The mechanism of action was also investigated. As asthma is an inflammatory condition our study P.betle Linn. [9] 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 ethnomedicinal properties – is bestowed with a unique position in the list of medicinal plants. [10] 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. [11]

Methodology

Male guinea pigs were selected for the experiments. Animals were weighed with the help of weighing machine. The male guinea pigs weighing 450gm Guinea pigs on average are selected for the experiment. The study was conducted in MIMS (Maharajah's Institute of Medical Sciences). Histamine is released from mast cells and basophiles by antigenic stimulation causing smooth muscle contraction, increased vascular permeability and mucus formation. Histamine can provoke bronchoconstriction by activating H_1 receptors, it is also responsible for bronchial hypersensitivity that is common feature of asthma=0.2% Histamine is administered by inhalation through aerosol in all groups. In control group animals, only vehicle (distilled water) was administered. Test drug was administered orally, according to the body weight, one hour before the histamine challenge. Preconvulsive Time (PCT) was determined from the time of exposure to onset of convulsions. Protection offered by treatment was calculated by using following formula.

Percentage of protection= (1-T1/T2) x 100

Where, T1 = the mean of PCT before administration of the drug and T2 = the mean of PCT after administration of the drug. The method is simple, easy and short lasting as well as reproducible. However it is difficult to examine the cells and their modification by anti-histaminic drugs.

Weight of the animals was measured before experiment. All male guinea pigs weighing 450gm on average are selected for the study. Guinea pigs were randomized into 4 groups (Control, Standard, and Test1 and Test 2 groups). Each group contains 6 animals. All animals were kept in overnight fasting. Prior to the experiment preconvulsive time for all the animals was noted by exposing to 0.2% histamine aerosol and was tabulated. To the control group guinea pigs 1ml of normal saline was administered orally 1 hour before exposing to 0.2% histamine aerosol and the preconvulsive time was noted. After 1 hour the second reading was noted.

To the standard group guinea pigs, diphenhydramine 25 mg/kg BW was administered orally 1 hour before exposing to 0.2% histamine aerosol and the preconvulsive time was noted, second reading was noted after 1 hour. To the test-1 group guinea pigs, piper betel Linn.100 mg/kg BW was 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. To the test-2 group guinea pigs piper betel Linn. 200 mg/kg BW was 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. Statically analysis of data was done using student't-test and ANOVA by SPSS software15th version.

Screening methods for anti asthmatic drugs: Screening methods used for evaluation of anti-inflammatory drugs were classified as: In vitro methods and In vivo methods

In vitro methods: Binding assays(Histamine Receptor Assay), Cell Culture Method, CULTEX Technique, WST Assay, Tests In Isolated Organs, Spasmolytic Acitivity in Guinea Pig Lungs, Vascular and Airway Responses to the Isolated Lung, and Reactivity of the Isolated Pefused Guinea Pig Trachea. **In vivo methods:** Bronchospasmolytic Activity in Anesthetized Guinea Pigs, Arachidonic Acid or PAF-induced Respiratory and Vascular Dsyfunction in Guinea Pigs, Anaphylactic Microshock in Guinea Pigs, Serotonin Aerosol-induced Asphyxia in Guinea Pig, Histamine-induced Bronchoconstriction in Anesthetized Guinea Pigs, Pneumotachography in Guinea Pigs, Microshock in Rabbits, Bronchial Hyperactivity in Guinea Pigs, Airway Microvascular Leakage in Guinea Pigs and Airway Inflammation in Mice. In this experiment we used Histamine-induced Bronchoconstriction model was used by histamine chamber for evaluation of anti-asthmatic property in guinea pigs.

Results and Discussion

In the present study was conducted in the Department of pharmacology, Maharajah's Institute of Medical Sciences (MIMS), Nellimarla, and Vizianagaram during the period of 2011 to 2013. 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 were selected and are divided into four groups each containing 6 guinea pigs (i.e., group I, II, III and IV respectively). The weight and normal pre-convulsive time of each guinea pig was 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 (Figure 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 (Figure II) and preconvulsive time of 99.80±0.33 sec with SD of 0.812 and SE of 0.3315 at 2nd hour (Figure III). In the II group (standard) of guinea pigs before administration of drug the mean of preconvulsive time is 117.5 sec (Figure 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 (Figure II) and mean preconvulsive time of 405±16.55 sec with SD of 40.54 and SE of 16.55 at 2nd hour (Figure III).

In the III group (test – 1) of guinea pigs before administration of drug the mean of preconvulsive time was 113.3 sec (Figure I) 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 (Figure II) and mean preconvulsive time of 295±2.14 sec with SD of 5.24 and SE of 2.141 at 2nd hour (Figure III). In the IV group (test – 2) of guinea pigs before administration of drug the mean of preconvulsive time is 112.5 sec (Figure I) 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 (Figure II) and mean preconvulsive time of 315±4.08 sec with SD of 10.00 and SE of 4.082 at 2nd hour (Figure III).

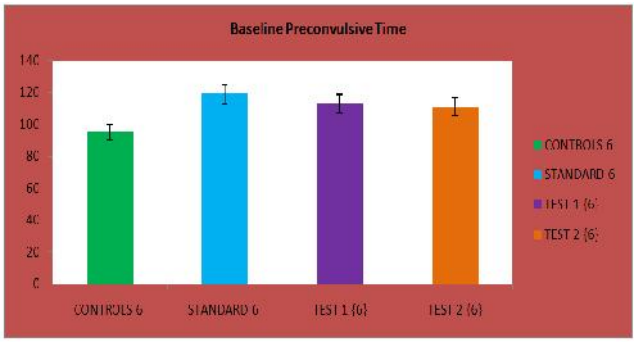

Figure 1: Characteristic of baseline preconvulsive time

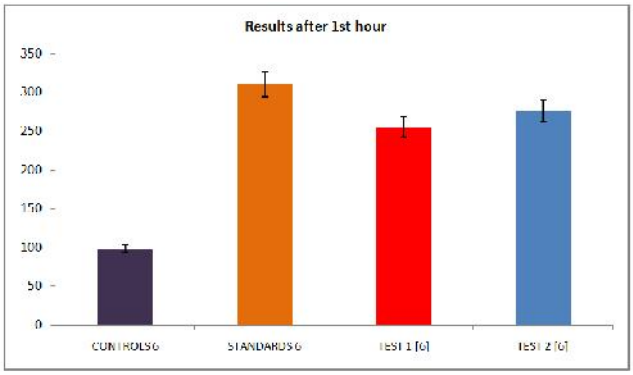

Figure 2: Shows Results after 1st hour

Bronchial asthma is an inflammatory condition so 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. May be responsible for reducing bronchial asthma. Histamine may cause bronchoconstriction so the antihistaminic activity of piperbetel linn. may be causative agent in reducing some bronchial asthma cases.

Comparision	Mean difference	95% Confidence interval		*p* Value
		From	To	
Control Vs Standard	-222.50	-237.26	-207.74	< 0.001
Control Vs Test – 1	-162.50	-177.26	-147.74	< 0.001
Control Vs Test – 2	-181.00	- 195.76	-166.24	< 0.001
Standard Vs Test-1	60.00	45.2	74.76	< 0.001
Standard Vs Test-2	41.500	26.73	56.261	< 0.001
Test-1Vs Test -2	-18.500	-33.26	-3.739	>.0.05

Table 1: Shows comparision of mean PCT 1st Hour

Source of variation	Degree freedom	Sum of squares	Mean square F	F
Treatments (Between columns)	3	171507	57169	
Residuals (Within the columns)	20	1668.9	83.447	685.09 IIS
Total	23	173176		

Table 2: Shows ANOVA 1st Hour

In comparison of control group with standard group the mean difference of preconvulsive time at 1st hour was -222.50 with 95% confidence interval from -237.26 to -207.74 with a p value of <0.001. In comparision of control group with test - 1 group the mean difference of preconvulsive time at 1st hour was -162.50 with 95% confidence interval from -177.26 to -147.74 with a p value of <0.001.In comparison of control group with test - 2 group the mean difference of preconvulsive time at 1st hour was -181.00 with 95% confidence interval from -195.76 to -166.24 with a p value of <0.001. In comparision 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.In comparision of standard group with test - 2 group the mean difference of preconvulsive time at 1st hour was 41.500 with 95% confidence interval from 26.73 to 56.261with a p value of <0.001. In comparision of test - 1 group with test - 2 group the mean difference of preconvulsive time at 1st hour was -18.500 with 95% confidence interval from -33.26 to -3.739 with a p value of <0.001 (Table – 1 and Table 2).

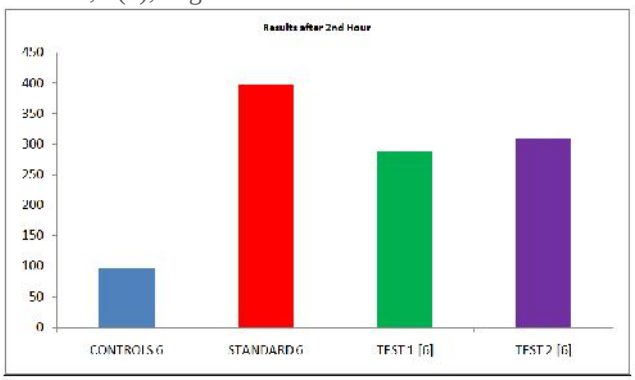

Figure 3: Shows Results after 2nd Hour

comparision	Mean difference	95% confidence interval		P - value
		From	To	
Control Vs Standard	-305.20	-339.21	-271.19	<0.001
Control Vs Test – 1	-195.20	-229.21	-161.19	<0.001
Control Vs Test – 2	-215.20	-249.21	-181.19	<0.001
Standard Vs Test-1	110.00	75.99	144.01	<0.001
Standard Vs Test-2	90.00	55.99	124.01	<0.001
Test-1Vs Test -2	-20.00	-54.00	14.00	>0.05*

Table 3: Characteristics of Comparison of mean PCT 2nd Hour

In comparison of control group with standard group the mean difference of preconvulsive time at 2nd hour was -305.20 with 95% confidence interval from -339.21 to -271.19 with a p value of <0.001. In comparision of control group with test - 1 group the mean difference of preconvulsive time at 2nd hour was -195.20 with 95% confidence interval from -229.21 to -161.19 with a p value of <0.001.In comparision of control group with test - 2 group the mean difference of preconvulsive time at 2nd hour was -215.20 with 95% confidence interval from -249.21 to -181.19 with a p value of <0.001. In comparision of standard group with test - 1 group the mean difference of preconvulsive time at 2nd hour

was 110.00 with 95% confidence interval from 75.99 to 144.01 with a p value of <0.001.In comparison of standard group with test - 2 group the mean difference of preconvulsive time at 2nd hour was 90.00 with 95% confidence interval from 55.99 to 124.01with a p value of <0.001. In comparison of test - 1 group with test - 2 group the mean difference of preconvulsive time at 2nd hour was -20.00 with 95% confidence interval from -54.00 to 14.00 with a p value of >0.05. Comparision 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 non significant p value of >0.05 (Table – 3).

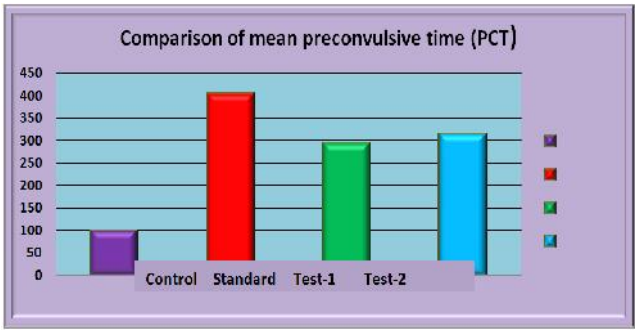

Figure 4: Shows Comparison of mean preconvulsive time (Percentage of protection)

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 mediators release. The present study shows protection against histamine induced experimental bronchial asthma in guinea pigs which may be due to Anti-inflammatory activity, Antioxidant action and Antihistaminic action.

Conflict of interest: None declared

Ethical approval: The study was approved by the Animal institutional ethics committee

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