

**“ANTI-ASTHMATIC STUDY OF ETHANOLIC LEAVES EXTRACT OF  
*BIOPHYTUM SENSITIVUM* L. IN ASTHMA INDUCED GUINEA PIGS”****Prof. Dr. Mathew George<sup>\*1</sup>, Prof. Dr. Lincy Joseph<sup>2</sup> and Umesh Kumar<sup>1</sup>**<sup>1</sup>Department of Pharmacology, Pushpagiri College of Pharmacy, Tiruvalla, Kerala, India.<sup>2</sup>Department of Pharmaceutical Chemistry, Pushpagiri College of Pharmacy, Tiruvalla,  
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Pharmacology, Pushpagiri  
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Tiruvalla, Kerala, India.**ABSTRACT**

The main objective of this study was to investigate the antiasthmatic activity of leaves of *Biophytum sensitivum* L. in guinea pigs. The ethanolic extract of *Biophytum sensitivum* L of two doses-100 mg/kg & 200 mg/kg body weight was selected for the study. The preliminary phytochemical studies showed the presence of steroids, phenolic groups, cardiac glycosides and flavonoids. The toxicity study showed that a oral dose of 2000 mg/kg body weight of ethanolic extract of leaves of *Biophytum sensitivum* L. is non toxic to guinea pigs and was compared with the standard drug 'Chlorpheniramine maleate' when given intraperitoneally. It was concluded from the studies that

ethanolic extract of leaves of *Biophytum sensitivum* L possess a significant dose dependent anti-asthmatic activity.

**KEYWORDS:** *Biophytum sensitivum* L, Antiasthmatic activity, Chlorpheniramine maleate.**INTRODUCTION**

Asthma is a chronic (long term) disease of the lungs.<sup>[1]</sup> It is one of the common disorders encountered in clinical medicine in both adults and children and it is characterized by inflammation of the airways which causes airway dysfunction.<sup>[2]</sup> The illness and mortality of the disease it a worldwide concern. The symptoms of bronchial asthma is characterized by wide blowout narrowing of the bronchial tube due to contraction of the smooth muscle in reply to stimuli subsequently in the release of histamine.<sup>[3]</sup> Asthma is a common disease nowadays and its prevalence rising worldwide with the highest prevalence in industrialized countries. 300 million people are affected by asthma worldwide and it has been estimated that

it will rise to a further 100 million by 2025. In the United States alone, asthma affects almost 17 million people, and this is a 75% increase in the last 20 years. This means that about 1 out of every 20 adults and close to 1 out of 13 children today have asthma. An alarming fact is that since 1980, asthma in children under age 5 has risen remarkably. In school age children, asthma has risen by 75%. India alone has an estimated 15–20 million asthmatics. Mortality data from developed countries show that the rates varies from 0.1- 0.8 per 100,000 persons aged 5–34. For managing asthma attacks, symptomatic relief is foremost requirement. In India, in various traditional systems like Ayurveda, Unani and Siddha, numerous herbs were mentioned for therapeutic use in asthma.<sup>[8]</sup>

It is a chronic lung disease which is caused by the increased airway hyper responsiveness and mucous production which leads to episodes of wheezing, coughing and shortness of breath. This may be due to liberation of endogenous and intrinsic mediators like bradykinin, chemokines, leukotrienes, nitric oxide, platelet activating factors and prostaglandins.<sup>[4]</sup> Histamine induced bronchoconstriction is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to convulsion in Guinea pigs and causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in cardiovascular system. A prominent effect caused by histamine leads to severe bronchoconstriction in the Guinea pigs that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms. The results of the study confirmed the bronchodilator properties of the plant, justifying its traditional claim in the treatment of asthma.<sup>[5]</sup>

*Biophytum sensitivum* L (Oxalidaceae) distributed throughout the tropical regions of South Asia, Africa and Madagascar.<sup>[6]</sup> It is a medicinal plant widely used in the treatment of various health ailments throughout the world. The plant extract showed the presence of flavonoids, saponins, tannins, terpenes, steroids, amino acids, essential oil, polysaccharides and pectin. The plant has been extensively studied by various researchers for its biological activities and therapeutic potentials.

In India, nine species of *Biophytum* are prominently found and out of these, three species viz. *Biophytum sensitivum* DC. Syn. *Oxalis sensitivum* Linn., *Biophytum reinwardtii* Edgew and *Biophytum umbraculum* Welw. Syn. *Biophytum petersianum* Klotzsch are reported to have ethnomedicinal potential. *Biophytum sensitivum* (L.) DC (*B. sensitivum*), commonly known as 'Life plant', is a mesophytic under-shrub growing in slightly moist places. The plant is

distributed up to an altitude of 1,800 m and is available during the rainy season in moist shady places.

The plant is bitter, thermogenic, diuretic, lithontriptic, suppurative, expectorant, stimulant and tonic. The leaves are astringent and antiseptic. It is useful in strangury, urinary calculi, hyperdipsia in bilious fevers, wounds, abscesses, asthma, phthisis, gonorrhoea, stomachalgia, insomnia and snake bite.<sup>[7]</sup>

In the present study the ethanolic leaves extract of *Biophytum sensitivum* L. was selected to treat asthma induced by histamine (0.2% w/v) in guinea pigs. It was found during the literature survey that the pharmacological work related to antiasthmatic activity has not been done so far and hence selected for studies.

## MATERIALS AND METHODS

### Collection of the plant material and preparation of extract

The whole plant was collected from Chengannur, Alappuzha district of Kerala. The leaves of the plant were taken and shade dried. It is then mechanically grinded to obtain coarse powder and defatted with pet ether (60-80°C) using soxhlet apparatus method. The defatted material is then again extracted using ethanol till it becomes colourless. The product is evaporated under dried vacuum to get rid of solvents.

**Phytochemical screening:** The crude ethanolic extract of *Biophytum sensitivum* is qualitatively tested for the presence of various phytoconstituents. The 22 positive screening demonstrated the occurrence of steroids, phenolic groups, cardiac glycosides, flavonoids, saponins, tannins and sterols in various extracts of *Biophytum sensitivum* L.<sup>[9]</sup>

**Experimental animals:** Studies were conducted using guinea pigs of either sex. They are caged prior to experiment for 5 days and is kept at a temperature of 22°C(±3°C) and relative humidity should be between 30%-70%. Conventional rodent diet is fed with an unlimited supply of water.

The animals are divided into six animals in each four group. Group 1 served as control and received 1% CMC(10ml/kg), Group 2 received standard drug Chlorpheniramine maleate (1mg/kg) and group 3 and 4 received ethanolic extracts of *Biophytum sensitivum* L. in 100mg/kg and 200mg/kg body weight doses.

**Acute toxicity studies:** Acute toxicity studies were conducted using Wistar albino rats of either sex (150-200g). Animals should be fasted overnight prior to experiment (food, but not water should be withheld). The ethanolic extract of *Biophytum sensitivum* L was administered using oral feeding tube in varying doses of 5, 50, 300 and 2000 mg/kg body weight. After administration of dose the animals are observed for first 30 minutes and then periodically for 4 hours and then upto 14 days. If 2/3 or 3/3 of the mortality rate was observed, then the dose is considered to be toxic. If mortality is not observed the test is conducted using higher doses. The oral dose of 2000mg/kg did not show any lethal effects in animals and hence the LD<sub>50</sub> value is considered to be 2000 mg/kg.

#### **Histamine Aerosol induced bronchoconstriction in Guinea pigs (in-vivo)<sup>[4]</sup>**

Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in *guinea pigs* by exposing histamine aerosol in a histamine chamber (30 x 15 x 15cm) made of Perspex glass. The required time for appearance of preconvulsive dyspnoea produced by the histamine was noted for each animal. Each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value.

Guinea pigs were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to four different groups of 4-5 animals per group. Animals in group 1 served as control and received distilled water. The animals of group 2 and 3 were given, by oral intubation, 200 and 500mg/kg of the plant extract, respectively, while group 4 received the standard drug - Chlorpheniramine maleate, intraperitoneally. After receiving the drugs, all the animals were again exposed to histamine aerosol in the chamber, one hour, four hours and 24 hrs, to determine pre convulsive time (PCT).

The protection undertaken by the treatment was calculated using the formula;

$$\text{Percentage protection} = \left( \frac{E_{ta} - E_{tb}}{E_{tb}} \right) * 100$$

Where: Eta is the mean of PCT (preconvulsion time) before administration of test drugs. Etb is the mean of PCT (preconvulsion time) after administration of test drugs at 1 hr, 4 hr and 24 hrs.

**Statistical analysis:** All the values of in vivo antiasthmatic studies of ethanolic extract of *Biophytum sensitivum* L. were expressed as mean and standard error of mean (S.E.M) and was examined for significance by ANOVA (analysis of variance) and groups were compared by Dunnett's test for individual comparison of groups with control. P Value were measured moderate significant at  $P < 0.01$ ,  $< 0.001$  level.

## RESULTS

**Phytochemical analysis:** The phytochemical analysis of ethanolic extract of *Biophytum sensitivum* L. revealed the presence of steroids, phenolic groups, cardiac glycosides and flavonoids. The ethanolic extract of leaves of *Biophytum sensitivum* L. yielded 7.45% w/w.

**Table No.1: Data showing effect of ethanolic extract of *Biophytum sensitivum* L. against Histamine induced bronchoconstriction in guinea pigs.**

GROUPS	Latent period of convulsion(in sec.) (MEAN±SEM)			
	Before	After 1 hour	After 4 hours	After 24 hour
Standard	16±0.21	56.65±0.33 <sup>**</sup>	63.15±0.32 <sup>**</sup>	28.65±0.45 <sup>**</sup>
EEBS (100mg/kg)	14.9±0.21	26.25±0.37 <sup>**</sup>	37±0.25 <sup>**</sup>	20.85±1.057 <sup>**</sup>
EEBS (200mg/kg)	15.65±0.26	40.46±0.56 <sup>**</sup>	48.21±0.47 <sup>**</sup>	21.13±1.053 <sup>**</sup>

**Table No.2: Data showing percentage protection of ethanolic extract of leaves of *Biophytum sensitivum* L. against Histamine induced bronchoconstriction in guinea pigs.**

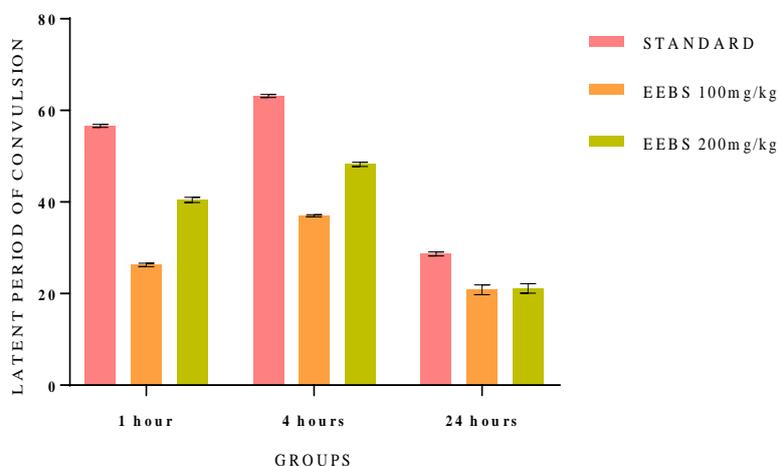
Groups	1 Hour	4 Hours	24 Hours
Standard	71.75%	74.66%	44.25%
EEBS (100mg/kg)	43.23%	59.72%	28.53%
EEBS (200mg/kg)	61.31%	67.53%	25.93%

## DISCUSSION

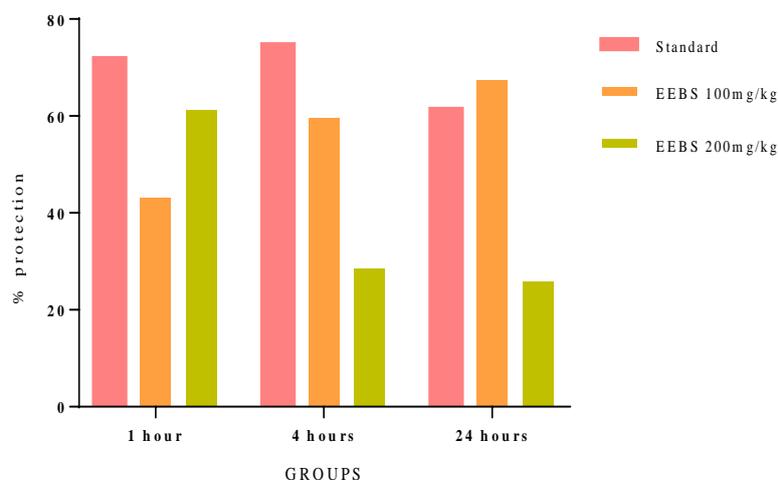
**Histamine induced bronchoconstriction in pigs:** The ethanolic extracts of leaves of *Biophytum sensitivum* L shows dose dependent effect in prolonging the preconvulsion time(PCT) in guinea pigs induced by histamine(0.2% w/v).

The dose of ethanolic extract of *Biophytum sensitivum* L. 200mg/kg p.o. extended the latent period of convulsions followed by exposure to Histamine aerosol at a time interval of 4 hours and showed the maximum percentage protection of 67.53% compared with the standard drug

Chlorpheniramine maleate (1mg/kg) p.o. which showed the protection of 74.66% at the same time interval (Table No.1 &2).



**Fig No.1: Effects of ethanolic extracts of *Biophytum sensitivum* L against the histamine induced bronchoconstriction in guinea pig.**



**Fig No.2: % protection of ethanolic extracts of leaves of *Biophytum sensitivum* L against Histamine induced bronchoconstriction in guinea pigs**

## CONCLUSION

The current study indicates that ethanolic extract of leaves of *Biophytum sensitivum* L possess highly significant antiasthmatic activity by inhibiting Histamine induced bronchoconstriction in guinea pigs by antagonist action in H1 receptors. The result from the present study indicates that the data obtained will be basis for further studies and application of this plant.

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