



## IN-VITRO AND IN-VIVO ANTI-ASTHMATIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF CAESALPINIA PULCHERRIMA

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### ABSTRACT

The ethanol extract of *Caesalpinia pulcherrima* was evaluated for antiasthmatic activity by using *in vitro* and *in vivo* models. *In vitro* models isolated guinea pig ileum preparation was studied to assess smooth muscle relaxant activity of *Caesalpinia pulcherrima*. The study showed that extract is effective against histamine-induced contraction. In isolated guinea pig ileum preparation extract exhibits maximum relaxant effect against histamine at concentrations. In *in vivo* antiasthmatic models, histamine induced bronchospasm in guinea pigs was adopted to study the antiasthmatic activity of *Caesalpinia pulcherrima* (200 & 400mg/kg). The preconvulsion time (PCT), i.e. the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion, was noted, followed by preconvulsion dyspnoea was calculated as basal value. Ethanolic leaf extract of *Caesalpinia pulcherrima* enhanced the time of preconvulsive dyspnoea against histamine induced dyspnoea. The results of these studies indicated that ethanolic leaf extract of *Caesalpinia Pulcherrima* exhibited antiasthmatic activity against both *in vitro* and *in vivo* animal models.

**Key words:** *Caesalpinia Pulcherrima*, Antiasthmatic Activity, Histamine and Dyspnoea.

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### INTRODUCTION

Asthma is defined as a common chronic disorder of the airways that is complex and characterized by variable and recurring symptoms, airflow obstruction, bronchial hyper-responsiveness (bronchospasm), and an underlying inflammation (Murray and Nadel, 2005).

It is estimated that over 300 million people around the world suffer from asthma globally and the number of people affected with asthma in the current

rising trends will grow by more than 100 million by 2025 (Holgate *et al.*, 2011). Asthma management involves both acute and long-term treatment. Medication selection hinges on the patient's age, disease severity, and comorbidities. Be sure to obtain a complete medication history before the patient starts taking asthma medications. Some drugs, including betablockers, angiotensin-converting enzyme inhibitors, cholinergics, and non potassium-sparing diuretics, may be contraindicated for patients receiving certain asthma agents (Kaufman, 2011). Long-term use can lead to high blood pressure, muscle weakness, cataracts, osteoporosis, decreased ability to resist infection, and reduced growth in children (Johnson, 2010). Despite the availability of a broad range of drugs for the treatment of asthma, the relief offered by them is mainly symptomatic. Moreover, the side effects of these drugs are also fairly disturbing.

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Hence, a continuous search is on going to identify effective and safe remedies to treat bronchial asthma. Therefore, it is necessary to look for new solutions to manage asthma. Although many drugs and interventions are available to manage asthma, in most instances these are expensive for a developing country like India and have several adverse effects. India is a country with a vast reserve of natural resources and a rich history of traditional medicine.

Keeping the above in the mind, and to justify the traditional claim, the study was aimed at investigating the anti-asthmatic property of the ethanolic leaf extract of *Caesalpinia pulcherrima*, by assessing its effect on histamine-induced bronchospasm. *Caesalpinia pulcherrima* belonging to the family Fabaceae, which grows well in all kinds of soil including sand, clay, loam, acidic or alkaline soils.

The wood is bitter, dry, sour, cooling; cure "vata" biliousness, fever, delirium, ulcers, strangury, urinary concentration and blood complaints. It is considered astringent and sedative. It is useful in vitiated condition of pitta. An infusion of the wood is a powerful astringent and emmenagogue. It is used in atonic diarrhea and dysentery, and its paste in rheumatism, hemorrhages and to treat wounds. Hot aqueous extract and chloroform extract of wood exhibited inhibitory action on cyclic AMP phosphodiesterase. The methanolic extract of the *Caesalpinia pulcherrima* lignum showed sleep time-elongation effect in mice and significant anti-hypercholesteramine activity. Brazilin dye is reported to have anti-inflammatory activity (Pawaret al., 2008). The trunk wood possesses antibacterial, demulcent and haemostatic properties. It is used in contusion, wounds, dysmenorrhoea, impetigo, leucorrhoea and anaemia (Kirtikar and Basu, 1987). El-Nashar HAS et al., 2015, investigated several pharmacological activities by using extracts of different plants of *Caesalpinieae* tribe were reported to possess a wide range of pharmacological activities, including anti-oxidant, anti-bacterial, anti-inflammatory, cytotoxic, anti-diabetic, antifungal, hepatoprotective, gastroprotective, analgesic, anti-arthritis, anti-filarial, antimalarial, anthelmintic, amoebicidal, diuretic, anti-psoriatic, anti-estrogenic, anti-fertility, wound-healing, anxiolytic, cardioprotective, immunomodulatory and anti-HIV activities. Traditionally the leaves of *Caesalpinia pulcherrima* was used for the treatment of breathing problems. The aim of the study is to evaluate the *invitro* and *invivo* anti-asthmatic activity of ethanolic leaf extract of *Caesalpinia pulcherrima* in guinea pigs.

## MATERIALS AND METHODS

### Plant Collection

The leaves of *Caesalpinia pulcherrima* were collected from the outskirts of Erode district. The plant was identified as *Caesalpinia pulcherrima* and

authenticated by the botanist, Botanical Survey of India, Agricultural University, Coimbatore. The voucher specimen (BSI/SRC/11/72/2017-18/Sci/01297) has been deposited in the herbarium for future reference.

### Preparation of Extract

The collected leaves were washed in running tap water to remove soil debris, shade dried and ground to coarse powder. The powder was then subjected to exhaustive extraction using 90% ethanol, at room temperature for 7 days by maceration. The ethanolic extract is concentrated by vacuum distillation to dry. The collected extract was stored in desiccators and used for further pharmacological study.

### Animals

Guinea pigs of either sex, weighing between 280 - 350 gm were used for this study. The animals were obtained from animal house, of Kerala Veterinary and Animal Science University, Mannuthy. On arrival, the animals were placed at random and allocated to treatment groups in stainless steel cages. Animals were housed at a temperature of  $24 \pm 2^\circ\text{C}$  and relative humidity of 30 - 70 %. A 12:12 light: day cycle was followed. All animals were permitted to free access to water containing Vitamin C and feed with standard pellet raw chaw. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines.

### *In-vitro* Antiasthmatic Activity

#### Isolated Guinea Pig Ileum Preparation

The guinea pig (overnight fasted) was sacrificed and ileum was mounted in an organ bath containing tyrode solution. The tyrode solution was continuously aerated and maintained at  $37 \pm 0.5^\circ\text{C}$ . The tissue was allowed to equilibrate for 30 min. under a load of 500 mg, contact time of 30 sec. and the response of histamine was recorded by 5 min time cycle. After obtaining a dose response curve of histamine ( $10\mu\text{g/ml}$ ) on ileum, ethanolic leaf extract of *Caesalpinia pulcherrima* ( $100\mu\text{g/ml}$ ) was added to the reservoir and same doses of histamine were repeated in presence of plant extract. Same procedure was repeated for standard drug (CPM  $10\mu\text{g/ml}$ ) as that of extract. 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 plant extract and standard drug (PranaliPandit et al., 2008).

### *In-vivo* Antiasthmatic Activity

Histamine Induced Bronchospasm in Guinea Pigs (Gokhale and Saraf, 1996)

Overnight fasted Guinea pigs were divided into four groups (n=6): All the animals are randomly divided into 4 groups each containing six animals. The animals of groups I received 0.1% Carboxy Methyl Cellulose (vehicle) and served as a control. Group II received Chlorpheniramine maleate 2 mg/kg p.o and served as standard. Group III and IV received 200 and 400mg/kg of ethanolic leaf extract of *Caesalpinia pulcherrima* respectively. The test drugs are administered orally using oral tubes by suspending the extract in 0.1 % CMC. Bronchospasm was induced in Guinea pigs by exposing them to histamine aerosol (0.2%) produced by an ultrasound nebulizer in an aerosol chamber (24×14×24 cm) made of Perspex glass. The time required for appearance of pre-convulsive dyspnoea caused by the histamine was recorded for each animal. Prior to drug treatment, 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 onset of dyspnoea leading to the appearance of convulsion, was noted. As soon as the preconvulsion dyspnoea (PCD) was noted, the animals were removed from the chamber and placed in fresh air to recover. This time for preconvulsive dyspnoea was recorded as basal value.

Percentage protection =  $(1 - T1/T2) \times 100$

where, T1 = the mean of PCT before administration of test drugs, and

T2 = the mean of PCT after administration of test drugs

### Statistical Analysis

The values are expressed as mean  $\pm$  SEM. The statistical analysis is carried out by paired 't' test for *invitro* studies and one way analysis of variance (ANOVA) followed by Dunnet's 't' - test for *invivo* studies using graphpad version I. P values <0.05 are considered as significant.

The table 1, shows the effect of Ethanolic Leaf Extract of *Caesalpinia pulcherrima* on histamine induced contraction in isolated guinea pig ileum preparation using

Tyrod as physiological salt solution. The responses were recorded against histamine and the influence of ELECP at various dose levels. The ELECP relaxed the ileum smooth muscles in dose dependent manner against the contraction induced by the histamine. The muscle relaxant effect produced by ELECP was similar as that of the reference control Chlorpheniramine maleate.

In response to the effect of ELECP against the histamine induced contraction in guinea pig ileum preparation, the concentration response curve was plotted and shown in the graph 1. In the control, due to histamine induced control, the graph was in the left side. In Chlorpheniramine and ELECP exposed, the graph was towards left side which, indicate the ELECP inhibited the histamine induced contraction and produced relaxation of guinea pig ileum.

Effect of ELECP on histamine induced bronchospasm in guinea pigs was studied and pre convulsive dyspnoea was observed at different time intervals. Additionally percentage protection was also measured as an antiasthmatic parameter and the results were shown on table.2. The preconvulsive dyspnoea of control at 1hr, 4hr and 24hr was 7.50 $\pm$ 0.43, 7.83 $\pm$ 0.48 and 7.50 $\pm$ 0.42 secs respectively. The preconvulsive dyspnoea of reference control Chlorpheniramine at 1hr, 4hr and 24hr was 20.50 $\pm$ 0.85, 26.00 $\pm$ 1.57 and 23.25 $\pm$ 1.18 secs respectively. The percentage protection at 24hr of reference control was 70.62%. The preconvulsive dyspnoea of ethanolic leaf extract of *Caesalpinia pulcherrima* 200mg/kg was at 1hr, 4hr and 24hr was 12.67 $\pm$ 0.49, 19.17 $\pm$ 1.25 and 19.00 $\pm$ 0.63 secs respectively. The percentage protection at 24hr of reference control was 61.42%. The preconvulsive dyspnoea of ethanolic leaf extract of *Caesalpinia pulcherrima* 400mg/kg was at 1hr, 4hr and 24hr was 18.33 $\pm$ 0.67, 21.50 $\pm$ 0.43 and 21.33 $\pm$ 1.02 secs respectively. The percentage protection at 24hr of reference control was 67.18%. Both the doses of ethanolic leaf extract of *Caesalpinia pulcherrima* showed equipotent effect as that of reference control Chlorpheniramine Maleate.

## RESULTS

### *In-vitro* Antiasthmatic Activity: Isolated Guinea Pig Ileum Preparation

**Table 1. Effect of Ethanolic Leaf Extract of *Caesalpinia Pulcherrima* (ELECP) on histamine induced contraction in isolated guinea pig ileum**

S.No	Concentration of Histamine	Isolated Guinea Pig Ileum (% Contraction)		
	2.5 $\mu$ g/ml	Control	Reference Control (CPM)	ELECP (2.5 $\mu$ g/ml)
1	0.1(0.25 $\mu$ g)	29.01 $\pm$ 1.06	14.55 $\pm$ 0.95***	9.09 $\pm$ 0.22
2	0.2(0.50 $\mu$ g)	38.18 $\pm$ 1.75	23.64 $\pm$ 1.05*	20.00 $\pm$ 0.62*
3	0.4(1.00 $\mu$ g)	70.71 $\pm$ 1.95	30.91 $\pm$ 1.63***	30.91 $\pm$ 1.66***
4	0.8(2.00 $\mu$ g)	83.64 $\pm$ 1.35	56.36 $\pm$ 1.87**	52.72 $\pm$ 2.85**
5	1.6(4.00 $\mu$ g)	98.18 $\pm$ 2.63	63.64 $\pm$ 2.22**	72.73 $\pm$ 2.37**
6	3.2(8.00 $\mu$ g)	100.00 $\pm$ 2.78	63.64 $\pm$ 2.16**	74.54 $\pm$ 1.22**

Values are in mean  $\pm$  SEM (n=6), \*P<0.05 , \*\*P<0.01, \*\*\*P<0.001 Vs Control

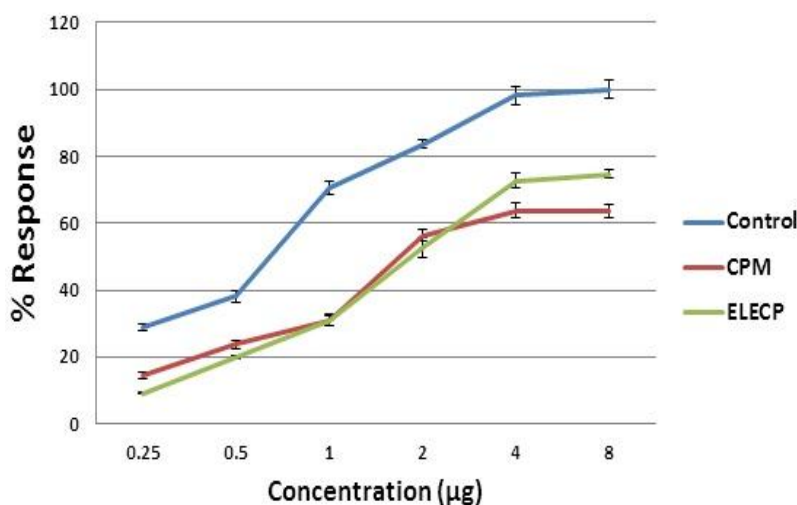
**Table 2. Effect of Ethanolic Leaf Extract of *Caesalpinia pulcherrima* (ELECP) on histamine induced bronchospasm in guinea pigs. (Percentage Protection was given in parentheses)**

Groups & Drug Treatment	Pre Convulsive Time (Secs)	Preconvulsion Dyspnea (secs)		
		1 hr	4 hr	24 hr
Group I 0.1 % CMC	7.17±0.60	7.50±0.43 (4.40%)	7.83±0.48 (8.42%)	7.50±0.42 (4.40%)
Group II Chlorpheniramine Maleate (2mg/kg)	6.83±0.49	20.50±0.85*** (66.68%)	26.00±1.57*** (73.73%)	23.25±1.18*** (70.62%)
Group III ELECP (200mg/kg)	7.33±0.71	12.67±0.49** (42.14%)	19.17±1.25*** (61.76%)	19.00±0.63*** (61.42%)
Group III ELECP (400mg/kg)	7.00±0.58	18.33±0.67*** (61.81%)	21.50±0.43*** (67.44%)	21.33±1.02*** (67.18%)

Values are in mean ± SEM (n=6), \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 Vs Control.

**Graph 1. Effect of Ethanolic Leaf Extract of *Caesalpinia Pulcherrima* (ELECP) on histamine induced contraction in isolated guinea pig ileum preparation**

Effect of Ethanolic Leaf Extract of *Caesalpinia Pulcherrima* (ELECP) on histamine induced contraction in isolated guinea pig ileum



## CONCLUSION

From the results of both *in-vitro* and *in-vivo* models, it was concluded that, *Caesalpinia pulcherrima* leaves possess antiasthmatic activity. *Caesalpinia pulcherrima* contains the phytoconstituents like flavonoids and phenolic compounds which may be responsible for its antiasthmatic activity. Additionally the

antioxidant property of *Caesalpinia pulcherrima* might also be one of the reasons for its above said activity. These findings scientifically validated the traditional claim of *Caesalpinia pulcherrima* for treating asthma in the folk medicine. Further the study may be extended to isolate and validate the active principal which is responsible for the antiasthmatic activity.

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