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"Evaluation of Anti-Asthmatic Potential Activity of *Azima Tetracantha Lam* Leaves Extract in Mice by Clonidine Induced Catalepsy Model"

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Abstract:
Azima tetracantha lam (family: Salvadoraceae), commonly known as Thella Uppichettu (Telugu) is used for its medicinal values in India since ancient time. The present study was under taken to evaluate the anti-asthmatic property of ethanolic extract of *Azima tetracantha* (EEAT) leaves at a dose range of 100 and 200 mg/kg using clonidine induced catalepsy in mice method. Phytochemical screening of EEAT evidenced the presence of alkaloids, flavonoids, glucosinolates, steroids, saponins and tannins. From the results it was controlled ethanolic extract of *Azima tetracantha* lam has shown significant in the anti-asthmatic activity. The resultant anti asthmatic activity may be due to the presence of alkaloids and glucosinolates.

Keywords: *Azima tetracantha lam*, anti-asthmatic activity, clonidine induced catalepsy in mice.

Introduction

One of the most typical illnesses seen in clinical practice is asthma. Around the world, 300 million people have asthma. and an additional 100 million new cases will be added by the year 2025[1]. It is unknown how prevalent asthma actually is in India. Although earlier studies reported a standard prevalence of 3% (more than 30 million asthmatics in India), there is growing evidence to suggest that this may be a gross under estimate[2]. The prevalence of childhood asthma across 16 different centers in India has been reported to range from 2.2% to 22% with a mean of around 5.5%.

Although asthma is frequently thought of as a lung disorder, recent research suggests that it may be a part of systemic airway disease involving the entire respiratory tract, and this is supported by the fact that asthma frequently coexists with other atopic disorders, particularly allergic rhinitis[3]. Asthma is defined as a chronic inflammatory disease of the airways. The chronic inflammation is along with airway hyper-responsiveness (an exaggerated airway constriction to specific stressors such as viruses, allergens and exercise) that leads to recurrent episodes of wheezing, breathlessness, chest tightness and/or coughing that can vary over time and in intensity.[4]

Materials and Methods

1.Plant material:

The fresh leaves of *Azima tetracantha lam* were collected from local areas in Anantapur, Andhra Pradesh. The plant material was taxonomically verified and authenticated by botanist Sara Pala party, Pithapur rajah's government college, Kakinada.

2. Extraction:

The fresh leaves of *Azima tetracnatha lam* were air dried under shade after 10 days of drying the leaves were powered using a mixer and passed through 40 mesh sieves. The ethanolic extract was prepared by using soxhlate apparatus, a t room temperature and then concentrated by using rotary evaporator at 50^0 C. The dried extract was stored in air tight container for further use.

3. Drugs and chemicals:

The drugs used were: clonidine (Unichem, India), and chlorpheniramine maleate (Pfizer Ltd.); all were purchased from a commercial source. Chemicals used were: ethanol AR (PCL, India) and Tween 80 AR (PCL, India).

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4. Experimental animal

All experimental procedures were carried out as per the guidelines prescribed by the committee for the purpose of control and supervision of experimentation on animals (CPCSEA) and were approved by the institutional animal ethics committee. Swiss albino mice weighing between 25 and 30 gm were used. It is housed in the Chalapathi Institute of Pharmaceutical Sciences, Guntur. Animals were divided into 5 groups (each group consists of 5), and maintained under standard laboratory conditions of temperature (25 2^0C); 12 hr. light and dark cycle. The distribution of animals into groups was carried out according to the experimental protocol. Separate groups of fresh animals were used for each experiment.

5. Ethical Approval

All the protocols were approved by Institutional Animal Ethical Committee (IAEC; Approval No: 09/IAEC/CLPT/2020-21; Dt: 05/12/2020) of Chalapathi Institute of Pharmaceutical Sciences, Guntur and aall the experimental procedures were carried out as per the guidelines prescribed by the committee for purpose of control and supervision on experimentation on animals (CPCSEA, Reg. no: 1048/PO/Re/S/07/CPCSEA.

6. Preliminary Phytochemical Analysis

Qualitative screening of EEAT leaves was performed for the identification of different classes of active chemical constituents. The tests were performed using standard procedures and the results of preliminary phytochemical study were tabulated in Table- 1

7. Clonidine induced catalepsy in mice: [5]

Bar test was used to study the effects of the test drugs on clonidine induced catalepsy. Mice were divided into five groups (n=5).Animals belonging to group I served as control and were administered the vehicle (ml/kg, p.o) Animals belonging to group II received chlorpheniramine maleate (10mg/kg intraperitoneal) as standard. Animals belonging groups to III and IV received *Azima tetracantha lam* doses (100,200 mg/kg, p.o) respectively[6]. Animal belonging to group V received only clonidine.

The forepaws of mice were placed on horizontal bar (1cm in diameter, 3cm above the table) and the time required to remove the paws from the bar was noted for each animal. All the groups received clonidine(1mg/kg, subcutaneously),1hour after receiving the drug the duration of catalepsy was measured at 15,30,60,90,120,150,and 180 min[7].

Bar test apparatus

Clonidine induced Catalepsy mice

8. Statistical analysis:

The data are presented as mean ± SEM. The data was analyzed by one-way ANOVA followed by bar test. Prism Graph Pad 3 was used for statistical analysis. P< 0.0001 was considered as significant.

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RESULTS AND DISCUSSION:

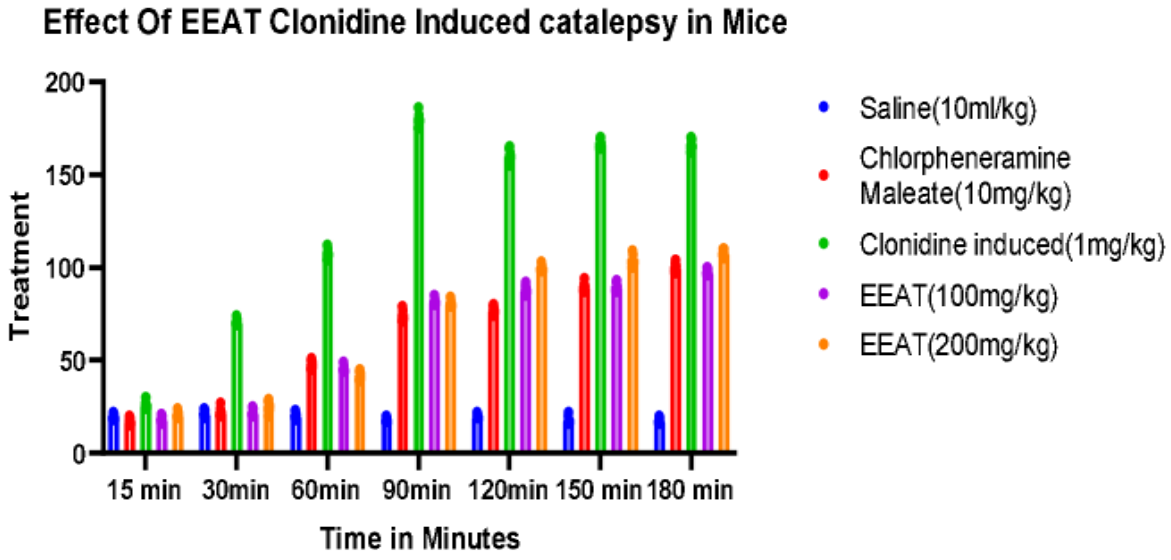
Table No – 1: Phytochemical analysis of EEAT

Phytoconstituents	Inference
Alkaloids	+
Glucosinolates	+
Saponins	+
Steroids	+
Flavonoids	+
Phenols	+
Tannins	+
Fats	-
Proteins and amino acids	+

Note: + Indicates present
- Indicates absent

Table No-2: Effect of Ethanolic Extract of *Azima tetracantha lam* By Clonidine Induced Catalepsy in mice.

Groups	15mins	30mins	60mins	90mins	120mins	150mins	180mins
Control	20±0.70	21.8±0.86	20.6±0.81	18.2±0.58	20.4±0.50	18.6±1.077	18±0.70
Standard	17.8±0.86****	22.8±1.24****	48.4±1.07****	75±1.51****	77.6±1.02****	90.2±1.15****	100.8±1.15****
Induced	26.4±12	71.2±0.86	108.2±1.35	180.4±1.80	161.2±1.77	167.2±1.06	166.6±1.43
EEAT 100mg/kg	18.8±0.86****	22.4±0.92****	46±0.89****	82.2±0.86****	88.8±1.15****	90.2±1.06****	98±0.70****
EEAT 200mg/kg	21.2±0.86****	25.6±1.56****	42.2±1.15****	81.2±0.86****	100.2±0.86****	104.4±1.63****	108.2±0.96****



The data represented in mean ± SEM (n = number of animals in each group = 5). The comparisons made by one-way ANOVA followed by bar test' test EEAT. ****p < 0.0001 is considered as mild significant compared to control group.

DISCUSSION:

Clonidine, a α_2 adrenoreceptor agonist, induces dose-dependent catalepsy in mice, which was impeded by the H_1 receptor antagonist but not the H_2 receptor antagonist. Clonidine releases histamine from mast cells, which are responsible for different asthmatic problems. The catalepsy caused by clonidine is mediated by histamine via H_1 receptors. The current study discovered that chlorpheniramine maleate (10mg/kg, ip) and EEAT doses (100, 200mg/kg P.O) inhibit catalepsy in a dose-dependent manner [9].

Clonidine produces catalepsy in mice. The positive control treatment group demonstrated the longest duration of catalepsy at 90 minutes following clonidine injection. There was significant inhibition of clonidine-induced catalepsy in the animals pretreated with EEAT. This demonstrates *Azima tetracantha* Lam's anti-asthmatic activity[10].
Clonidine (1 mg/kg, i,p) produced catalepsy in mice, which remained for 3 hr. The vehicle treated group showed maximum duration of catalepsy (180.4±1.80.) at 90 minute after the administration of clonidine. Significant (****p< 0.0001) inhibition of catalepsy is seen in EEAT doses (100, 200mg/kg P.o) of mice

CONCLUSION:
Present study concluded that the drugs having anti asthmatic potential inhibits clonidine induced catalepsy, EEAT leaves possesses anti asthmatic activity for future scope of present investigation is isolate active phytoconstituents which is responsible for anti-asthma tic activity. The results of preliminary phytochemical investigation of ethanol extract revealed the presence of an array of active constituents including alkaloids, glucosinolates, flavonoids, steroids. The anti-asthmatic activity may be due to the presence of alkaloids, glucosinolates.

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