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Original Article

EFFECT OF METHANOLIC EXTRACT *CALOTROPIS PROCER* (Ait) R.Br AGAINST CHLORINE INDUCED ASTHMA IN MICE

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ABSTRACT

Among several respiratory diseases affecting man, bronchial asthma is the most common disabling syndrome. The therapy of asthma usually employs steroids or disease modifying drugs. The long-term use of these, however, may not limit the disease progression. All of these drugs have side effects and the search for a novel anti-asthmatic drug continues. There is a dire need to identify effective and safe remedies to treat bronchial asthma. The present study deals with the effect of methanolic extract of leaves by using animal model. This study is based on the effect of *Calotropis procera* leaves extract on chlorine induced asthma in Albino mice. The study shows that extract is effective against chlorine induced asthma. CPME at 250 mg/kg showed a significant affect against Cl_2 induced asthma when compared to all other groups.

Keywords Potential Antiasthmatic, Chlorine, *Calotropis Procera* leaves.

INTRODUCTION

Asthma is a chronic inflammatory disease that may occurs in the smaller airways of the lungs. The occurrence of asthma increases as communities adopts western lifestyles and become urbanized, and the prevalence is expected to increase to 400 million by 2025 [1, 2]. *Calotropis procera* has been reported as a traditional folkloric medicine in treatment of asthma in the Indian literature. Phytochemical studies on *Calotropis procera* have afforded several types of compounds such as Cardenolide, triterpenoids, alkaloids, resins, anthocyanins and proteolytic enzymes in latex, flavonoids, tannins, sterol, saponins, and cardiac glycosides. Flowers contain

-terpenes, multiflorenol, and cyclisadol.The leaves contain mainly the amyrin, amyrin acetate, ß-sitosterol, urosolic acid, Cardenolide, calotropin, calotropagenin [3-6].

MATERIALS AND METHODS

Plant material

The Leaves (2 kg) of *Calotropis procera* was collected from surrounding field of Guntur district of Andhra Pradesh between October-November, 2014.The leaves was authenticated by Dr.P.SATYANARAYANA RAJU,Plant Taxonomist, Department of Botany and microbiology, Acharya Nagarjuna University, Nagarjuna nagar, Guntur. The shade-dried leaves were ground. It was stored in an airtight, hard polyethylene container 10-12 days.

Preparation of Methanolic extracts

Leaves were shade dried and powdered. The powder and the solvent were poured into a separating funnel. Allowed it to drain down. Filtrate was collected into a conical flask and concentrate using evaporating dish over boiling water. Extract was collected in weighing bottle and stored.

Experimental animals

All animals were housed at ambient temperature (22±1°C), relative humidity (55 ±5° %) and 12:12 hrs light/dark cycle. Animal had free access to standard pellet diet and water ad libitum. The Protocol approved by the institutional animal ethics committee (IAEC) as per the guidance of committee for the purpose of control and Supervision of Experiments on animals (CPCSEA), Government of India.

SCREENING OF ANTI ASTHMATIC ACTIVITY:

Experimental design

Male Swiss albino mice weighing range of 25-35g were used and divided into four groups, six animals in each group.

- Group I - Control (Normal air) **[7]**
- Group II - Disease control (100 ppm of chlorine)
- Group III -Standard (Dexamethasone) 50mg/kg [8]
- Group-V - Methanolic extracts of *Calotropis procera* 250 mg/kg [9]

The animals will be treated with the selected doses of test and standard compound one hour before the induction of asthma. After 1 hr of the administration of the dose of treatment, the animals were sensitized by chlorine at the dose of 100 ppm (parts per million) for 5 min by using a nebulizer, that is connected to a closed chamber. After 24 hrs of exposure the animals were sacrificed.

Collection of broncho alveolar lavage fluid

The animals were anaesthetized under diethyl ether as anesthesia. Then expose the trachea and introduce a 0.5ml of sterile saline into lungs via a needle/ a Polyethylene tube. Withdraw the BALF into a test tube. Collected fluid was centrifuged at 3000 RPM for 10 min. Supernatant was collected and used for estimations.

Cell counts in Broncho alveolar lavage fluid

Total cells were counted using Neubauer chamber and the differential cell count is by making the smears. These slides were stained with Geimsa stain and eosin stain. A differential cell count was determined on a minimum of 300 cells.

Lung histology

After24 hrs of Chlorine sensitization lung tissues was removed. Following harvesting, the lungs were perfused with saline until the effluent was clear. Kept in formalin at 4^0C for 24h.

Embedding in paraffin vacuum

Hard paraffin was melted and poured into L-shaped blocks. The lung pieces were then dropped into the molten paraffin quickly and allow cooling.

Sectioning

The blocks were cut using microtome to get sections of thickness of 4µ. The sections were taken on a microscopic slide on which sticking substance was applied.

Staining

Eosin is an acid stain; hence it stains all the cell constituents pink which are basic in nature. Hematoxylin, a basic stain which stains all the acidic cell components blue. The strained sections were observed microscopically for its histological changes produced by chlorine induced asthma in albino mice. The ability of the extracts to prevent the Chlorine induced changes was determined histologically.

RESULTS AND DISCUSSION

Table 1: Effect of extracts of *C.Procera* on differential cell count on Cl_2 induced asthma in mice.

Types of cells	Groups(n=6) Mean±SEM			
	Control	Disease control(Cl_2)	Dexamethasone	CPME
Macrophages	8.667±2.512***	18.33±1.11	7.00±0.57***	7.333±1.80***
Eosinophils	4.667±0.8819***	19.00±2.76	4.83±0.65***	14.17±1.66
Neutrophils	8.833±0.8724***	15.83±0.98	7.16±1.74***	8.000±0.57***
Lymphocytes	10.76±1.022***	20.83±2.28	11.17±1.57***	5.667±1.02***
Epithelial cells	4.667±0.889**	14.50±1.94	14.67±1.58	7.833±2.05*
Total cells	46.17±6.44	70.17±9.79	43.33±5.82*	44.83±5.75*

Values are Mean±SEM, n=6, one way ANOVA followed by Dunnett multiple comparison test. *, **, ***P<0.05, when compared with asthmatic control.

Table 2:Histopathological analysis :(Qualitative method)

Group	Dose	Route of administration	Scoring grade(0-4)
			Inflammation
Control	-	-	0.33± 0.33**
Disease control(Chlorine)	100 ppm	Inhalational	3.00± 0.57
Dexamethasone	50mg/kg	Per oral	1.00± 0.57*
CPME	250mg/kg	Per oral	1.66± 0.33

Values are Mean±SEM, n=6 one way ANOVA followed by Dunnett multiple comparison test. *P<0.05, when compared with asthmatic control.

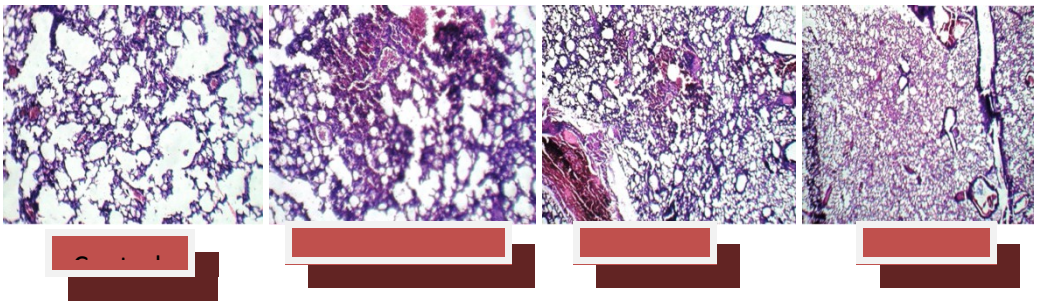

Table 3: Histological analysis (Quantitative method)

Group	Dose	Route of administration	Scoring grade(0-4)
			Eosinophils
Control	-	-	0.66± 0.33
Disease control(Chlorine)	100 ppm	Inhalational	2.00± 0.57
Dexamethasone	50mg/kg	Per oral	0.66± 0.33
CPME	250mg/kg	Per oral	1.00± 0.57

DISCUSSION

The present study deal with the screening of antiasthmatic activity of various extracts of leaves of Calotropis procera. Bronchial asthma is a chronic inflammatory disease, characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyper responsiveness to various stimuli, in which many type of cells play a role such as macrophages/monocytes, lymphocytes, epithelial cells, eosinophils, and neutrophils and also total cells.

Chlorine is a very toxic gas and exposure to levels as low as 1 ppm for a few minutes can irritate the eyes, nose, and throat. Chlorine also has a strong irritant effect on the airways and may increase BHR [Bronchial hyper responsiveness].The occupational exposure standard for chlorine is 0.5 ppm time-weighted average over 8 hours, or 1 ppm over 10 minutes. Chlorine can be detected by smell at around 1 ppm.

The selected plant species have been used in the traditional systems of medicine for treating various ailments including asthma. Importantly, there was no scientific evidence for the anti-asthmatic activity of these plants. The present study validates traditional claims of these plants.

In my study, thr methanolic extract is prepared to evaluate the anti asthmatic activity. For that evaluation the mice are classified into 4 groups (n=6).The groups are Control, Disease control, Standard (Dexamethasone), test group (methanolic extracts of C. procera).CPME at 250 mg/kg showed a significant affect against Cl2 induced asthma when compared to all other groups.

However, further investigation should be carried out to isolate active chemical constituents that are responsible for Anti-asthmatic activity and includes analysis of active principles by UV, IR, MS and NMR spectroscopy along with separation techniques by HPTLC

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