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Research Article

EVALUTION OF ANTIASTHMATIC ACTIVITY OF CALOTROPIS GIGANTEA ROOTS

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ABSTRACT

The present study deals with the effect of ethanolic extract of roots Calotropis gigantea by using various in vivo and in vitro animal models. In vitro model like isolated guinea pig ileum preparation was studies to know basic mechanism by which extract shows relaxant activity. The study shows that extract is effective against histamine induced contraction. Animal studies involve use of histamine induced bronchocontraction. These studies showed significant protection at lower doses while further increase in dose level showed reduced activity. The results of these studies indicated usefulness of ethanol extract of calotropis gigantean in asthma.

Key words: Antiasthmatic, Bronchoconstriction, *Calotropis gigantea*.

INTRODUCTION

Asthma is very commonly occurring condition that is most difficult to control in chronic stage. In the united state alone asthma affects almost 17 million people & this is a 75% increase in the last 20 yrs. This means that about one out of every 20 adults & close to one 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 has alone an estimated 15-20 million asthmatics. Mortality data from developed countries show that the rates varies from 0.1 – 0.8 per 10,000 persons aged 5-34. For managing asthma attack symptomatic relief is foremost requirement. In India, in various traditional systems like Ayurveda, Unani & Siddha numerous herbs were mentioned for therapeutic use in asthma[1].

Calotropis gigantea (Asclepeaceae) is one of the important plants mentioned in Ayurveda Unani for asthma. According to Ayurveda dried whole plant is good tonic, expectorant, depurative, and anthelmintic. The dried root bark is febrifuge, anthelmintic, depurative, expectorant, and laxative. The powered root is used in asthma, bronchitis, and dyspepsia. The leaves are useful in the treatment of paralysis, arthralegia, swelling and intermitant fever. Flowers are bitter, astringent, stomachic, anthelmintic and tonic[2, 3].

Four new chemical constituents including one naphthalene derivative, named calotropnaphthalene, two terpene derivatives, namely calotropises juiterpenol and calotropisesterterpenol and an aromatic product designated as calotropbenzofuranone along with a known compound, sucrose, have been isolated from the roots of the Calotropis gigantea. [4].

MATERIALS AND METHOD

Plant material

The plant of calotropis gigantea was collected from the roadside locations of Aurangabad (Maharashtra) region and was authenticated by department of Botany, BAMU, Aurangabad. Plant material was preserved in pharmacognosy department of Dr. Ved Prakash Patil, college of pharmacy, Aurangabad. The root of the plant was separated, dried and coarsely powdered.

Preparation of plant extract

The dried root bark powder (1.5 kg) was subjected to hot extraction with MeOH by Soxhlet extractor and after evaporation of the solvent 140 g crude extract was found. Twenty gram of the crude MeOH extract was fractionated into petroleum ether fraction (15 g), chloroform fraction (2 g), ethyl acetate fraction (1 g) and aqueous fraction (2 g). After complete extraction, the solvent was removed by distillation under reduced pressure and extract was concentrated to dryness in vacuum. The percentage of ethanol soluble extractives was calculated with reference to air-dried plant material and the yield was found to be 11.18 ± 0.70 % w/w.

Experimental animals

Guinea pigs of either sex (350-450 g) were selected for present study. Six animals were taken in each group and maintained under standard laboratory conditions. They were allowed free access to standard dry pellet diet and water ad libitum during the experiment. Wister rats weighing 150 -250 and Swiss mice of either sex bread at Dr. Ved Prakash Patil College of pharmacy, Aurangabad were housed at standard condition of temperature (22±1º) and 12/12 h light / dark cycle. They were allowed free access to standard dry pellet diet and water ad libitum during the experiment. All experimental procedures were followed in strict accordance with the guideline prescribed by the Committee for the Purpose of Control and Supervision on Experimental on Animals (CPCSEA).

Screening of anti-asthmatic activity

In vitro studies on isolated guinea pig ileum Preparation[4-5]

Overnight fasted guinea pigs were sacrificed using cervical dislocation method. Ileum was quickly dissected out and mounted in an organ bath maintained at 30±0.5°C and containing 20 ml Tyrode's solution under basal tension of 500 mg. The solution was continuously bubbled with air. The responses to drug were recorded on student physiograph using isotonic transducer, which exerted a basal tension equivalent to 500 mg load on tissues. The tissues were allowed to equilibrate for 30 minutes, during which, the bathing solution was changed at every 10 minutes. The contractile responses of ileum to Histamine were recorded in presence and absence of extract of drug.

In vivo studies on Acetylcholine and Histamine induced bronchospasm in guinea pigs

Guinea pigs of either sex (350-450 g) were selected and randomly divided into four groups each containing six animals. The animals were kept on fasting overnight before treatment. The ethanolic extract and standard drug were administered orally in 0.5 % CMC. The single dose treatment was given one and half an hour before the study. Later the animals were exposed to an aerosol of 0.25 % histamine and time for preconvulsion state was observed for each animal as described by Sheth *et al.* (1972)[6]. After 15 days of washout period, the same animals were treated with the above treatment and time for preconvulsion state was observed for 0.5% acetylcholine bromide aerosol spray[7-8].

Haloperidol-induced catalepsy[9]:

Swiss mice were divided into 8 groups (n=6), control group received saline and other groups received single dose of extract (100, 200, 300, 500, 1000, 2000 mg/kg p.o.), respectively. Chlorpheniramine maleate (10 mg/kg) was used as positive control. The entire group received haloperidol (1 mg/kg i.p.) 1 h after the drug administration and the duration of catalepsy was measured at0, 30, 60, 90,120 and 150 min.

Table 1: **Effect of the ethanol extract of calotropis gigantea on histamine-induced contractions**

Dose of Histamine(2.5 ug/ml)	Isolated guinea pig ileum preparation	
ml	Control group % maximum contraction	Test group % maximum contraction
0.1	14.776±0.925	9.058±1.188
0.2	28.748±0.501	20.534±1.618
0.4	58.488±2.511	38.806±2.163
0.8	83.33±2.988	64.446±1.384
1.6	83.33±2.988	64.446±1.384

Effect of EE of calotropis gigantea on the histamine induced contraction on the isolated guinea pig preparation was tabulated. All values are expressed as mean SEM of sample size of n= 6. All treated groups were compared with controlled group.

Table 2: Effect of the ethanol extract of *Calotropis gigantea* on histamine-induced bronchoconstriction

Groups	Dose in mg/kg p. o.	PCT (before) T1	PCT (after) T2	Mean exposition time	% protection
1	Control	1.428±0.029	1.505±0.013	0.077±0.034	5.101
2	50	0.923±0.014	1.581±0.074	0.727±0.006	41.44
3	100	1.156±0.015	2.327±0.111	1.166±0.100	50.95
4	200	1.145±0.010	8.595±0.163	7.448±0.076	86.67
5	300	1.287±0.037	4.411±0.138	3.124±0.148	72.24
6	500	1.336±0.032	3.208±0.014	1.873±0.028	58.331
7	1000	1.215±0.065	1.266±0.018	0.074±0.043	7.00
8	CPM(2mg/kg)	0.907±0.003	10.796±0.103	9.898±0.099	91.59

All values are expressed as mean SEM of sample size of n= 6. All treated groups were compared with control group.CPM is Chlorpheniramine maleate (2mg/kg)

Table 3: Effect of the ethanol extract of *Calotropis gigantea* on haloperidol-induced catalepsy

Group	Dose mg/kg	Duration of catalepsy (sec)at mean SEM				
		30min	60min	90min	120min	150min
1	CONTRL	216.27±0.36	251.5±2.87	265.57±0.27	280.83±3.92	238.45±28.92
2	100	206.16±0.24	234.06±0.71	215.01±0.67	229.00±0.59	205.45±0.47
3	200	198.82±0.44	228.13±0.37	198.17±0.42	208.67±0.51	190.95±1.45
4	300	102.21±0.37	83.35±1.00	70.73±0.27	52.63±0.37	42.2±0.29
5	500	170.83±0.98	207.55±0.42	195.13±0.84	198.67±0.49	180.33±1.09
6	1000	199.27±0.90	216.45±0.73	184.27±0.76	208.65±0.48	192.65±0.75
7	2000	203.48±0.40	235.5±0.54	219.53±0.39	226.25±0.33	208.33±0.54
8	CPM(10mg/kg)	89.55±0.54	66.46±0.39	52.53±0.28	32.75±0.35	53.23±0.53

All values are expressed as mean SEM of sample size of n= 6. All treated groups were compared with control group.CPM is Chlorpheniramine maleate (2mg/kg)

Table 4: Effect of *Calotropis gigantea* ethanol extract on passive paw anaphylaxis

Group	Dose mg/kg	Paw edema volume (ml) mean SEM			
		1h	2h	3h	4h
1	Control	0.923±0.02	0.75±0.01	0.626±0.09	0.56±0.05
2	50	0.521±0.06	0.401±0.06	0.343±0.04	0.311±0.01
3	100	0.731±0.03	0.535±0.03	0.433±0.03	0.358±0.02
4	200	0.535±0.03	0.321±0.03	0.331±0.02	0.246±0.02
5	300	0.64±0.03	0.361±0.03	0.431±0.03	0.43±0.03
6	500	0.55±0.02	0.465±0.01	0.486±0.01	0.426±0.01
7	1000	0.587±0.15	0.408±0.11	0.482±0.14	0.401±0.12
8	Dextromethazone (0.27mg/kg)	0.426±0.12	0.239±0.06	0.258±0.08	0.245±0.07

All values are expressed as mean SEM of sample size of n= 6. All treated groups were compared with control group.CPM is Chlorpheniramine maleate (2mg/kg)

Passive paw anaphylaxis in rats [10]

Wistar rats were given subcutaneously in the doses of 100 mg of egg albumin on day 1, 3 and 5. On day 10 of sensitization, blood was collected and centrifuged to separate serum. Animals were divided into eight groups (n=6). Control group received saline and other groups received single dose of extract 50, 100, 200, 300, 500, 1000 mg/kg p.o. Dexamethasone was used as standard (0.27 mg/kg p.o). Prior to drug treatment animals were sensitized with serum. Next 24 h, after drug treatment animals again challenged with 10 mg egg albumin and edema inhibition was calculated.

RESULTS AND DISCUSSION

The present study dealt with screening of antiasthmatic activity of ethanol extract of roots of calotropis gigantea. 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 cell types play a role, more important being mast cells, eosinophils and T- lymphocytes. Different agonists like acetylcholine, histamine, 5-hydroxyltryptamine and bradykinin are responsible for contractile responses[12]. In isolated guinea pig ileum preparation,

there is a right side shift of dose response curve of histamine in the presence of ethanol extract of calotropis gigantea indicating antiasthmatic action [Table1].

Histamine is one of the major inflammatory mediators in the immediate phase of asthma, causing airway hyper responsiveness and bronchial airway inflammation. The study regarding involvement of H1 and H2 receptors has been done in experimental asthma in guinea pig using respiratory smooth muscle and it was confirmed that there is prominent involvement of H1 receptors as compared to H2 receptors especially in asthma [12].

The maximum percentage protection i.e. 86.67 % observed at 200mg/kg dose for bronchorelaxant study comparable with that of standard Chlorpheniramine maleate 91.59%. Statistical significance in post treated exposition time and mean exposition time also showed 200 mg/kg as effective dose. Further increase in the dose showed decreased activity [Table 2].

Haloperidol induces catalepsy by inhibiting dopamine D2 receptors and inhibits dopamine secretion. Dopamine is agonist for adrenaline. Adrenaline is physiological antagonist of histamine. So as there decrease in dopamine there is imbalance in neurotransmitters means high level of histamine [13]. In this study significant protection against haloperidol-induced catalepsy at dose 300 mg/kg. Further increase in the dose showed decreased activity [Table 3].

Allergic asthma is a chronic inflammatory process occurring due to exposure of allergen resulting in the activation of T-lymphocyte with subsequent release of inflammatory mediators. Immuno-modulating agents are useful in the treatment of asthma by inhibiting the antigen-antibody (AG-AB) reaction and there by inhibiting the release of inflammatory mediators [13]. Calotropis gigantea has been reported to possess anti-inflammatory activity [14]. Percent inhibition of paw edema volume was calculated and maximum effective dose was observed at 200 mg/kg. at different hour intervals it was found that effect of dose 200 mg/kg was maximum up to 24 h, further percent inhibition goes on decreasing. But still that percent inhibition in paw edema was significantly effective as compare to other doses. Where as, in statistical analysis of paw edema volume it was observed that 200 mg/kg dose had significant effect comparable that with Dexamethasone. Here also observed that further increase in dose decreased activity [Table 4].

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