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IN-VITRO AND *IN-VIVO* EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF CALOTROPIS PROCERA ROOT EXTRACTS OF *CALOTROPIS PROCERA*

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Calotropis procera, Anti-asthmatic, passive paw, Leukocytosis, Goat tracheal chain, Guinea pig.

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ABSTRACT: Objective: The *Calotropis procera* (Ait.) R.Br. (Ascelpidaeaceae) has been used traditionally as anthelmintic, depurative, diaphoretic, emetic, expectorant, febrifuge, and purgative, but no scientific reports are available in the regulation of asthmatic condition. In this present study, *Calotropis procera* (Ait.) R.Br. was selected to investigate scientifically to establish scientific data for its traditional claim. **Materials and Methods:** The anti-asthmatic activity of MECP (Methanolic extract of roots of *Calotropis procera*) and AECP (Aqueous extract of roots of *Calotropis procera*) was studied by using *in-vitro* and *in-vivo* models. The reference drug used was chlorpheniramine (CPM) and Dexamethasone. The *in-vitro* models included isolated goat tracheal chain and guinea pig ileum preparation. Further, the *in-vivo* included histamine-induced bronchospasm, milk-induced leukocytosis, passive paw anaphylaxis model, and haloperidol-induced catalepsy. **Results:** In the *in-vitro* and *in-vivo* models, the oral dose of both MECP and AECP showed significant results as compared to control. Among both extracts, MECP showed better activity in the prevention of asthma. **Conclusion:** Based on phytochemical analysis of the root's extracts of *Calotropis procera* (Ait.) R.Br showed the presence of alkaloids, steroids, glycosides, flavonoids, tannins, and carbohydrates. The methanolic (MECP) and aqueous (AECP) extracts are effective in all the models of asthma as an order of MECP > AECP except AECP > MECP in isolated guinea pig ileum preparation. Therefore it can be concluded that the plant *Calotropis procera* (Ait.) R.Br was found to possess potential anti-asthmatic activity.

INTRODUCTION: Asthma word is derived from a Greek word meaning 'breathless [1]. It is a chronic inflammatory disorder induced by inflammation of the airways and triggered by a genetic predisposition or antigen sensitization.

The main symptoms of asthma include bronchial hyper-responsiveness, increase in mucus production and remodeling and congestion of airways which are due to the infiltration of the immune cells into the lungs, causing lung inflammation [2].

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Eosinophils, neutrophils, mast cells, T lymphocytes and dendrite neuron cells are the inflammatory mediators which play a major role in the development of asthma.

Acute lung inflammation is followed by lung injury leading to pulmonary fibrosis, turning into impairment of gas exchange. Epithelial damage is due to the influx of eosinophils, exudation of plasma wall, airway wall, and basement membrane thickening [3]. The 60 million individuals worldwide are suffering from this disease. In India, it constitutes 0.2% of all deaths and 0.5% of the National burden of diseases [4]. Current therapeutic agents in disease include β2 agonists, anticholinergics, methylxanthines, mast cell stabilizers, anti-inflammatory drugs (inhaled corticosteroids), leukotriene antagonists, anti-immunoglobulin E (anti-IgE) antibody. These agents are administered for a long duration which leads to various adverse effects, like restlessness, muscle tremors, hypotension, hyperglycemia, tachycardia, flushing, convulsions, mood changes and adrenal crisis.

Also, irrational usage of these drugs is a chief hindrance in the treatment of asthma. Primarily, the irrational use of inhaled corticosteroids is known to cause abnormalities in bone growth, and the use of long-acting β2-adrenergic agonists alone may increase morbidity. Moreover, complicated treatment regimens, high cost of treatments, poor inhalation technique, and late results lead to non-compliance and non-adherence to these current anti-asthmatic drug therapies. Thus, to reduce these adverse effects and to improve patient compliance, there is a need for complementary therapies for asthma [5, 6].

It is a fact that many asthmatic drugs are of plant origin, herbal medicine is a promising approach for finding a good solution for this disease [7]. The *Calotropis procera*. (Ascelpidaeaceae) is a soft-wooded, evergreen, perennial, laticiferous shrub and distributed in tropical and subtropical regions of Asia and Africa [8, 9]. It is an evergreen xerophytic plant, generally found in arid and semi-arid habitats [10]. *Calotropis procera* was known as "Rakta arka" in ancient Ayurvedic medicines [11]. The *Calotropis procera* is also known AS Swallow-Wart, Milk Weed, Apple of Sodom (purple-flowered), Vellerukku, Erukku in Siddha, and Tamil [12, 13]. The whole plant contains α and β amyrin, teraxasterol, gigantin, giganteol, isogiganteol, β-sitosterol, and a wax [14]. Roots of the plant contain triterpenes, a new norditerpenyl ester, named Calotropterpenyl ester, mundarol isovalerate and quercetin -3- rutinoside apentacyclic triterpinoids such as Calotropurseny acetate and calotropfriedelenyl acetate, akundarol isovalerate [15]. The roots are alterative, Anthelmintic, depurative, diaphoretic, emetic, expectorant, febrifuge, and purgative; used to treat anasarca, asthma, ascites, and bronchitis, cough, cutaneous diseases, intestinal worms, leprosy, and eczema [16]. The powdered root is used in asthma, bronchitis, and dyspepsia [17]. *Calotropis procera* (Ait.) R. Br. is a wild-growing plant of family Asclepiadaceae that exhibit different pharmacological activities like analgesic [18], antifertility [19], antitumor [20], Anthelmintic [21], spasmolytic [22], anticancer [23], anti-inflammatory activity [2]4, antibacterial activity [25], anti-ulcer activity [26], hepatoprotective [27,] CNS depressant activity [28], anticonvulsant activity [28]. The current pharmacotherapeutic approaches to asthma have several limitations [29, 31]. Also, C. procera shares a close homology with C. gigantea, its con-generic plant [32], which has anti-asthmatic activity [33]. Hence the *Calotropis procera* (Ait.) R.Br. has been selected to scientifically investigate scientific data for its traditional claim for use in asthma [8].

TABLE 1: THE TAXONOMICAL HIERARCHY OF *CALOTROPIS PROCERA* (AIT.) R. BR. (1) IS SHOWN IN AS BELOW

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Asteridae
Order	:	Gentianales
Family		Asclepiadaceae
Genus	:	Calotropis R. Br
Species	:	*Calotropis procera*

MATERIALS AND METHODS

Procurement and Authentication: Roots of *Calotropis procera* were collected from wastelands of Kurukshetra and authentified as *Calotropis procera* Ait. R.Br by Dr. H.B. Singh, Scientist Incharge, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi, where a

voucher specimen (NISCAIR/RHMD/Consult/-2012-13/12/2153/159) has been deposited for further reference. The coarse powder of the plant was then extracted with methanol in the Soxhlet apparatus until it also became color less. A semisolid mass was obtained after evaporating the solvents under reduced pressure and then vacuum dried to yield solid residues. The aqueous extract was also prepared by macerating the 1kg of coarse powder in water for 12 h.

Drugs and Chemicals: Dexamethasone, histamine, chlorpheniramine maleate, and haloperidol were obtained from Ostar Laboratory, India, and various chemicals *i.e.*, calcium chloride, egg albumin, potassium chloride, sodium chloride, sodium hydrogen carbonate, glucose, sodium dihydrogen phosphate, were purchased from Hi-Media, India and Sigma Aldrich laboratory. All the above-mentioned chemicals were used for experimentation.

Phytochemical Screening of Various Extracts: All the extracts of *Calotropis procera* (Ait.) R.Br was subjected to qualitative chemical tests to identify the Phytoconstituents present in the respective extract [34, 35].

Procurements of Animals: Dunkon-Hartley Guinea pigs (350-400 gm), Wistar rats (150-250 gm) and Swiss mice (20-25 gm) of either sex were used for evaluating anti-asthmatic activity. Dunkon-Hartley Guinea pigs (350-400 gm) of either sex were bought from the animal house of NIPER, Mohali. Wistar rats (150-250 gm) and albino mice (20-25 gm) of either sex were bought from Animal House NIPER, Mohali. They were acclimatized in the Animal House of Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, and housed at standard conditions of temperature (22 ± 1 °C) and 12/12 h light/dark cycle kept in polypropylene cages. They were fed with a standard pellet diet and *ad libitum* water. The animals were fasted overnight for the conduction of the experiment.

Acute toxicity Study: The acute toxicity study was carried out on female mice by administering (MECP and AECP) extracts orally at one dose level (5, 50, 300, 2000 mg/kg body weight) only. The dose that shows toxicity signs/mortality is the toxic dose, and $1/10^{th}$ of this toxic dose is considered for therapeutic explorations. Toxicity study of all extracts of *Calotropis procera* roots was performed as per OECD guideline 423. The animals were observed for changes in the skin, fur, eyes and, mucous membranes. The animals were also observed changes in the respiratory, circulatory, autonomic nervous system, central nervous system, somatomotor activity, and behavior patterns. Attention was given to the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma [36].

***In-vitro* Anti-asthmatic Evaluation:**

Isolated Goat Tracheal Chain Preparation: The goat trachea was collected from the slaughterhouse from freshly slaughtered goat and was immediately transferred to a thermostat flask containing cold Krebs's Hansleit solution (4 °C). The trachea was kept in Krebs's Hansleit solution at 4 °C in the refrigerator until used on the next day. The Goat trachea was cut transversely between cartilage segments to give several rings of tracheal muscle. The bioassays of goat tracheal preparation in 10 µg/ml of histamine in Krebs's Hansleit solution and presence of 100 µg/ml of plant extract were carried out to obtain the differences in the dose-response curve. The percentage maximum contractile response was plotted to generate the dose-response curve of histamine in the absence and presence of the plant extract [37].

Isolated Guinea Pig Ileum Preparation: Overnight fasted guinea pigs of either sex weighing 400-600 g were sacrificed using the head blow stunning method. The animal was quickly dissected, and the ileum was taken out, mounted in an organ bath containing 10 ml Tyrode solution under the basal load of 500 mg. The solution was continuously aerated with 6-8 bubbles/minute at 37.5 °C temperature. The responses of the ileum to the drug were recorded on smoked kymograph paper on Sherrington rotating drum. The tissue was allowed to equilibrate for 30 min, during which the bathing solution was changed every 10 min. The bioassays of ileum preparation in 10 µg/ml of histamine in Tyrode solution and 100 µg/ml of plant extract were carried out to obtain the differences in the dose-response curve. The contractile responses of the ileum to agonist

acetylcholine were also recorded in the presence and absence of plant extract [38].

In-vivo Anti-asthmatic Evaluation

Histamine Induced Bronchospasm: Dunkon-Hartley Guinea pigs were divided into 8 groups (n=5). The Control group received distilled water and other groups received a single dose of methanolic and aqueous extracts (50, 100 and 200 mg/kg p.o.). CPM (2 mg/kg) was used as the positive control. Before and after drug treatment, each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. Pre-convulsive time (PCT) was noted from the time of histamine exposure to the onset of dyspnoea leading to the appearance of pre-convulsive dyspnoea (PD) in a sec. The percentage age protection offered by drugs in PCT was calculated for each dose and positive control was calculated by using the following formula

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

Where, T1 = the mean of PCT before administration of test drugs and T2 = the mean of PCT after administration of test drugs [39].

Milk Induced Leukocytosis: Albino mice were divided into 8 groups (n=6). The Control group received distilled water, and the group which received only milk served as an intoxicant. After 1hr of drug treatment, except for control, all other groups received sterilized and cooled milk injection in a dose of 4 ml/kg (s.c.). Total leukocyte count was done in each group before drug administration and 24 h after milk injection [40].

Haloperidol Induced Catalepsy: Albino mice were divided into 8 groups (n=6).The Control group received distilled water and other groups received a single dose of methanolic and aqueous extracts (50, 100 and 200 mg/kg p.o.). CPM (10 mg/kg) was used as the positive control. The entire group received haloperidol (1 mg/kg, i.p.). 1 hr after the drug administration, and the duration of catalepsy was measured at 0, 30, 60, 90, 120 and 150 min 41.

Passive Paw Anaphylaxis: The 100 µg of egg albumin (subcutaneously) were given to Wistar rats subcutaneously, three doses of albumin on day 1st, 3rd and 5th On the 10th day of sensitization, blood was collected from the retro-orbital plexus, and collected blood was allowed to clot, and the serum was separated by centrifugation at 1500 rpm. Animals were divided into 8 groups (n=6). The Control group received distilled water, and other groups received a single dose of methanolic and Aqueous extracts (50, 100, and 200 mg/kg, p.o.). Dexamethasone was used as standard (0.27 mg/kg, p.o.). Before drug treatment, animals were sensitized with serum. The next 24 h, after drug treatment animals, were again challenged with 10 µg egg albumin [42]. Edema volume was represented as a difference in the reading before and after antigen challenge, and the percent inhibition of volume was calculated by using the following formula [41].

$$\text{Percentage inhibition} = 1\text{-} (Vt/ Vc) \times 100$$

Where Vt = Mean relative change in paw volume in test group Vc = Mean change in paw volume in the control group.

Statistical Analysis: All values were expressed as mean ± SEM, and data were analyzed by ANOVA followed by Dunnett's t-test.

RESULTS: The methanolic extract of *Calotropis procera* (MECP) tested positive for alkaloids, carbohydrates, flavonoids, glycosides (anthraquinone, saponin, cardiac), steroids, and protein. The aqueous extract of *Calotropis procera* (AECP) tested positive for alkaloids, carbohydrates, flavonoids, glycosides (saponin), and tannins. All the extracts of *Calotropis procera* (Ait.) R.Br was found safe at the dose of 2000 mg/kg as per OECD guidelines 423.

Evaluation of Anti-Asthmatic Activity Using Goat Tracheal Chain: In the present study, there was a decrease in %age response in the presence of MECP and AECP at a dose of 100 μgmL^{-1} of *Calotropis procera* when compared to histamine (10 μgmL^{-1}) alone but less than standard drug chlorpheniramine (100 μgmL^{-1}). The percentage decrease in response was more in MECP as compared to AECP at a dose of 100 μgmL^{-1} as shown in **Table 2** and **Fig. 1.**

Evaluation of Anti-Asthmatic Activity Using Isolated Guinea Pig Ileum Preparation: The MECP and AECP are subjected to a dose-response

curve using isolated guinea pig ileum preparation. The percentage decrease in response was more in AECP as compared to MECP at a dose of 100 μgmL^{-1} as shown in **Table 3** and **Fig. 2.**

TABLE 2: THE %AGE RESPONSES OF VARIOUS EXTRACTS OF *CALOTROPIS PROCERA* (AIT.) R.BR ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION IN PRESENCE OF HISTAMINE

Groups	Drug(Dose)	% Response					
		0.1 ml	0.2 ml	0.4 ml	0.8 ml	1.6 ml	3.2 ml
Control	Histamine (10µg/ml)	5.2	6.5	26	65	93.5	100
Standard	Histamine (10µg/ml) + CPM (100µg/ml)	2.5	5	9.09	18.18	30	33.7
MECP	Histamine (10µg/ml) + MECP(100µg/ ml)	3.8	5.7	11.03	24.67	30.06	41.05
AECP	Histamine (10µg/ml) + AECP(100µg/ml)	4.4	6.1	13.11	31.8	43.11	50.64

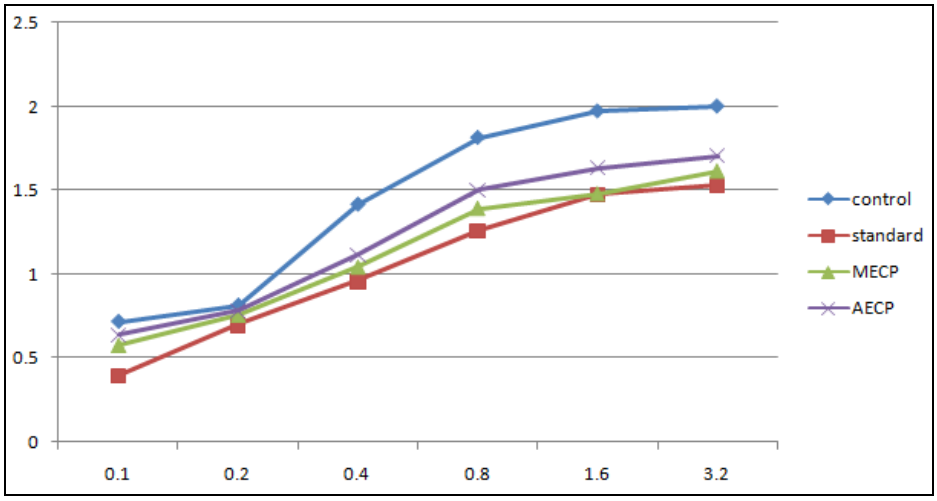

FIG. 1: SHOWING LOG-DOSE RESPONSE CURVE OF EXTRACT AND STANDARD USING GOAT TRACHEAL CHAIN IN PRESENCE OF HISTAMINE.

TABLE 3: SHOWING RESPONSES OF VARIOUS EXTRACTS OF *CALOTROPIS PROCERA* (AIT.) R.BR ON ISOLATED GUINEA PIG ILEUM PREPARATION IN PRESENCE OF ACETYLCHOLINE

Groups	Drug(Dose)	% Contraction				
		0.1 ml	0.2 ml	0.4 ml	0.8 ml	1.6 ml
Control	Acetylcholine(10µg/ ml)	88.23	97.14	100	97.14	100
MECP	Acetylcholine (10µg/ml) + MECP(100µg/ ml)	62.55	73.52	77.14	71.14	74.2
AECP	Acetylcholine (10µg/ml) + AECP(100µg/ml)	60.00	65.21	51.42	37.14	37.14

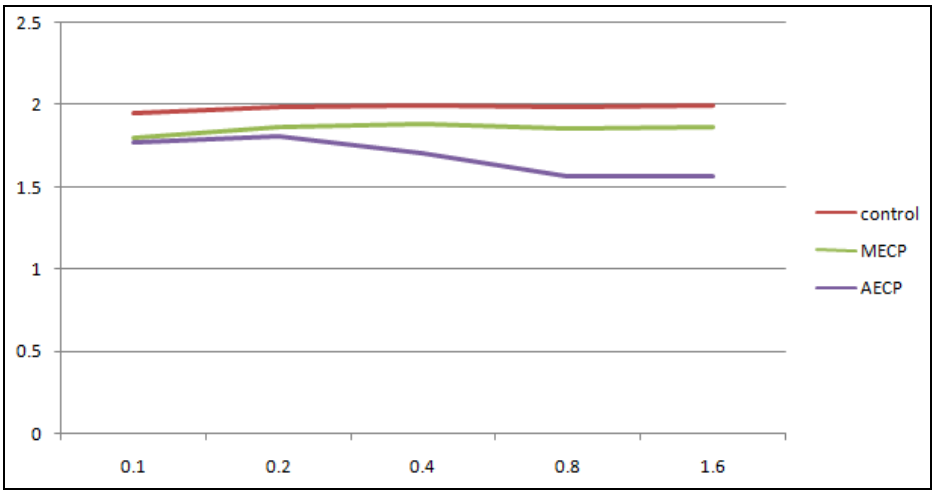

FIG. 2: SHOWING THE LOG-DOSE RESPONSE CURVE OF ACETYLCHOLINE IN THE PRESENCE AND ABSENCE OF GUINEA PIG ILEUM

Histamine-Induced Bronchospasm: All doses of MECP (50 mg/kg, 100 mg/kg and 200 mg/kg) significantly ($p<0.01$) prolonged the mean exposition time followed by exposure of histamine

aerosol (0.2%) whereas AECP (200 mg/kg) ($p<0.01$) and AECP (100 mg/kg) ($p<0.05$) significantly prolonged the mean exposition time The maximum percentage protection provided among all extracts was 50.34% which was offered by MECP 200 mg/kg, comparable to standard chlorpheniramine (2 mg kg^{-1}) 67.10 % as shown in **Table 4.**

TABLE 4: EFFECTS OF VARIOUS EXTRACTS OF *CALOTROPIS PROCERA* (AIT.) R.BR ON HISTAMINE INDUCED BRONCHOSPASM

Groups	Drug (Dose), route	PCT(Before)	PCT (After)	MET	% Protection
Control	Distilled Water (10 ml/ kg)	8.08 ± 1.77	84.8 ± 1.80	4 ± 1.26	4.11
Standard	CPM (2 mg/kg), i.p	100.4 ± 3.66	305.2 ± 3.83	204.8 ± 2.57**	67.10
MECP	50 mg/kg, p.o	77.2 ± 2.26	101.2 ± 4.39	24 ± 2.7 **	23.71
MECP	100 mg/kg, p.o	72.4 ± 2.5	111.4 ± 7.13	39 ± 5.66 **	35.00
MECP	200 mg/kg, p.o	71 ± 1.14	143 ± 6.21	72 ± 7.22**	50.34
AECP	50 mg/kg, p.o	91 ± 4.33	105.4 ± 3	15.8 ± 2.51	14.9
AECP	100 mg/kg, p.o	84.8 ± 5.23	106.6 ± 5.1	21.8 ± 1.49*	20.45
AECP	200 mg/kg, p.o	65.4 ± 2.69	88.4 ± 4.83	23 ± 3.71**	26.01

Values are in Mean ± SEM. One-way ANOVA followed by Dunnett's t-test. Where **p<0.01, * p< 0.05 as compared to control, n=5

Effect of Plant Extracts On Milk Induced Leukocytosis: The mean difference in the number of leukocytes before and after milk treatment were calculated in various groups control, standard, MECP (50, 100 and 200 mg/kg), AECP (50, 100, and 200 mg/kg). All groups showed a significant ($p<0.01$) decrease in the number of leukocytes as compared to the control group, among all the extracts doses, the maximum decrease in the number of leukocytes was observed in MECP 200 mg/kg (1480 mm3), but it was less than standard revealed from **Table 5.**

TABLE 5: SHOWING THE DIFFERENCE IN LEUKOCYTES OF VARIOUS GROUPS IN MICE BEFORE AND AFTER MILK TREATMENT

Treatment	No. of Leukocytes (cu mm)		Difference in Leukocyte count
	Before Treatment	After Treatment	
Control	5180 ± 185.47	8960 ± 128.84	3780
Standard	4660 ± 289.14	5400 ± 300	740**
MECP(50 mg/kg)	4460 ± 283.90	6960 ± 354.4	2700**
MECP(100 mg/kg)	5680 ± 451	7260 ± 471.5	1940**
MECP(200 mg/kg)	4560 ± 280.36	6420 ± 385.49	1480**
AECP (50 mg/kg)	4140 ± 128.84	7340 ± 478.2	3200**
AECP (100 mg/kg)	4640 ± 254.17	7100 ± 273.86	2460**
AECP (200 mg/kg)	5500 ± 234.52	7180 ± 241.60	1680**

Values are in Mean±SEM. One-way ANOVA followed by Dunnett's t-test. Where **p<0.01 as compared to control, n=5

Effect of Plant Extracts On Passive Paw Anaphylaxis: MECP at (100 and 200 mg/kg) showed significant ($p<0.01$) activity at all intervals *viz*. 1 h, 2 h, 3 h and 4 h. MECP at 50 mg/kg showed significant ($p<0.01$) activity at all intervals except at 3 h. AECP showed significant ($p<0.01$) activity at 200 mg/kg (1 h, 2 h, 3 h and 4 h) and 100 mg/kg (1 h). Paw edema volume of various groups at different time intervals is illustrated in **Table 5.**

TABLE 6: SHOWING PAW EDEMA VOLUME OF VARIOUS GROUPS AT DIFFERENT TIME INTERVALS:

Group	1 hour	2 hour	3 hour	4 hour
Control	0.684 ± 0.003	0.570 ± 0.001	0.508 ± 0.01	0.326 ± 0.032
Standard	0.194 ± 0.032**	0.114 ± 0.005**	0.168 ± 0.02**	0.152 ± 0.02**
MECP (50 mg/kg)	0.554 ± 0.038**	0.504 ± 0.01**	0.374 ± 0.05	0.238 ± 0.01*
MECP (100 mg/kg)	0.49 ± 0.002**	0.47 ± 0.009**	0.316 ± 0.012**	0.226 ± 0.05**
MECP (200 mg/kg)	0.374 ± 0.007**	0.35 ± 0.006**	0.274 ± 0.008**	0.206 ± 0.05**
AECP (50 mg/kg)	0.638 ± 0.006	0.55 ± 0.009	0.484 ± 0.012	0.31 2± 0.01
AECP (100 mg/kg)	0.504 ± 0.01**	0.528 ± 0.02	0.470 ± 0.01	0.310 ± 0.08
AECP (200 mg/kg)	0.498 ± 0.01**	0.414 ± 0.007**	0.366 ± 0.08**	0.234 ± 0.023**

Values are in Mean ± SEM. One-way ANOVA followed by Dunnett's t-test. Where **p<0.01, * p< 0.05 as compared to control, n=5

The percentage inhibition of paw volume was more in the case of MECP as compared to AECP. Among all the extracts, maximum inhibition offered 46%, by MECP 200 mg/kg at 3 h followed by standard drug (80% at 2 h). Percentage inhibition of paw volume at various time intervals is illustrated in **Table 7.** and paw edema volume is shown in **Table 6.**

TABLE 7: TABLE SHOWING THE PERCENTAGE INHIBITION OF PAW VOLUME AT VARIOUS TIME INTERVALS

Groups	1 hour	2 hour	3 hour	4 hour
Standard	71.00	80	70	53.37
MECP (50 mg/kg)	19.00	11.5	26.37	26.9
MECP (100 mg/kg)	28.36	17.5	37.7	30.6
MECP (200 mg/kg)	45.53	38.59	46	42
AECP (50 mg/kg)	6.7	3.5	47	4.9
AECP (100 mg/kg)	20.31	7.4	6.2	5
AECP (200 mg/kg)	27.19	27.36	27.9	28.3

Effect of Plant Extracts On Haloperidol Induced Catalepsy: Both MECP and AECP showed significant ($p<0.01$) at the dose of (50, 100, 200 mg/kg) at all the *intervals i.e.* 30, 60, 90, and 120 min. Among all doses of the extracts, MECP and AECP at 200 mg/kg showed maximum activity against haloperidol-induced catalepsy at 30 min *i.e.,* 106 ± 3.62 sec and 106.6 ± 2.08 sec respectively, which was comparable to standard 97 ± 1.28 sec. The duration of catalepsy of various groups at different time intervals is shown in **Table 8.**

TABLE 8: SHOWING THE INFLAMMATION REDUCTION IN HALOPERIDOL-INDUCED CATALEPSY MODEL

Treatment	Time (seconds)				
	30 min	60 min	90 min	120 min	150 min
Control	202.8 ± 15.1	243.4 ± 14.38	266 ±13.43	273 ± 15.91	230 ± 18.70**
Standard	97 ± 1.28**	122.2 ± 2.74**	133 ±3.07**	124 ± 4.01**	98.8 ± 2.88**
MECP(50 mg/kg)	138 ± 5.34**	158 ± 3.89**	180.8 ±5.01**	198 ± 5.40**	184 ± 6.18**
MECP (100 mg/kg)	131.4 ± 3.60**	152.6 ± 4.40**	165.2±3.83**	180.2 ± 3.27**	152.6 ± 5.38**
MECP (200 mg/kg)	106 ± 3.62**	126.6 ± 1.2**	141±1.94**	132.2 ± 3.13**	110.4 ± 3.01**
AECP (50 mg/kg)	144 ± 5.22**	165.2 ± 3.8**	182±5.28**	199 ± 4.72**	175 ± 8.19**
AECP (100 mg/kg)	136 ± 3.0**	154.2 ± 2.92**	168.2±3.89**	183.6 ± 3.40**	155.6 ± 4.06**
AECP (200 mg/kg)	106.6 ± 2.08*	126.8 ± 2.24**	144±2.34**	126.2 ± 3.44**	113.4 ± 1.503**

Values are in Mean ± SEM. One-way ANOVA followed by Dunnett's t-test. Where **p<0.01, * p< 0.05 as compared to control, n=5

DISCUSSION: The present study deals with the screening of anti-asthmatic activity of *Calotropis procera* (Ait.) R.Br using *in-vitro* and *in-vivo* models such as goat tracheal chain isolated preparation (*in-vitro*), isolated guinea pig ileum preparation (*in-vitro*), histamine-induced bronchospasm in guinea pigs, milk-induced leukocytosis in mice, haloperidol-induced catalepsy in mice & passive paw anaphylaxis in rats (*in-vivo*). Histamine is one of the biological mediators of allergy, inflammation, and primary bronchoconstriction that leads to narrowing the lumen in airways and consequently releases a number of secondary inflammatory mediators such as mast cells and leukotrienes prostaglandins, and cytokines. The goat tracheal smooth muscle has histamine (H1), muscarinic (M3), and beta-adrenergic (β2) receptors. Serotonin, histamine and acetylcholine are the contractile agents used in the bioassay of drugs. In the isolated goat tracheal chain isolated preparation, there was a decrease in the percentage of response in the presence of the methanolic extract of *calotropis procera* (MECP) and decoction aqueous extract (100 µg/ml) of *Calotropis procera* (Ait.) R.Br when compared to histamine (100 µg/ml) alone, but it was less than that of standard drug chlorpheniramine (10 µg/ml). In the early stage of asthma, inflammatory mediators like histamine, acetylcholine, leukotrienes and prostaglandins release triggered by exposure to allergens, irritants, cold air, or exercise. Some of these mediators directly cause bronchoconstriction. The spasmolytic drugs like beta-adrenergic agonists, xanthine derivatives (caffeine, theophylline and methylxanthine) and cholinolytics (propranolol, timolol, betaxolol) are

used as quick-relief medications in such as acute asthmatic conditions attacks [42]. In isolated guinea pig ileum preparation, all the AECP and MECP decrease the percentage contractions produced by acetylcholine as compared to acetylcholine alone. Thereby the extract could be used as an adjuvant to a main therapeutic regimen for dosage regulation and enhanced symptomatic relief. Histamine, when inhaled, causes hypoxia and leads to convulsion in guinea pigs, and causes very strapping smooth muscle contraction, profound hypotension, and capillary dilation in the cardiovascular system. A well-known effect caused by histamine leads to severe bronchial constriction in the guinea pigs that causes asphyxia, suffocation, and ultimately death. Bronchodilators can delay the occurrence of these symptoms [40].

In the histamine-induced bronchospasm model, all extracts showed significant activity in a dose-dependent manner except AECP (50 mg/kg) in which the results were non-significant. The maximum % age protection was shown by MECP (200 mg/kg) *i.e.* 50.34% but it was less than the protection offered by that of standard chlorpheniramine (2 mg/kg) *i.e.* 67.10%. Herbal formulations used in the treatment of asthma include some stress-relieving herbs to facilitate adoption to stress since nervous debility may exacerbate symptoms of asthma. The normalization effect of an individual can be observed in milk-induced leukocytosis *i.e.* increase in leukocyte count) after parenteral administration of milk. Therefore, in the milk-induced leukocytes count, all the extracts *i.e.* MECP and AECP showed significant activity ($p < 0.05$). The maximum decrease in leukocyte count was observed in MECP (200 mg/kg) *i.e.* 148 mm3, but it is less than the standard drug *i.e.* 740 mm3.

Allergic asthma is a chronic inflammatory process occurring due to exposure to an allergen resulting in the activation of T-lymphocytes with subsequent release of inflammatory mediators. Immunomodulatory agents are useful in the treatment of asthma by inhibiting the antigen-antibody (Ag-Ab) reaction and thereby inhibiting the release of inflammatory mediators [37]. Further, in the passive paw anaphylaxis model, all the extracts showed a significant decrease in paw volume with time when compared to the control group except AECP (50 mg/kg). It is observed that paw volume decreased with time. The MECP (200 mg/kg) shows maximum percentage inhibition in paw volume, but it was less than standard Dexamethasone. Catalepsy is induced by haloperidol by inhibiting dopamine D2 receptors, which inhibits dopamine secretion. Dopamine is an agonist for adrenaline [37]. Adrenaline is the physiological antagonist of histamine. So as there decreases in dopamine, an imbalance in neurotransmitters means a high level of histamine. In haloperidol-induced catalepsy, all extracts showed significant ($p<0.01$) activity. The MECP and AECP extracts are effective in all the models of asthma as an order of MECP> AECP except AECP in isolated guinea pig ileum preparation.

CONCLUSION: The most commonly used drugs in the treatment of chronic asthmatic conditions are steroids *i.e.* glucocorticoids. These cause dependence and weaken our immune system. The *Calotrpois procera* extracts either methanolic or aqueous showed the presence of glycosides, flavonoids, steroids, carbohydrates, and other secondary metabolites.

The methanolic extract is found to be effective in all the models of asthma over the aqueous extract. Therefore, it can be concluded that the methanolic extract of *Calotropis procera* has therapeutic potential and can be evaluated further for the bioactive compounds responsible for elucidating the anti-asthmatic activity. Furthermore, the present study may be useful in the identification, characterization, and standardization of potential biomolecules having anti-asthmatic properties and revalidate the use of *Calotropis procera* plant parts in the ayurvedic system of medicine.

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Ethical Approval: The study protocol was approved by the Institutional Animal Ethics Committee of the Institute of Pharmaceutical Sciences, Kurukshetra University Kurukshetra, with ref no: 659.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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