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Anti-histaminic and bronchodilatory activities of aqueous and methanol extracts of *Calotropis procera* (Ait) R.Br. root bark on allergic asthma in rodents

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Abstract
The root bark of *Calotropis procera* (*C. procera*) (Asclepiadaceae) has been reported to be a part of herbal remedies for the management of allergic conditions including asthma. However, there is paucity of data on its anti-histaminic and bronhodilatory activity in asthma. This study therefore aimed to provide some pharmacological rationale for the ethnomedical use of *C. procera* as an anti-histamine and bronchodilator in asthma. The aqueous and methanol extracts of *C. procera* were investigated for anti-histaminic and bronchodilatory activities using histamine induced contraction of isolated guinea pig tracheal chain (at 0.5 ml, 1 ml and 2 ml, and stock concentration of 0.5 mg/ml), histamine induced contraction of isolated guinea pig ileum strip test (at 0.1, 0.2, 0.4, 0.8 and 1 ml, and stock concentration of 10 mg/ml), and haloperidol induced catalepsy test in rats (at 200 mg/kg and 300 mg/kg doses). Both extracts of *C. procera* significantly relaxed ($p<0.01$) histamine induced contraction of isolated guinea pig trachea. The extracts also significantly inhibited ($p<0.001$) histamine induced contraction of isolated guinea pig ileum. The aqueous extract did not significantly inhibit haloperidol-induced catalepsy. However, methanol extract significantly inhibited ($p<0.05$) haloperidol-induced catalepsy at 300 mg/kg. The aqueous and methanol root bark extract of *C. procera* was found to poses anti-histaminic and bronchodilatory activities in *in vivo* and *in vitro* anti-asthmatic test on animal models, with the methanol extract having greater activity than the aqueous extract, thus support the folkloric use of the plant in inflammatory and allergic conditions including asthma.

Keywords: *Calotropis procera*, Anti-histaminic, Bronchorelaxant, Anti-asthmatic, Histamine.

INTRODUCTION

According to the Global Strategy for Asthma Management and Prevention asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role and is associated with airway hyper-responsiveness, widespread, variable, and often reversible airflow limitation that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing [1]. The World Health Organisation (WHO) defines asthma as a chronic inflammatory disease of the airways characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person.

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Symptoms may occur several times in a day or week in affected individuals, and for some people become worse during physical activity or at night [2].

Asthma affects an estimated 300 million individuals worldwide [3]. It is a global health problem affecting all age groups, with increasing prevalence in many developing countries, rising treatment costs, and a rising burden for the patients and the community [3]. Asthma still imposes an unacceptable burden on health care systems, and on society through loss of productivity in the workplace and, especially for pediatric asthma, disruption to family [3]. Asthma is a worldwide problem, with increasing prevalence in both children and adults; a prevalence rate of 5 – 10% has been reported for Nigeria [4]. Despite advanced understanding of the pathophysiology of asthma, affected patients continue to incur significant morbidity from the disease [5].

Healthcare providers managing asthma face different issues around the world, depending on the local context, the health system, and access to resources [3]. Asthma occurs in all countries regardless of level of development. Over 80% of asthma deaths occur in low and lower-middle income countries [6]. During the sixty-first World Health Assembly in May 2008, the WHO Director-General warned that asthma is on the rise "everywhere", regardless of a country's income status [7]. Therefore, there is a need for newer or alternative approach to the control of asthma.

In asthma, bronchoconstriction or intermittent airway constriction is a major hallmark of the disease, giving rise to the characteristic symptoms of wheezing, coughing, chest tightness and shortness of breath [8]. Broncho-constriction is the main component of the immediate phase of asthmatic response on exposure to allergen [9, 10]. Spasmolytic effect or relaxation of bronchial smooth muscles may provide relief in diseases of the airways (such as asthma) where spasm of bronchial smooth muscles is the major cause of increased airway resistance [4]. Currently available therapeutic options employed in the treatment of bronchoconstriction include bronchodilators such as β2 agonists, muscarinic receptor antagonists and methylxanthines [11]. Besides their role in the management of bronchoconstriction in asthma, these bronchodilators are also the cornerstone of therapy in chronic obstructive pulmonary disease [12]. Bronchodilators are the mainstay of quick-relief medications for an asthma attack. In combination with corticosteroids, they also form part of controller medications for asthma [13]. However, with regard to morbidity, there is considerable evidence that a regular use of beta-agonist drugs as a class could potentially lead to worsening asthma control because of the effects on bronchial hyper responsiveness, the development of tolerance, reduced protection against provoking stimuli, an increased allergen load, and masking of the symptoms of deteriorating asthma [14]. Muscle tremor and hypokalemia are also major adverse effects of β_2 agonists [15, 16]. Methyl xanthines have narrow therapeutic index and require monitoring of drug levels [17, 18]. Therefore, there is the need for the development of better and safer drugs for asthma. Antihistamines have been shown to have bronchodilatory effects on allergen, exercise, and adenosine-monophosphate-challenge testing and to prevent allergen-induced nonspecific airway hyper responsiveness [19]. Antihistamines have also been shown to delay or prevent the development of asthma in a subgroup of atopic children. These data suggest that antihistamines may have beneficial effects in the management of asthma [19]. Adverse effects of anti-histamines include sedation, dry mouth and throat, blurred vision, urinary retention, constipation and rarely liver injury [20].

In 2008, a bulletin of the World Health Organisation (WHO) stated that "Traditional herbal medicines are getting significant attention in global health database" [21]. WHO (1998) encourages the use of herbal medicine of proven safety and efficacy as phytomedicine in societies where orthodox medicines are economically unobtainable. Medicinal plants constitute the cornerstone of traditional / herbal medicine [22]. Important medicinal uses of the various parts of *Calotropis procera* (*Asclepiadaceae*) have been widely reported [23]. Roots of *Calotropis procera* are used as hepato-protective agents, against colds and coughs, asthma, syphilis and elephantiasis, as an anti-inflammatory, analgesic, antimalarial and antimicrobial [24]. Sen and Behra reported the traditional use of root of the plant in asthma [25]. Singh and Pandey in "Medicinal plantore of the tribals of eastern Rajasthan." also reported the beneficial use of the root of the plant in asthma [26]. Geoffroy *et al.* revealed the aqueous and hydro-alcoholic root bark extract of the plant to be relatively safe for oral consumption in rodents [27]. However, there is paucity of data to validate the ethnobotanical claim of the use of the plant as an anti-histaminic and bronchorelaxant in the management of asthma. This research was therefore undertaken to scientifically evaluate the aqueous root bark extracts of *Calotropis procera* for anti-histaminic and bronchorelaxant activities on asthma in rodents.

EXPERIMENTAL

Experimental animals. Wistar rats weighing 150 - 200 g and guinea pigs (500 - 600 g) of both sexes bred at Animal House Facility, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used. The animals were maintained in a well-ventilated room, fed on standard rodent feed (Grand Cereals Ltd, Jos) and granted access to water *ad libitum*. They were kept in clean cages under normal light / dark cycle. All experimental procedures followed the ethical guidelines for the care and use of laboratory animals as provided by Ahmadu Bello University Research Policy (Revised, 2010) and accepted internationally (NIH 1985, Revised 1996).

Plant material. The whole plant of *Calotropis procera* was collected from the roadside locations of Saye Village of Zaria Local Government Area, Kaduna State, Nigeria. It was identified and authenticated by a Botanist in the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, by comparing with existing specimen (Voucher specimen number 900219). The root bark of the plant was separated, air dried to constant weight and coarsely powdered at the Department of Pharmacognosy and Drug development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

Preparation of plant extract. Dried root powder (450 g) was extracted by cold maceration with distilled water for 48 h. Another 450 g sample of the powdered plant was extracted with 90% $^{v}/_{v}$ methanol for 72 h using Soxhlet extraction apparatus. The extract obtained was concentrated in a rotary evaporator under reduced pressure and completely dried over a regulated water bath maintained at 40 - 60^{O}C.

Acute toxicity studies. The oral median lethal doses of both aqueous and methanol extracts were determined using Lorke's method [28]. The study was carried out in two phases. Prior to the commencement of the studies, rats were deprived of food overnight. In phase 1, three groups of three animals were used. The extract was administered orally in geometrically increasing doses (10 mg/kg, 100 mg/kg and 1000 mg/kg). The treated animals were observed for twenty-four hours for signs and symptoms of toxicity and death. In phase 2, three groups of one animal each were given

the extract orally at doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg (since none of the phase 1 animals died). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24 hours. The geometric mean of the lowest lethal dose (for which the animals died) and highest non-lethal dose (for which the animals survived) was taken as the median lethal dose (LD_{50}).

Anti-asthmatic studies

Effect of drug on guinea pig tracheal chain. A guinea pig weighing 300 g was killed by blow on the head and the throat was cut. The entire trachea was dissected out and transferred to a dish containing Krebs solution, cleaned from connective tissue and was cut into individual rings. Twelve rings were tied together with silk threads and mounted in an organ bath containing oxygenated Krebs solution and maintained at 37°C [29]. Tissues were placed under an initial tension of 0.3 g and were washed three times at 15 min interval. They were then equilibrated under a 2 g tension and after a 15-20 min equilibration period, experiments were initiated. Isometric contractions were recorded using a micro dynamometer. 0.5 ml of histamine (30 µg/ml) alone was administered; the responses were allowed to plateau and then recorded. The tissue was rinsed thoroughly and contractions were induced again by adding 0.5 ml of histamine (30 µg/ml). When the contraction has reached its maximum (after 10 minutes), 0.5 ml of aqueous extract of *Calotropis procera* root bark (5 mg/ml) was administered. The responses were allowed to plateau and then recorded. The tissue was rinsed thoroughly, the process was repeated for 1 ml, and 2 ml for both extracts [30].

In vitro studies on isolated guinea pig ileum preparation. Overnight fasted guinea pig was sacrificed using cervical dislocation method. 3 cm long 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 aerated with air. The responses were recorded on a microdynamometer using isotonic transducer, which exerts a basal tension equivalent to 500 mg load on tissues. The tissue was allowed to equilibrate for 30 minutes, during which, the bathing solution was changed at every 10 minutes. The contractile response of ileum to histamine 10µg/ml was recorded in presence and absence of *Calotropis procera* extract 10 mg/ml. Maximum contractions were recorded for each response, in the presence and absence of the extracts [31].

Haloperidol-induced catalepsy in rats. Thirty-six (36) adult rats were grouped into six (6) groups of six (6) rats each. Group 1 received 2 ml/kg normal saline and served as control. Groups 2 and 3 received single doses of aqueous extract, 200 and 300 mg/kg orally, respectively. Groups 4 and 5 received doses of methanol root extracts 200 and 300 mg/kg orally, respectively. Group 6 received Chlorpheniramine maleate at a dose of 10 mg/kg served as positive control. All groups received haloperidol at a dose of 1 mg/kg, intraperitoneally 1 h after the drug administration. The severity of catalepsy was measured at 0, 30, 60, 90,120 and 150 min. Catalepsy of an individual rat was measured in a stepwise manner by a scoring method as follows:

Step I: Each rat was taken out of the cage and placed on a table. It was then pushed forward by a gentle touch on the back. If it failed to move when touched gently on the back or pushed, a score of 0.5 was assigned.

Step II: the front paws of the rat were placed alternately on a 3cm high block. If the rats failed to correct the posture within 15 seconds a score of 0.5 for each paw was added to the score of step I.

Step III: the front paws of the rats were placed alternately on a 9cm high block. If the rat failed to correct the posture within 15 seconds, a score of 1 for each paw was added to the scores in I and II. Thus for an animal, the

highest possible score was 3.5 (cut-off Score) and this reflects total catalepsy [32, 33].

Statistical analysis. Results were expressed as mean ± SEM. Analysis was done using Statistical Package for the Social Sciences (SPSS) Version 19. One-way analysis of variance (ANOVA) followed by Dunnett Post Hoc test were used for isolated guinea pig trachea and ileum preparation, while Friedman test followed by Wilcoxon signed rank test for was used for haloperidol induced catalepsy test. Differences in mean were considered to be significant when $p \leq 0.05$.

RESULTS

Percentage yield. A 450g sample of powdered *C. procera* yielded 61.91 g after extraction with water, while the other 450 g root bark powder yielded 70.37 g after extraction with 90% $^{v}/_{v}$ methanol. The percentage of methanol and aqueous soluble extractives were calculated with reference to air-dried plant material and the yield were determined to be 15.64 % $^{w}/_{w}$ and 13.76 % $^{w}/_{w}$, respectively.

Acute toxicity studies. The oral, median lethal doses of the aqueous and methanol root bark extracts of *C. procera* were estimated to be greater than 5,000 mg/kg in rats (Table 1).

Anti-asthmatic studies.

Effect of aqueous Calotropis procera root bark extract on histamine-induced contraction of isolated guinea pig trachea. 0.5 ml of histamine (30 µg/ml) resulted in contraction of the smooth muscle of the trachea and 0.5 ml of aqueous and methanol extracts *C. procera* (0.5 mg/ml) produced a significant relaxation ($p< 0.01$) of the pre-contracted tracheal smooth muscles. 1 ml of histamine (30 µg/ml) also exhibited contractions, which were significantly relaxed ($p < 0.005$) by administration of 1 ml aqueous, and methanol extracts of *C. procera* (0.5 mg/ml) respectively (Figure 1 and Table 2).

Histamine-induced contractions of isolated guinea pig ileum preparation. Both aqueous and methanol root bark extracts of *C. procera* at 10 mg/ml, significantly inhibited ($p < 0.001$) histamine induced contractions of isolated guinea pig ileum preparation (Figure 2 and Table 3).

Table 1: The oral median lethal dose (LD_{50}) of crude aqueous and methanol extracts of *C. Procera* in Wistar rats

Dose(mg/kg)	Route	No. of death/No. used	Phase	% Mortality
10	Oral	0/3	I	0
100	Oral	0/3	I	0
1,000	Oral	0/3	I	0
1,600	Oral	0/1	II	0
2,900	Oral	0/1	II	0
5,000	Oral	0/1	II	0

LD_{50} was calculated to be > 5,000 mg/kg (oral route)

Table 2: Effect of aqueous and methanol root bark extracts of *C. procera* on histamine-induced contractions of isolated guinea pig trachea chain preparation.

Volume (ml)	Maximum Contraction (mm)		
	Control (Histamine 10 µg/ml)	Methanol ext (0.5 mg/ml)	Aqueous ext (0.5 mg/ml)
0.5	3.80 ± 0.49	1.00±0.55[b]	1.20 ± 0.37[b]
1.0	5.20 ± 0.37	1.60 ± 0.51[a]	1.40 ± 0.51[a]
2.0	5.20 ± 1.11	1.70 ± 0.44	2.10 ± 0.40

Values are Mean ± SEM, n=5. One way ANOVA, "a" and "b" are significantly different from control at $p< 0.005$ and $p< 0.01$ respectively. F (2, 12) = {10.77 at $p< 0.005$(0.5 ml), 20.79 at $p< 0.005$ (1.0 ml) and 6.93 at $p< 0.01$ (2.0 ml)}. Ext = Extract, mm = millimeter.

Table 3: Effect of aqueous and methanol root bark extracts of *C. procera* on histamine-induced contractions of isolated guinea pig ileum preparation

Volume (ml)	Maximum contraction (mm)		
	Control (Histamine 10µg/ml)	Methanol ext (10mg/ml)	Aqueous ext (10mg/ml)
0.1	13.600 ± 0.87	2.600 ± 0.40[a]	3.00 ± 0.32[a]
0.2	18.400 ± 0.51	4.800 ± 0.58[a]	6.400 ± 0.68[a]
0.4	22.000 ± 0.89	7.200 ± 0.37[a]	12.200 ± 1.02[a]
0.8	27.200 ± 0.58	8.800 ± 0.49[a]	15.60 ± 0.51[a]
1.0	26.600 ± 0.51	7.800 ± 0.58[a]	16.00 ± 0.51[a]

Values are Mean ± SEM, n=5. One way ANOVA, "a" is significantly different from control at $p < 0.001$. $F\{(6, 28) = 20.48; p < 0.001\}$.

Table 4: Effect of aqueous and methanol root bark extracts of *C. procera* on haloperidol-induced catalepsy in rats

Treatment	Dose (mg/kg)	Total cataleptic score				
		30 min	60 min	90 min	120 min	150min
D/water	2ml/kg	3.20 ± 0.20	3.50 ± 0.00	3.50 ± 0.00	3.50 ± 0.00	3.50 ± 0.00
ACPE	250	2.50 ± 0.55	3.50 ± 0.00	3.10 ± 0.40	3.50 ± 0.00	3.50 ± 0.00
ACPE	350	2.00 ± 0.55	3.10 ± 0.29	3.30 ± 0.20	2.30 ± 0.58	2.90 ± 0.40
MCPE	250	0.90 ± 0.40	2.10 ± 0.51	2.20 ± 0.25	2.75 ± 0.75	2.60 ± 0.40
MCPE	350	1.20 ± 0.37	1.50 ± 0.47	2.30 ± 0.37[a]	3.30 ± 0.20[a]	2.10 ± 0.37
CPM	10	0.00 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00

Values are Mean ± SEM, n=5. "a" is significantly different at $p<0.01$ respectively. Friedman Test, followed by Wilcoxon Signed-rank test with Bonferroni correction factor applied; n=5. CPM is Chlorpheniramine, D/Water is distilled water, ACPE is aqueous *Calotropis procera* extract, and MCPE is methanol *Calotropis procera* extract.

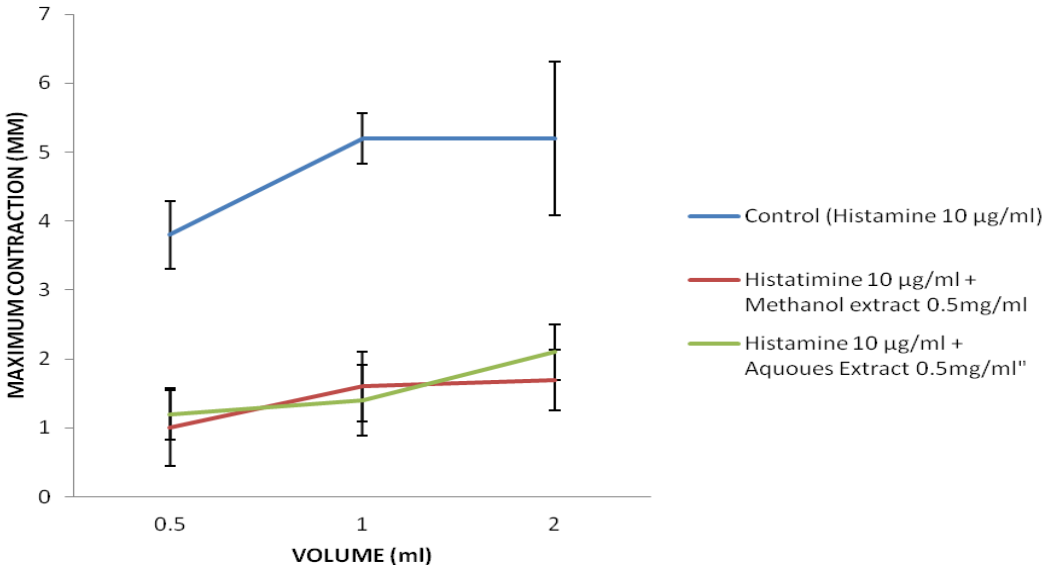

Figure 1: Effect of aqueous and methanol *Calotropis procera* root bark extracts on histamine-induced contraction of isolated guinea pig trachea.

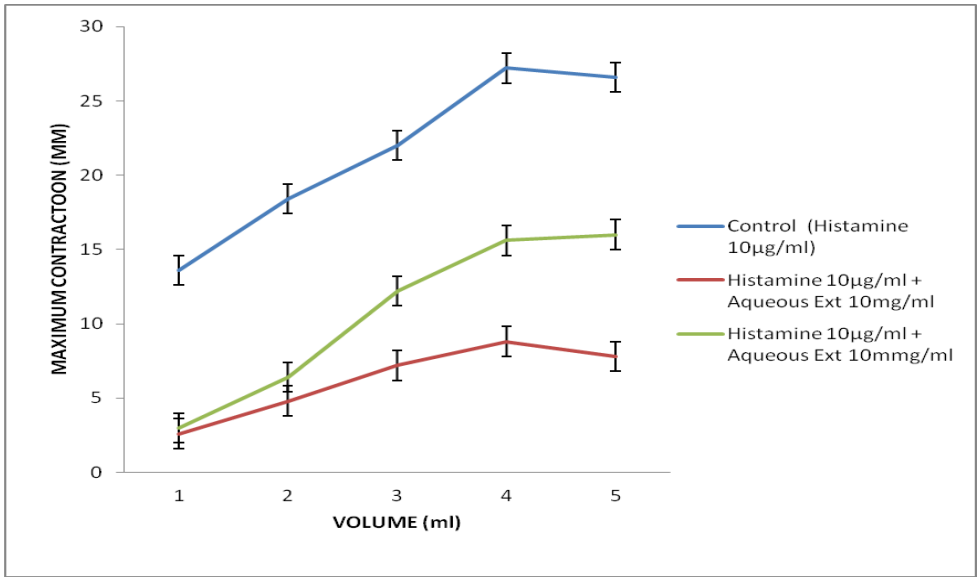

Figure 2: Effect of aqueous and methanol root bark extracts of *C. procera* on histamine-induced contraction of isolated guinea pig ileum.

Haloperidol-induced catalepsy in rats. In vehicle-treated group, (distilled water) haloperidol induced total catalepsy after 30 minutes of intraperitoneal injection. Thirty (30) minutes in each group was taken as control and compared with other times. Both aqueous and methanol extracts of *C. procera* prolonged the time for development of full catalepsy in all the treatment groups. Significant protection against haloperidol-induced catalepsy ($p<0.01$) was observed at 90 minutes and 120 minutes post haloperidol exposure at dose of 350 mg/kg in methanol extract group. The aqueous extract did not afford any significant protection against haloperidol-induced catalepsy (Table 4).

DISCUSSION

The present study attempts to investigate the anti-asthmatic activity of the aqueous and methanol extracts of *C. procera* in rodents. Syndrome of bronchial asthma is characterized by widespread narrowing of the bronchial tree due to contraction of the smooth muscles in response to multiple stimuli resulting in the release of chemical mediators such as histamine [34]. The stimulation of H_1 receptors produces graded dose related contractions of isolated guinea pig trachea preparation. Histamine acts by binding to G-protein coupled histamine receptors (H_1, H_2, H_3, and H_4); in this case H1 receptors found on smooth muscles of the bronchial tree. The H_1 receptor is linked to an intracellular G-protein (Gq) that activates phospholipase C and phosphatidylinositol (PIP2) signaling pathway thus leading to smooth muscle contraction [35].

In the present study, the aqueous and methanol root bark extracts of *Calotropis procera* (5mg/ml) were involved in inhibition of contractions produced by histamine; either by directly preventing the binding of histamine to its receptor, specifically by acting as a receptor antagonist of peripheral histamine H1 receptors [36] thus inhibiting the contractions; or by acting as beta 2 adrenergic receptor agonist, stimulation of beta 2 receptors leads to activation of enzyme adenylcyclase that form cyclic adenosine monophosphate (AMP) from adenosine triphosphate (ATP). This high level of cyclic AMP relaxes bronchial smooth muscles and decreases airway resistance. Also high levels of cyclic AMP inhibits bronchoconstricting mediators such as histamine and leukotriene from the mast cell in

the airway; or the extracts acted as anti-cholinergic agents which causes relaxation by specifically blocking the M_3 type muscarinic acetylcholine receptors in the smooth muscle of the bronchial tree [37]; or by acting as phosphodiesterase 4 inhibitors preventing cyclic AMP. The bronchospasmolytic activity of *Calotropis procera* exhibited by relaxing pre-contracted isolated trachea provides evidences to support its traditional use as an anti-asthmatic agent.

The stimulation of H_1 receptors produces graded dose related contractions of isolated guinea pig ileum preparation [38, 31]. In the present study, significant inhibition ($p<0.001$) by the aqueous and methanol extracts of contractions of the isolated guinea pig ileum induced by histamine depicts its H_1 histamine antagonistic activity. This confirms its bronchospasmolytic potential and therefore supports the anti-asthmatic property of the plant.

Catalepsy is an extrapyramidal side effect of drugs that inhibits dopamine transmission or histamine release in the brain. Haloperidol is an antipsychotic drug that inhibits dopaminergic transmission in the brain. Haloperidol induces catalepsy by inhibiting dopamine D_2 receptors and dopamine secretion. Dopamine is an agonist on beta-adrenergic receptors, it releases adrenaline from storage vesicles and it is a biosynthetic precursor for adrenaline. Adrenaline is a 1physiological antagonist of histamine [39]. Therefore, the delay in development of catalepsy by methanol extract suggests that they may act as a physiological antagonist of histamine.

Conclusion. The anti-histamine and bronchodilatory activities exhibited by aqueous and methanol root back extracts of *C. procera* in rodents support the folkloric use of the plant in allergic conditions including asthma.

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