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Research Article

Immune-mediated Anti-inflammatory Activity of Root Bark Extracts of *Calotropis procera* (Ait) R.Br. in Rodents

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Background: The root bark of *Calotropis procera* has been reported to be a part of herbal remedies for the management of allergic diseases like asthma. However, there is paucity of data on its anti-inflammatory activity in allergic disorders.

Objectives: This study is aimed to investigate the immune mediated anti-inflammatory activity of root bark extracts of *Calotropis procera* in rats.

Materials and Methods: Aqueous and methanol root bark extracts of *Calotropis Procera* were subjected to preliminary phytochemical screening and their oral median lethal doses were estimated in rats. The aqueous and methanol root bark extracts were investigated for anti-allergic activity using carrageenan-induced leucocytosis (100 and 200 mg/kg doses) and egg albumin induced passive paw anaphylaxis (250 and 350 mg/kg doses) test in rats.

Results: The oral median lethal doses of both extracts were found to be greater than 5000 mg/kg in wistar rats. Both extracts were found to contain alkaloids, flavonoids, tannins, saponins, cardiac glycosides, and triterpenes. Both extracts significantly ($p < 0.001$) decreased leucocyte count in carrageenan induced leucocytosis test at the dose of 100 and 200 mg/kg, with both aqueous and methanol extracts exhibiting the same level of decrease in leucocyte count. Equally, there was a statistically significant decrease ($p < 0.05$) in paw size at 250 mg/kg and 350 mg/kg in egg albumin-induced passive paw anaphylaxis compared to the peak increase for both standard and test groups, but with the aqueous extract exhibiting a greater level of decrease in paw size than methanol extract.

Conclusion: The aqueous and methanol root bark extracts of *Calotropis procera* possesses Anti-inflammatory activity in *in vivo* anti-allergic tests on animal models, thus support the folkloric use of the plant in inflammatory and allergic conditions including asthma.

Key words: *Calotropis procera*; Anti-inflammatory; Allergy; Carrageenan; Egg albumin.

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1. Introduction

Allergies, also known as allergic diseases, are a number of conditions caused by hypersensitivity of the immune system to something in the environment that usually

cause little problem in others (McConnell, 2007). Substances that often cause allergic reactions are called allergens and may include: Pollen, dust mites, mold spores, pet dander, food, insect stings, latex and medicines. In clinical practice, allergy manifests in form

of various different conditions such as anaphylaxis, urticaria, angioedema, allergic rhino-conjunctivitis, allergic asthma, serum sickness, allergic vasculitis, hypersensitivity pneumonitis, atopic dermatitis (eczema), contact dermatitis and granulomatous reactions, as well as the colorful spectrum of food- or drug induced hypersensitivity reactions.

According to World Health Organization (WHO, 2011), Worldwide sensitization (IgE antibodies) to foreign proteins in the environment is present in up to 40% of the population. Worldwide, the rise in prevalence of allergic diseases has continued in the industrialized world for more than 50 years (WHO, 2011). In 2012, 10.6% or 7.8 million children reported respiratory allergies in the past 12 months. In 2012, 12% or 8.8million children reported skin allergies in the past 12 months (NHIS, 2012).

There is increasing patronage of herbal medicine among populace of developing countries. This has led the World Health Organisation to encourage 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. Important medicinal uses of the various parts of *Calotropis procera* (Asclepiadaceae) have been widely reported (Kumar and Basu, 1994). Roots of *Calotropis procera* are used against colds and coughs, asthma, syphilis and elephantiasis, and in the treatment of liver disease, pain, malaria and infections (Sharma and Sharma, 2000). Sen and Behra (2007) reported the traditional use of root of the plant in asthma. Singh and Pandey (1980) in "Medicinal plantore of the tribals of eastern Rajasthan.", also reported the beneficial use of the root of the plant in asthma. Geoffroy et al, (2013) revealed the aqueous and hydro-alcoholic root bark extract of the plant to be relatively safe for oral consumption in rodents. However, there is paucity of data on scientific validation of the ethnobotanical claim of the use of the plant in the management of allergic conditions. This research was therefore undertaken to scientifically evaluate the aqueous and methanol root extracts of *Calotropis procera* for anti-inflammatory activity in rodent models of allergy.

2. Materials and Methods

2.1 Plant Material

The whole plant of *Calotropis procera* was collected from the roadside locations of Saye Town in Zaria local government area, Kaduna State - Nigeria. It was identified and authenticated by a Taxonomist in the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State - Nigeria by comparing with existing specimen (Voucher specimen number of 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 Science, ABU, Zaria, Kaduna - Nigeria. 450 g of the dried root powder was extracted by cold maceration with distilled water for 48 h. Another 450 g 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 Rotavapor

under reduced pressure and completely dried over a regulated water bath maintained at 40 - 60°C.

2.2 Phytochemical Screening

Phytochemical screening was carried out on both the aqueous and methanol leaf extracts using simple chemical tests to detect the presence or absence of chemical constituents as detailed in the literature (Evans, 2009).

2.3 Experimental Animals

Wistar rats (150 -200 g) of either sex bred at Animal House Facility, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used in the study. The animals were maintained in a well-ventilated room, fed on standard rodent feed 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).

2.4 Acute Toxicity Studies

The oral median lethal doses of both aqueous and methanol extracts were determined using Lorke's method (1983). The study was carried out in two phases. Prior to the commencement of the studies, rats were deprived of food over night. 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 (LD₅₀).

2.5 Pharmacological Studies

Passive paw anaphylaxis in rats

Ten (10) rats were sensitized by administering subcutaneously three doses of 100 mg of egg albumin on days 1, 3 and 5. On day 10 of sensitization, blood was collected and centrifuged to separate serum. A fresh set of thirty (30) rats were divided into six (6) groups each containing five (5) rats and were initially immunized with 0.5 mls of the previously collected serum subcutaneously. Group 1 (Control) received distilled water at a dose of 2 ml/kg orally. Group 2 was administered with Dexamethasone at a dose of 0.27 mg/kg orally. Groups 3 and 4 received single oral doses of 250 and 350 mg/kg aqueous extracts, while group 4 and 5 were administered methanol extracts of the plant at 250 and 350 mg/kg doses orally respectively. Twenty four (24) hours past drug treatment, animals again challenged with 10 mg egg albumin subcutaneously on

the right hind paw and edema inhibition was calculated at 0, 1, 2, 3 and 4 hours by measuring the paw diameter using a vernier calliper (Patil, 2010).

Carrageenan-induced leucocytosis in rats

Forty two (42) albino rats were divided into seven (7) groups of six (6) rats each. Group 1 was the standard reference group. Group 2 (Control group) received distilled water (2 ml/kg), Chlorpheniramine (2mg/kg i.p.) was administered to group 3 (represents the standard drug). Groups 4 and 5 received single doses of aqueous extracts (100 and 200 mg/kg p.o.), while group 6 and 7 (100 and 200 mg/kg p.o.) received methanol extracts of the plant. Distilled water-administered group served as a negative control. After 1 h of drug treatment, all groups received carrageenan injection at a dose of 1 mg/kg subcutaneously. Total leucocyte count (TLC) for group 1 was determined initially so as to ascertain and compare the normal WBC count for rats. Blood samples were collected from each rat 24 h after carrageenan injection and total leukocyte count were done using Sysmex Hematological Analyser (Bhargava and Singh, 1981; Horn and Robin, 1975).

2.6 Statistical Analysis

Results were expressed as mean \pm 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 for carrageenan induced leucocytosis test; and repeated measures ANOVA followed by Bonferroni test for multiple comparisons in passive paw anaphylaxis test. Differences in mean were considered to be significant at $p \leq 0.05$.

3. Results

450 g 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.

Phytochemical screening revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, triterpenes, and saponins, while anthraquinones were found absent in both extracts.

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.

Passive Paw Anaphylaxis in Rats

In the vehicle-treated group, egg albumin (antigen) increased the paw volume in the sensitized animals, which was measurable up to the time period of 4 hr. Maximum inflammation of paw volume (i.e. the peak of passive paw anaphylaxis) was obtained after 1 hr of antigen challenge for all treatment groups. 1 hr in each group was taken as control and compared with other times. Aqueous extract of *C. procera* (250 mg/kg and 350 mg/kg), significantly inhibited ($^a p < 0.001$) anaphylactic inflammation 1 hr post antigen exposure (**Table 1**). Significant inhibition ($^b p < 0.05$) by methanol extract was obtained for 250 mg/kg after 4 hr and for 350 mg/kg after 3 hr ($^b p < 0.05$) of antigen exposure. Although both extracts statistically significantly inhibited paw inflammation, but the aqueous extract was more effective in the inhibition of paw inflammation compared with methanol extract. Dexamethasone (Dexa, 0.27 mg/kg) also, significantly reduced ($^a p < 0.001$) paw volume after one hour of antigen exposure (**Table 1**). The aqueous extract (at 250 mg/kg and 350 mg/kg doses) was as effective as dexamethasone (at 0.27 mg/kg dose) in inhibiting paw inflammation.

Table 1: Effects of Methanol and Aqueous Root Bark Extracts of *Calotropis procera* on Passive Paw Anaphylaxis in Rats

Treatment	Dose (mg/kg)	Paw Volume (mm ³)				
		0 h	1 h	2 h	3 h	4h
D/water	2 ml/kg	3.96 \pm 0.14	5.90 \pm 0.26	5.94 \pm 0.26	6.03 \pm 0.25	6.23 \pm 0.23
Dexa	0.27	3.87 \pm 0.14	5.71 \pm 0.26	5.06 \pm 0.26 ^a	4.93 \pm 0.25 ^a	4.64 \pm 0.23 ^a
ACPE	250	3.71 \pm 0.14	6.72 \pm 0.26	5.55 \pm 0.26 ^a	5.34 \pm 0.25 ^a	4.97 \pm 0.23 ^a
ACPE	350	3.49 \pm 0.14	6.10 \pm 0.26	5.37 \pm 0.26 ^a	5.32 \pm 0.25 ^a	4.92 \pm 0.23 ^a
MCPE	250	3.26 \pm 0.14	5.06 \pm 0.26	4.91 \pm 0.26	4.71 \pm 0.25	4.42 \pm 0.23 ^b
MCPE	350	3.42 \pm 0.14	5.22 \pm 0.26	4.98 \pm 0.26	4.70 \pm 0.25 ^b	4.54 \pm 0.23 ^b

Values are Mean \pm SEM, n=5. "a" and "b" are significantly different at $p < 0.001$ and $p < 0.05$ respectively. F (5, 24) = {4.09 (0 hr), 5.42 (1 hr), 2.42 (2 hrs), 3.99 (3 hrs) and 8.24 (4 hrs) at $p < 0.05$ }. Repeated Measure ANOVA followed by Bonferroni test. ACPE = Aqueous *Calotropis procera* Extract, MCPE = Methanolic *Calotropis procera* Extract, mm = Millimeter, D/ water = Distilled water.

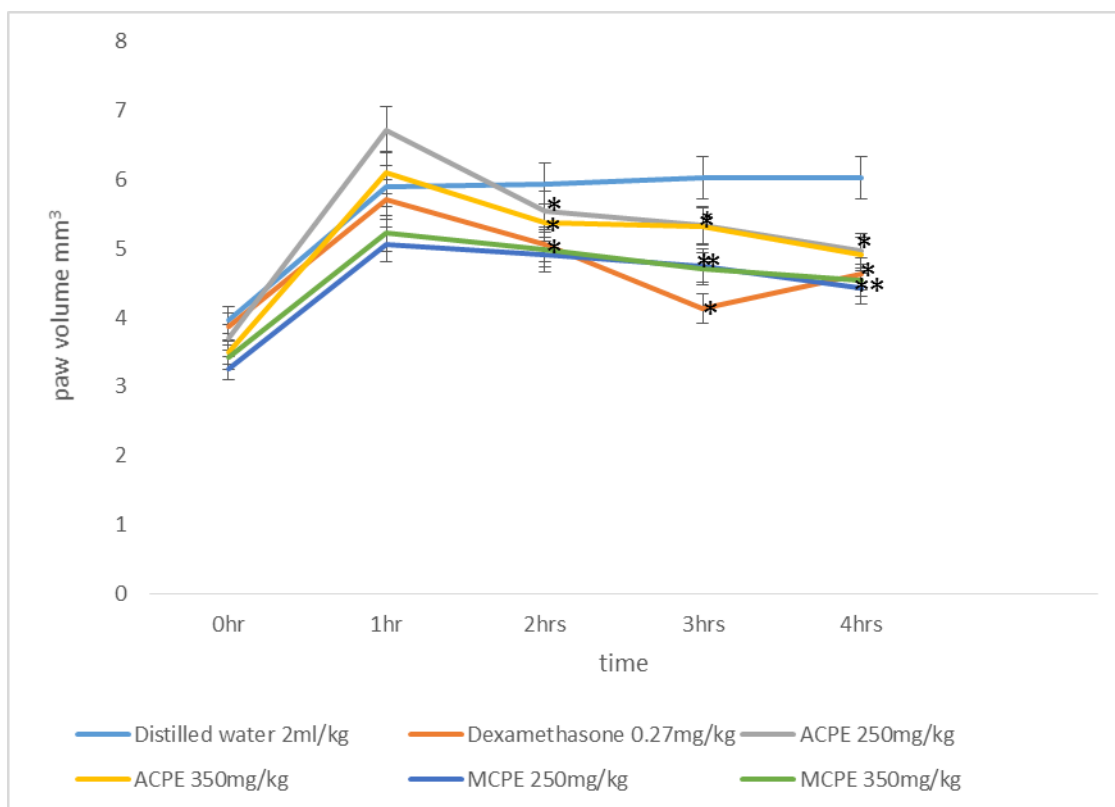


Figure 1: Effect of *Calotropis procera* on Egg Albumin-induced Passive Paw Anaphylaxis in Rats.

Carrageenan induced leucocytosis in rats

The mean TLC of rats in the group that was neither pretreated with any drug nor challenged with carrageenan (Group 1) fell within the normal total leucocyte count for wistar rats as recommended by the Zoological Education Network ($6.6 - 12.6 \times 10^3/\text{mm}^3$) (Table 2). Carrageenan increased the mean Total

Leucocyte Count (TLC) above the normal TLC for rats as seen in the negative control (Group 2). Control group was compared with other groups. Both 100 mg/kg and 200 mg/kg doses of aqueous and methanolic extracts produced a statistically significant inhibition ($^a p < 0.001$) of carrageenan induced increase in TLC. Chlorpheniramine maleate (2 mg/kg), significantly inhibited ($^a p < 0.001$) increase in TLC (Table 2).

Table 2: Effects of Methanol and Aqueous Root Extracts of *Calotropis procera* on Carrageenan Induced Leucocytosis in Rats

Treatment Groups	Doses	Total Leucocytes Count (TLC) ($\times 10^3/\text{mm}^3$)
SRG (for TLC in Rats)	—	7.100 ± 0.81
D/water	2ml/kg PO	12.920 ± 0.58
Chlorpheniramine Maleate	2mg/kg IP	7.840 ± 0.61^a
ACPE	100mg/kg PO	5.580 ± 0.79^a
ACPE	200mg/kg PO	5.300 ± 0.54^a
MCPE	100mg/kg PO	7.600 ± 0.66^a
MCPE	200mg/kg PO	12.840 ± 0.85

One way ANOVA, $n=6$. All treated groups were compared with control (Normal Saline). "a" is significantly different from control at $p < 0.001$. $F \{(6, 28) = 20.48; p < 0.001\}$. PO = Oral, IP = Intraperitoneal, SRG = Standard Reference Group.

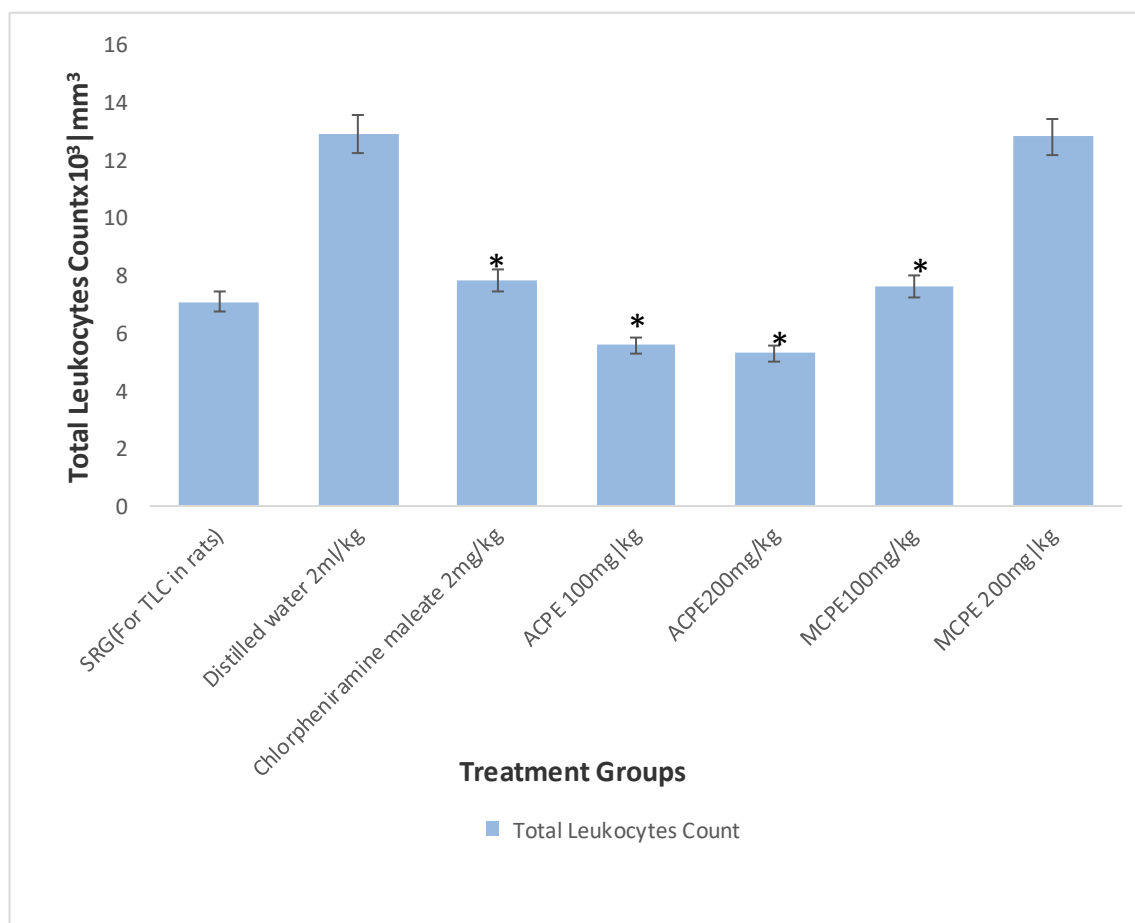


Figure 2: Effects of Aqueous and Methanol Root Bark Extracts of *Calotropis procera* on Carrageenan Induced Leucocytosis in Rats

4.0 Discussion

The present study attempted to provide some scientific rationale for the ethnomedical use of the root bark of *C. procera* in the management of some allergic conditions.

The high oral median lethal dose (LD₅₀), above 5,000 mg/kg showing the possible safety of aqueous and methanol *Calotropis procera* extracts when used orally.

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 (Agrawal and Mehta, 2007; Dhawan et al, 2003). Subcutaneous administration of egg albumin to rats raises the anti-serum to egg albumin in the plasma and sub planter. Injection of plasma containing these antibodies then challenged with egg albumin leads to passive anaphylaxis in rats (Pungle et al, 2003).

Significant inhibition of egg albumin induced inflammation by both aqueous and methanol extracts implies that they have activity against anaphylactic inflammation. The protective effects of the extracts in reducing paw volume in passive paw anaphylaxis may be due to inhibition of antigen-antibody reaction and

thereby inhibiting release of inflammatory mediators (Pandit, 2008).

Significant reduction of carrageenan induced total leucocyte count by both extracts at doses of 100 mg/kg and 200 mg/kg demonstrates their anti-inflammatory activity, further corroborating its anti-allergic potential. Most allergic and non-allergic asthmatics, including those with mild asthma, have bronchial eosinophilia and there is a significant association between eosinophil activation and asthma severity as well as bronchial hyper responsiveness (Horn and Robin, 1975).

Although there may be multiple "triggers" for an inflammatory response (such as mast cell secretion), there is general agreement that a lymphocyte-directed eosinophilic bronchitis is a hallmark of asthma (Ranjeeta et al, 2009). Total leucocyte count in this context is used as a function of eosinophil count, because an increase in eosinophilia will reflect as an increase in total leucocyte count. The test standard reference obtained ($7.100 \pm 0.58 \times 10^3/\text{mm}^3$) falls within the range recommended by the Zoological Education Network for rats ($6.6 - 12.6 \times 10^3/\text{mm}^3$).

Nagore and co in 2009 found out that flavonoids are found to be active at both the phases of allergic response. The presence of flavonoids identified in both extracts could be responsible for the observed anti-asthmatic activity of *C. procera*.

5.0 Conclusion

The anti-inflammatory activity of the root bark extracts of *C. procera* in murine-model of allergic asthma provide some scientific basis for the ethnomedical use of the plant in allergic inflammatory conditions including asthma.

Conflict of Interest declaration

The authors declare no conflict of interest.

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