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Phytochemical Screening and Anti-Inflammatory Properties of the Hydroethanolic Leaf Extract of *Calotropis procera* (Ait). R. Br. (Apocynaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Author EO designed the work, did some lab work and drafted and edited the final manuscript. Author EOA supervised it and edited the final manuscript. Author RPB provided minor supervision and statistical analysis of the work. Author ITH managed the literature searches, some lab work and the analysis of the study. Author FJE did the phytochemical screening aspect of the work. Author EW supervised the work and edited the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Inflammation has been implicated in many disorders and currently available therapies elicit adverse effects. The coarse shrub of *Calotropis procera* has been used in folk medicine to treat several ailments such as asthma, rheumatism and also fever. The anti-inflammatory potential of *Calotropis procera* was assessed in this study to evaluate its effectiveness.

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Place and Duration of Study: University of Cape Coast, Ghana between June 2016 and March 2017.
Methodology: This study was carried out to screen for the phytochemical constituents and investigate the anti-inflammatory properties of *Calotropis procera* extract (CPE) using four *in vitro* assays (heat-induced haemolysis, hypotonic-induced haemolysis, albumin denaturation and the bovine serum albumin assay (BSA)) and, two *in vivo* models (carrageenan and formalin-induced paw oedema models). Three graded concentrations of the plant extract (CPE) and a standard anti-inflammatory agent, diclofenac: 100, 300 and 1000 µg/mL were used in the *in vitro* models while 30, 100 and 300 mg/kg were used in the *in vivo* experiments. Qualitative tests for tannins, alkaloids, flavonoids, carbohydrates, triterpenoids, steroids, proteins, reducing sugars, glycosides, saponins and amino acids were performed to determine the phytochemical constituents of the extract.
Results: The phytochemical screening revealed presence of alkaloids, tannins and triterpenes, reducing sugars, sterols, amino acids, and glycosides. CPE 1000µg/mL demonstrated anti-inflammatory characteristics by significantly stabilizing RBCs exposed to heat and hypotonic solution and also inhibiting protein denaturation in heat-induced denaturation (69.24%); hypotonic-induced (85.09%); albumin denaturation (87.80%) and BSA (96.86% assays). Similar results were observed for the two lower doses of CPE as well as diclofenac. In the *in vivo* tests, CPE was able to significantly reduce both carrageenan-induced *(P*=0.0105*)* and formalin-induced inflammation *(P*<0.0001*)* in rats. Diclofenac the reference anti-inflammatory agent also showed similar effects in both tests. The percentage inhibition of paw oedema produced by CPE 30, 100, and 300 mg/kg in the formalin test were 19.61% 35.88% and 47.66% respectively while diclofenac (10 mg/kg) produced 85.16% inhibition of paw oedema.
Conclusion: From the present study, it can be observed that CPE possessed anti-inflammatory properties and thus vindicates the folkloric use of this plant in inflammatory conditions.

Keywords: Formalin test; Calotropis procera; heat-induced haemolysis; phytopharmaceutical; inflammation; folk medicine.

1. INTRODUCTION

Inflammation is the response of the body to injury or infection depicted as redness, pain, swelling, heat and the loss of function [1]. This response by the body against the aetiological agents is mediated by activated inflammatory cells such as neutrophils, macrophages, plasma cells and lymphocytes [2]. Key participants in inflammation include the blood vessels, circulating leukocytes, connective tissue cells (mast cells, resident macrophages and fibroblasts) and chemical mediators produced from plasma and leukocytes [3].

Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, aspirin and indomethacin are the mainstay in the management of inflammatory disorders. Other classes include the corticosteroids and the disease-modifying anti-inflammatory agent. However, the use of these drugs is limited by their numerous deleterious side effect such as gastric ulcerations which limit their use [4]. This underscores the need to search for newer, more effective and safer drugs.

For centuries, the coarse shrub of *Calotropis procera* has been known to be a very important source of ascaricidal, schizonticidal, nematocidal, anti-microbial, antihelmintic, molluscicidal, insecticidal, anti-inflammatory, anti-diarrhoeal, larvicidal, anticancer agents and cytotoxic chemicals [5,6]. The leaf biomass of the plant is potentially a good adsorbent for the removal of crystal violet (a cationic dye) from aqueous solution and is being used in textile industry [7].

Even though there is widespread usage of the plant in folk medicine for the treatment of several inflammatory conditions, there is still paucity of scientific report validating its usage. The few anti-inflammatory reports on the plant used the latex of the plant [8, 9] which is far from its traditional usage where aqueous or alcoholic leaf extracts are used. The other study [10] that also reported on anti-inflammatory activity of the leaf extract of the plant did only one acute model which is inadequate to make a case on the anti-inflammatory effect of an agent. It is therefore imperative that additional evaluation of the anti-inflammatory activity tests of the leaf extract is conducted using several *in vitro* and *in vivo* models to authenticate the folkloric usage and give insight for the potential mechanisms of action of this plant. A significant anti-inflammatory effect in a battery of anti-

inflammatory models in addition to multi-faceted mechanisms of action would be a strong justification for further research on the plant to discover novel phytoconstituents that can serve as scaffolds for novel therapeutic agents that can be used to treat arthritis and other inflammatory diseases.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extract Preparation

The leaves of *Calotropis procera* were collected from the University of Cape Coast Botanical Gardens. The leaves were identified by a botanist at the School of Biological Sciences Herbarium, University of Cape Coast and its voucher specimen was deposited in the herbarium. It was dried for seven days under shade and powdered using a hammer mill. An amount of 200 g of the powdered leaves was extracted with 2 L of 70% ethanol for 48 h using a Soxhlet apparatus (Aldrich® Soxhlet Extraction Apparatus, Z556203, St. Louis, MO, USA). The extract obtained was subsequently concentrated using a rotary evaporator (Rotavapor R-215 model, BÜCHI Labortechnik AG, Flawil, Switzerland) under reduced pressure and temperature (50°C). This was further dried to powder on a water bath, labelled as CPE (*Calotropis procera* extract) and then preserved in a desiccator containing activated silica until it was ready for use. The yield obtained was 4.1 % w/w.

2.2 Chemicals and Reagents

Diclofenac sodium, formalin, carrageenan, bovine serum albumin used were of analytical grade and were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA.

2.3 Animals

Sprague-Dawley rats (170 – 250 g) of both sexes were be bought from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon – Ghana. They were kept in stainless steel cages (34×47×18 cm^3) in groups of five at the animal house facility of the Department of Biomedical Sciences, University of Cape Coast. The animals were given normal commercial pellet diet (Agricare Ltd, Kumasi, Ghana) and provided water *ad libitum*. The animals were kept under normal laboratory conditions with regards to room temperature and humidity. All the techniques and protocols used in the study were done in accordance with established public health guidelines in “Guide for Care and Use of Laboratory Animals” [11]. Also, all protocols used in the study were approved by the Department of Pharmacology Ethical Review Committee.

2.4 Collection of Blood Samples

To obtain erythrocytes for the various *in vitro* experiments, whole blood was collected from a healthy volunteer who had not taken any non-steroidal anti-inflammatory drug (NSAID) for two weeks prior to the experiment and the blood kept in heparinized vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 min at 3000 rpm. The packed cells were washed with 0.9% saline and 10% v/v RBC suspension was made using isotonic solution which was composed of 0.9 g NaCl in 100 mL of distilled water.

2.5 Phytochemical Screening

Qualitative chemical tests were conducted on the leaf extract of *Calotropis procera* (Apocynaceae) to check presence or absence of the following phytoconstituents; tannins, alkaloids, flavonoids, carbohydrates, triterpenes, steroids, proteins, reducing sugars, glycosides, saponins and amino acids. For qualitative investigation, 500 mg of the extracts were dissolved in hydro-alcoholic solvent. Phytochemical screening of the extracts was carried out by a procedure that was based on earlier reports [12,13].

2.6 *In vitro* Models of Inflammation

2.6.1 Hypotonic solution-induced haemolysis

A method earlier described by Rahman et al. [14] was used. The test sample was made up of 0.5 mL stock erythrocyte (RBC) suspension mixed with 5 mL of hypotonic solution (0.45% NaCl) and varying concentration of *Calotropis procera* extract (100, 300 and 1000 µg/mL). The negative control was made of 0.5 mL RBC suspension mixed with hypotonic buffered solution alone. The positive control was made of 0.5 mL of the RBC suspension, 5 mL of the hypotonic solution and diclofenac (100, 300 and 1000 µg/mL). The mixtures were incubated at 10 min at room temperature, centrifuged for 10 min at 3000 rpm and haemoglobin content of the supernatant was measured spectrophotometrically at 540 nm. The experiment was carried out in triplicate. The

percentage inhibition of haemolysis was calculated by the following equation:

$$\% \, Inhibition = 100 \times (\frac{Absorbance\ of\ sample}{Absorbance\ of\ negative\ control} - 1)$$

2.6.2 Heat-induced Haemolysis

The test was performed as previously described [14]. The test sample consisted of 2.0 mL of 10% RBC suspension and 2.0 mL of CPE (100, 300 and 1000 μg/mL). The negative control consisted of 2.0 mL of 10% RBC suspension and 2.0 mL of normal saline. The positive control samples were made of 2.0 mL of 10% RBC and 2.0mL of diclofenac (100, 300 and 1000 μg/mL). The experiment was carried out in triplicate. The samples were heated at 56°C for 30 min then cooled to room temperature followed by centrifugation at 2500 rpm for 10 min. The supernatant was collected, and absorbance was measured at 560 nm. Percent membrane stabilization was calculated by the method of Rahman, et al. [14] as follows:

$$\% \, Inhibition = 100 \times (\frac{Absorbance\ of\ sample}{Absorbance\ of\ negative\ control} - 1)$$

2.6.3 Albumin denaturation assay

The test was performed as described previously [14]. The reaction mixture (5 mL) for the test sample consisted of 0.2 mL of egg albumin (from fresh egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2.0 mL CPE (100, 300 or 1000 μg/mL). The negative and positive control samples contained the same volume of the egg albumin and PBS but the extract was replaced with 2.0 mL of distilled water and 100, 300 or 1000 μg/mL diclofenac respectively. The mixtures were incubated at 37 ± 2°C for 15 min and then heated at 70 °C for 5 min. After cooling, the absorbance was measured at 660 nm. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated with the formula:

$$\% \, Inhibition = 100 \times (\frac{Absorbance\ of\ sample}{Absorbance\ of\ negative\ control} - 1)$$

2.6.4 Bovine Serum Albumin (BSA) Model of Inflammation

The test was performed using an earlier described method [14]. The reaction mixture of the test sample consisted of 0.5 mL of 1% BSA fraction and 0.5 mL CPE (100, 300 or 1000 μg/mL). The negative and positive control samples contained same volume of 1% BSA and 0.5 mL of normal saline and diclofenac (100, 300 or 1000 μg/mL) respectively. The samples were incubated at 37°C for 20 min and then heated at 51°C for 20 min. The samples were cooled and the turbidity was measured spectrophotometrically at 660 nm. The percentage inhibition was calculated using the formula:

$$\% \, Inhibition = 100 \times (\frac{Absorbance\ of\ sample}{Absorbance\ of\ negative\ control} - 1)$$

2.7 *In Vivo* Models of Inflammation

2.7.1 Carrageenan-induced Paw oedema model

Sprague- Dawley rats were randomly divided into 5 groups (n=5). Carrageenan-induced paw oedema was performed according to the method described by Nonato, et al. [15]. The paw thickness of each rat was measured initially as the baseline using the digital calipers and recorded. The rats were then treated orally as follows: negative control (normal saline, 10 mL/kg, p.o), positive control (diclofenac, 10 mg/kg, p.o) and CPE (30, 100 or 300 mg/kg, p.o). All rats were intraplantarly injected with 0.1 mL of 1% w/v carrageenan in sub-plantar tissues and the paw thickness measured after every hour for 5 hours post-carrageenan treatment.

Percentage inhibition of oedema was calculated using the relation;

$$\% \, Inhibition = \left(\frac{Paw\ diameter\ at\ time\ T}{Paw\ diameter\ at\ time\ 0}\right) \times 100$$

2.7.2 Formalin-induced Paw Oedema

The test was performed as described previously by Choudhary, et al. [16]. Twenty (20) male Sprague-Dawley rats randomly assigned to five groups (n=5) were used in this study. Rats in three of the groups were administered with either CPE 30, 100 or 300 mg/kg orally. Diclofenac was administered to one group as the positive control group (10 mg/kg, p.o) whereas the negative control was received distilled water (10 mL/kg p.o). Prior to drug administration, the left hind paw volumes of all animals were measured and recorded using digital calipers. One hour after

the drug administration, 0.05 mL of 1% w/v solution of formalin was injected into the left hind paws of each animal. Three hours after each formalin injection, the paw diameters were measured again and thereafter, every 24 h for 10 consecutive days. Results were expressed as percentage inhibition in paw thickness at various intervals in comparison to the initial values as follows:

$$\% \, Inhibition = \left(\frac{Paw \ diameter \ at \ tme \ T}{Paw \ diameter \ at \ time \ 0}\right) \times 100$$

2.8 Statistical Analysis

Data were expressed as mean ± SEM. Graph Pad® Prism for Windows Version 7.0 (Graph Pad Software, San Diego, CA, USA, 2016) was used for all statistical analysis. P < 0.05 was considered statistically significant for all tests. Differences between treatment groups were compared using one-way ANOVA with a Bonferroni's post hoc test.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The result of the phytochemical screening of hydroethanolic leaf extract of *Calotropis procera* revealed the presence of alkaloids, tannins and flavonoids, reducing sugars, triterpenoids, sterols, amino acids and glycosides. This is shown in Table 1.

Table 1. Phytochemical screening of *C. procera* extract

Test	Results
Tannins (Ferric Chloride Test)	Present
Alkaloids (Dragendoff's Test)	Present
Reducing sugars (Fehlings' Test)	Present
Steroids (Liebermann-Burchard Test)	Present
Saponins (Frothing Test)	Present
Flavonoids (Ferric Chloride Test)	Present
Carbohydrates (Molisch's Test)	Absent
Proteins (Biuret's Test)	Absent
Triterpenoids (Salkowski Test)	Present
Amino acids (Ninhydrin Test)	Present

3.2 *In vitro* Models of Inflammation

3.2.1 <u>Hypotonic solution – induced Haemolysis</u>

CPE significantly protected the membranes of human red blood cells against haemolysis ($F_{6,14}$ = 120.1 P<0.0001) with a maximum mean inhibitory effect of 85.09% at a concentration of 1000 µg/mL. Similarly, diclofenac similarly exhibited a significant protection of the membranes of human red blood cell by a mean inhibition of 84.21% at 1000 µg/mL as shown in Fig 1 (a).

3.2.2 <u>Heat-induced haemolysis</u>

CPE markedly protected the membranes of human red blood cells against haemolysis ($F_{6,14}$ = 82.15 P<0.0001) with a maximum mean inhibitory effect of 69.24% at a concentration of 1000 µg/mL. Also, diclofenac sodium similarly exhibited a similar protection of the membranes of human red blood cell by a mean inhibition of 84.05% at 1000 µg/mL as depicted in Fig. 1(b).

3.2.3 <u>Albumin denaturation assay</u>

CPE protected the proteins in fresh egg albumin against denaturation ($F_{6,14}$= 107.9 P<0.0001) by exhibiting a maximum mean inhibitory effect of 87.80% at a concentration of 1000 µg/mL. Also, diclofenac similarly exhibited a significant protection of the proteins in the fresh egg albumin by a mean inhibition of 72.23 % at 1000 µg/mL as illustrated in Fig. 1 (c).

3.2.4 <u>Bovine serum albumin assay</u>

Results shown in Fig. 1(d) indicates that CPE could protect the proteins in the Bovine serum albumin against denaturation ($F_{6,14}$= 95.74 P<0.0001) by exhibiting a maximum mean inhibitory effect of 96.86% at a concentration of 1000 µg/mL. Also, diclofenac sodium similarly exhibited a significant protection of the proteins in the Bovine serum albumin by a mean inhibition of 98.06% at 1000 µg/mL.

3.3 *In vivo* Models of Inflammation

3.3.1 <u>Carrageenan-induced Paw Oedema</u>

Results presented in Fig. 2(a) represents a time-course curve of the percentage change in paw oedema following administration of CPE (30, 100 and 300 mg/kg), diclofenac (10 mg/kg) and saline 10 mL/kg. CPE at 300 mg/kg significantly (P<0.05) reduced paw oedema produced by carrageenan at the second hour and this continued to the fifth hour. Diclofenac (10 mg/kg) however significantly (P<0.01) decreased paw oedema at the first hour and was sustained throughout the entire duration of the experiment.

Fig. 2(b) shows total oedema calculated as AUC of the time curse curve of the various treatments groups. Diclofenac 10 mg/kg produced the smallest total oedema compared to the control group (Ctrl). CPE 30, 100 and 300 mg/kg also respectively reduced total oedema significantly ($F_{(4,15)}$ = 6.24; *P*=0.0105) in a dose-dependent fashion.

3.3.2 Formalin-induced Paw Oedema

Intraplantar injection of formaldehyde into rat's hind paw produced a marked increase in paw volume in all animals injected (Fig 3a and b). This is indicated by marked paw oedema on the first day which was sustained in the negative control group throughout the entire duration of the experiment (i.e. from day 1 to day 10). CPE (30, 100 and 300 mg/kg, p.o.) significantly reduced paw thickness by 19.60% 35.88% and 47.65% respectively while diclofenac (10 mg/kg) produced 85.15% inhibition (Fig 3a). CPE also produced a significant and dose-dependent anti-oedematous effect at the 3 doses tested ($F_{(4,15)}$=100.9; *P*<0.0001) (Fig. 3b).

4. DISCUSSION

Results of the present study confirm the anti-inflammatory potentials of *Calotropis procera* and indicate other novel mechanisms by which the reported anti-inflammatory effects could be mediated.

The human red blood cell membrane stabilization method has been employed as one of the fast *in vitro* methods for the rapid screening of the anti-inflammatory properties of newly discovered plants or molecules [17]. The test is based on red blood cell because the membrane of the red blood cell is highly analogous to the membrane of lysosomes which are involved in the inflammatory processes [18]. The ability of the novel plant extract to stabilize the membrane of red blood cells shows that extract will be able to stabilize the membrane of lysosomes as well [18]. Stabilizing the membrane of lysosomes is very crucial in limiting inflammatory response since this process prevents the release of lysosomal contents of activated neutrophils which causes further tissue inflammation and

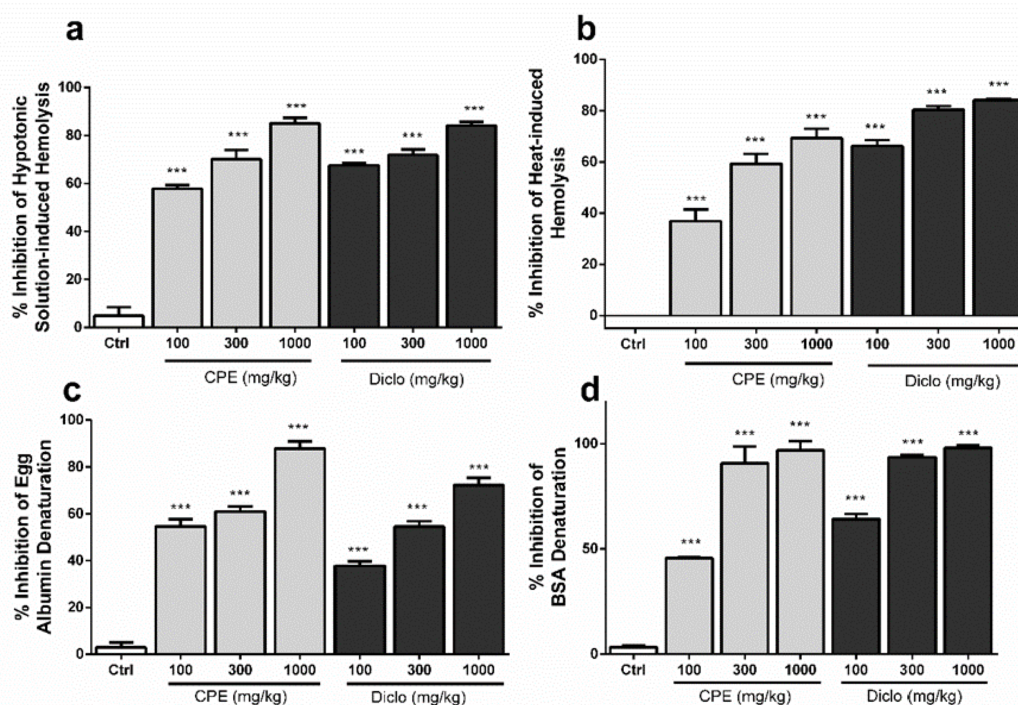

Fig. 1. The effect of *Calotropis procera* extract (100, 300 and 1000 µg/kg, p.o.) and diclofenac (100, 300 and 1000 µg/kg, p.o.) on % inhibition of (a) hypotonic solution-induced haemolysis (b) heat-induced haemolysis (c) egg albumin denaturation (d) BSA denaturation. Data indicate mean ± SEM (n=6)

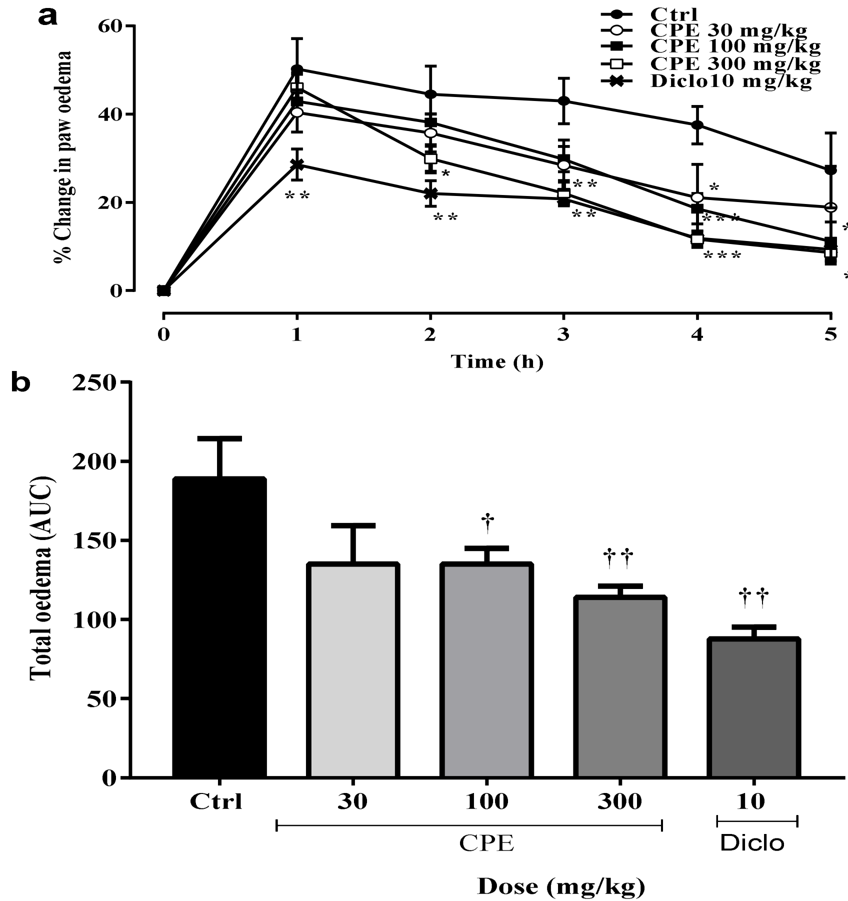

Fig. 2. The effect of Calotropis procera extract (30, 100 and 300 mg/kg.) and diclofenac (10 mg/kg, *p.o.*) on (a) % change in paw oedema and (b) total oedema in carrageenan-induced acute inflammation model in rats

damage following extracellular release [1, 18]. The extracellular activity of these enzymes are said to be related to acute or chronic inflammation. The Non-Steroidal Anti-inflammatory Drugs (NSAIDs) used in the treatment of inflammation and its related disorders act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. The hydroethanolic *C. procera* extract at concentrations of 100, 300 and 1000 µg/mL exhibited membrane stabilization activity by inhibiting membrane lysis when RBCs were exposed to heat and hypotonic solution. Since the red cell membrane can be likened to that of lysosomes, [1,19] its stabilization by CPE in both heat and hypotonic solution induced membrane destabilization indicates CPE may as well stabilize the membrane of lysosomes thereby preventing the likelihood of inflammation.

Protein denaturation is the process by which proteins lose their tertiary and secondary structure by the application of external stress such as strong acids and bases, a concentrated organic or inorganic salt, organic solvent and heat [1]. Denaturation of proteins is a well-documented cause of inflammation [1] and rheumatoid arthritis hence inhibition of protein denaturation may prevent or control inflammation. It is probably due to alteration in the hydrogen, hydrophobic, electrostatic and disulphide bonding which also results in the production of auto-antigens that stimulate auto-immune responses leading to some inflammatory disorders such as rheumatoid arthritis [14, 20] With this mechanism of inflammation in mind, the present study, investigated the anti-inflammatory potential of *C. procera* by assessing its effects on disruption of proteins from fresh egg albumin and bovine serum albumin (BSA). The hydroethanolic

extract of *C. procera* at concentrations of 100, 300 and 1000 μg/mL inhibited the disruption of the protein structures dose-dependently. Since protein denaturation is a well-documented cause of inflammation [1], it is speculated that CPE's anti-inflammation properties could be due in part to the inhibition of protein denaturation and the sequelae that follows it.

To confirm the results obtained from the *in vitro* studies and lead further evidence to support the use of *C. procera* traditionally for the management of inflammatory conditions, the carrageenan-induced paw oedema model was also used. This is a useful model to assess the contribution of inflammatory mediators involved in acute inflammation. The development of oedema in the rat hind paw after the injection of carrageenan has been described as a biphasic event in which various mediators operate in sequence to produce this inflammatory response. The initial phase of oedema (0 – 1 h) is attributed to the release of histamine and serotonin [21] while the late accelerating phase of swelling (2 – 6 h) correlates with the elevated production of bradykinins, prostaglandins and leukotrienes [22] The extract possibly inhibited the release of inflammatory mediators such as bradykinin and prostaglandins which are implicated in the second phase of inflammation in these models for anti-inflammatory agents.

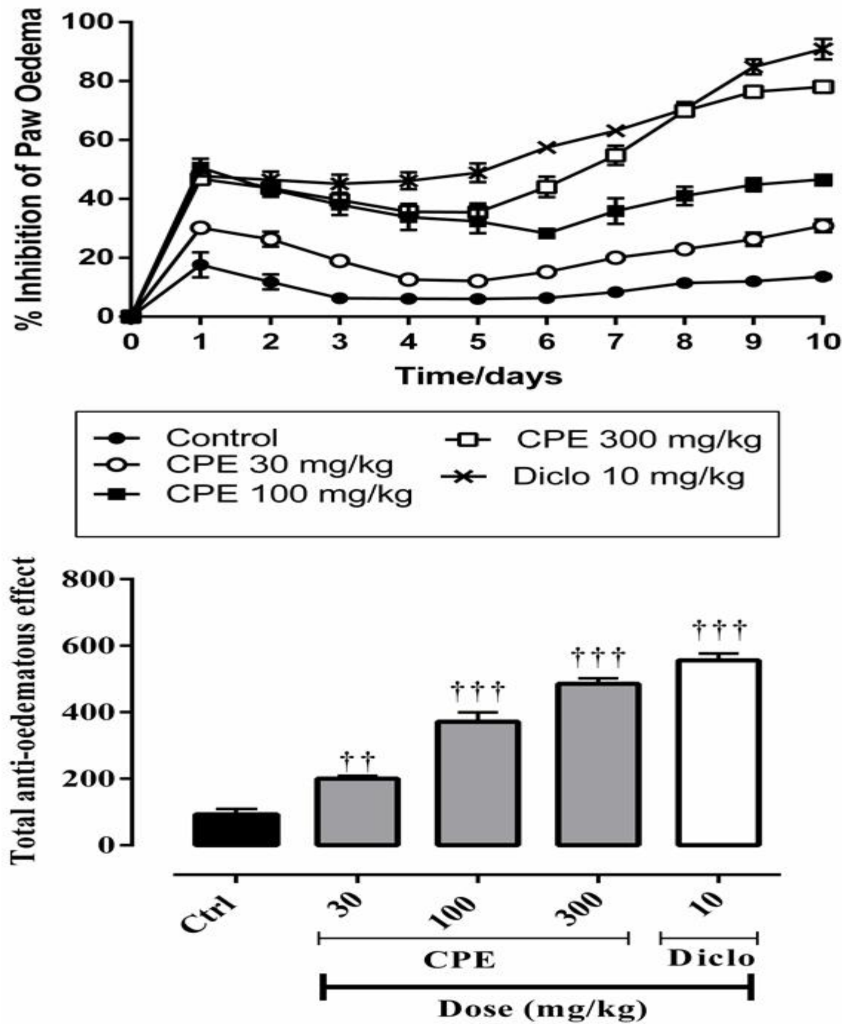

Fig. 3. The effect of *Calotropis procera* extract (30, 100 and 300 mg/kg, p.o.) and diclofenac (10 mg/kg, p.o.) on (a) % inhibition of paw oedema and (b) total anti-oedematous effect in the formalin induced chronic inflammation model in rats

To further investigate the anti-inflammatory effect of the extract, the formalin-induced paw oedema model was also employed. Intraplantar injection of formalin is known to produce a biphasic inflammatory response – early and late phase [23]. In the early phase, histamine, bradykinin and other inflammatory mediators are released and this occurs within two hours after intraplantar injection of formalin. The second phase which occurs after two hours involves the release of prostaglandins, cytokines, bradykinins etc. The effect produced by these mediators last throughout the ten days of the test. CPE (30, 100, 300 mg/kg) ameliorated the induced oedema like the standard analgesic drug used, diclofenac (10 mg/kg). Diclofenac has been known to exhibit this activity via inhibition of cyclooxygenase metabolism of arachidonic acid to produce prostaglandins. It is possible that the extract also exhibited its anti-oedematous activity through similar pathways. This view is supported by Arya and Kumar [9] who reported earlier that the latex extract of the plant exhibited its anti-inflammatory activity through inhibition of prostaglandins and other inflammatory mediators such as bradykinin, serotonins and histamines.

The presence of phytochemicals such as alkaloids, tannins saponins, and triterpenes, reducing sugars, sterols, amino acids and glycosides which were identified to be present in CPE could also contribute to the observed anti-inflammatory effect of the *C. procera*. Phytochemicals like the tannins and flavonoids have been recognised as responsible for the anti-inflammatory activities of medicinal plants [24]. Manthey, et al. [25], also reported that a number of flavonoids act by inhibiting key enzymes that are important in the synthesis of prostaglandins. Triterpenoids may perform their anti-inflammatory action by reducing the number of cells that express the inducible nitric acid synthase (iNOS) [26], or through the inhibition of nitric oxide production by decreasing the expression of iNOS [27]. Alkaloids are also said to reduce the intensity of oedema caused by carrageenan by inhibiting vascular permeability induced by histamine [28].

5. CONCLUSION

The inhibition of red cell haemolysis and protein denaturation and the reduction of oedema by the hydroethanolic extract of *Calotropis procera* from this study provides further evidence that *C. procera* has an anti-inflammatory potential. This supports the folkloric use of the plant as a potent anti-inflammatory agent. Further studies need to be done to isolate the pure compounds producing this effect. These can serve as scaffolds for novel anti-inflammatory agents with better therapeutic effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the ethics committee of the Department of Pharmacology, KNUST, Ghana and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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