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Anti-inflamatory Effects of Aqueous Extract of *Alchornea cordifolia* in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The World Health Organization (WHO) ranks chronic inflammatory diseases as the greatest threat to human health. The current study investigated the anti-inflammatory effects of aqueous leaf extract of *Alchornea cordifolia* on Wistar rats. Twenty Wistar rats of both genders weighing between 110-178g were used for this study. Egg albumin was used for induction of inflammation. The rats were grouped into five groups of 4 per group. Groups 3, 4 and 5 received 400, 800mg/kg b.w of aqueous extract and reference drug respectively after induction. Groups 1 and 2 served as normal and negative controls. Rats were sacrificed and blood samples collected for hematological and biochemical analyses. Phytochemical screening of the plant revealed the presence of alkaloid, phenolic compounds, Tannins and Quinine. Result showed significant in ($p \leq 0.05$) in paw size, rectal temperature and number of writhing in group 2 and non-significant reduction ($p \geq 0.05$) in group 4 were seen when compared to group 1 and 2 respectively. Significant ($p \leq 0.05$) increase in C-reactive protein and nitric oxide concentrations were observed in groups 3 and 4 when contrasted to group 2 in the studies. Non-significant differences in all hematological parameters in all treated groups were observed when compared to group 2. Aqueous leaf extract of *Alchornae cordifolia* displayed anti-inflammatory effects at 800 mg/ kg b.w after 4 hours of treatment.

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1. INTRODUCTION

Disease conditions leading to inflammation and pains are major healthcare problem worldwide. Incidences of pain and inflammation-related disorders have been on the increase worldwide. Although, several synthetic agents are available in treatment of these disorders, long-term usage has been reported by Qandil [1] to potentially lead to undesired effect. Management of painful inflammatory diseases (which in some cases elicit fever) whether with steroidal or non-steroidal medication is a common practice clinically [2]. The non-steroidal anti-inflammatory drugs (NSAIDs) have been found to block some stages in the biosynthesis of prostaglandins through the inhibition of cyclooxygenase (COX) [1]. NSAIDs are crucial drugs in reduction of inflammation especially when accompanied with pain. The often use of NSAIDs have been reportedly linked with heart, stomach, and nephro-toxicity [3,4]. In same manner, corticosteroids regular intake as reported by Gautam and Jachak (2009) could trigger hypertension, hyperglycemia and osteoporosis. Undesired out-comes and recurrence of symptoms characterized some of these synthetic drugs. Bioprospecting better anti-inflammatory, anti-pyretic and analgesic agents remains a subject of great interest [2], which solution may lie in plants [5]. Plants have a reputation as remedies in ethnomedicine for the management of inflammatory disorders and pain [6]. Recently, scouting for anti-inflammatory phytoconstituents has been increased. Extracts from plant reportedly possess vast secondary metabolites with myriad biological activities [7]. Plants are interesting focal point for novel anti-inflammatory compounds. This necessitated the need for this study which was designed to evaluate the anti-inflammatory properties of aqueous leaf extract of *Alchornea cordifolia* on Wistar rats.

2. MATERIALS AND METHODS

2.1 Source of Experimental Animal

Wistar rats were purchased from the Department of Pharmacy, Faculty of Pharmaceutical Science, University of Port Harcourt, Rivers State, Nigeria. They were housed in different cages by groups with renewable bedding, and were fed with standard rat feed and clean water, allowing them to acclimatize for fourteen days under normal temperature, humidity and light-dark cycle for acclimatization.

2.2 Plant Collection and Identification

The plant was obtained from the botanical garden of the University of Port Harcourt, Rivers State, Nigeria. After collection, the plant was sent to the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt, where it was properly identified.

2.3 Extract Preparation

The leaves were properly washed in running tap water and allowed to air dry for 2 weeks, and blended into fine powder form. Twenty five gram of the powder was macerated in 100 ml of deionized water for 24 hours under mechanical agitation at room temperature. The suspension was filtered using Whatman filter paper (25 mm) and dried in a water-bath at approximately 55^{o}C. Crude extract gotten was store at 4^{o}C before usage.

2.4 Experimental Design

Wistar rats of both sex weighing 110g to 178g were divided into five groups (n=4rats/group) of 20 rats for anti-inflammatory studies and anti-pyretic studies.

Chart 1. Experimental design for anti-inflammatory study

Groups	Description	Dose
Group 1	Normal control	No induction
Group 2	Negative control	1 ml of 0.2% egg albumin
Group 3	*Alchornea cordifolia* aqueous extract	1 ml of 0.2% egg albumin + 400 mg/kg
Group 4	*Alchornea cordifolia* aqueous extract	1 ml of 0.2% egg albumin + 800 mg/kg
Group 5	Reference drug	1 ml of 0.2% egg albumin + 50 mg Aspirin

2.5 Phytochemical Screening

Alkaloid: The aqueous *Alchornea cordiolia* leaves crude extract was dissolved in 2MHCl. The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with a few drops of Mayer's reagent; another portion with a few drops of Dragondroffs reagent and the other portion was treated with equal amount of Wagner's reagent. The creamy precipitate, orange precipitate and brown precipitate respectively indicated the presence of alkaloids (Salehl-Surmaghi et al., 1992).

Saponnin: The presence of saponnin was determined by frothing test. 0.5g of the extract was vigorously shaken with distilled water and was allow to stand for ten minutes and was classified for saponnin content as follows: Absence of froth indicate absence of saponnin and the presence of stable froth of more than 1.5 cm indicated the presence of saponnins (Kapor et al 1969).

Coumarin: One ml of plant extract in a test tube was treated with 3-4drops of 1%potassium hydroxide solution (obtained by 1g of KOH in ethanol) and the appearance of a yellow color indicates the presence of Coumarin.

Tannins: Ferric Chloride test:0.5g of plant extract was stirred with distilled water and filtered. Two drops of 5%Ferric Chloride solution was added to the filtrate. Formation of a blue black, green blue green precipitate was taken for evidence of tannins (Trease and Evan 1989).

Phenolic compound: 0.5g of plant extract was stirred with distilled water and filtered .2-3,drops of, 1% neural Ferric Chloride solution was added ,(obtained by adding dilute ammonia until precipitate just begin to form and filtered).The appearance of violet color with ferric ion indicates the presence of phenolic compounds.(shindo's test).

Quinine: To the test sample in a test tube dilute sodium hydroxide was added .formation of blue green or red color indicates the presence of quinine (shiondo' test).

Steroids: Liebermann-Burchard reaction was performed to assess the presence of steroids. Drops of chloroform, 3-4drops of acetic anhydride and drop s concentrated sulphuric acid were added down the side, appearance of purple colour that changes to blue or green color shows the presence of steroids.

Cardiac glycosides; Keller: Kiiani test was performed to access the presence of cardiac glycosides .the crude dry powder of the plant was treated with I ml of Ferric chloride reagent (mixture of 1 volume of5% $FeCl_3$ solution and 99volume of glacial acetic acid). To the solution a few drops of concentrated sulfuric acid was added. Appearance of greenish blue color within a few minutes indicates the presence of cardiac glycosides (Ajalyeobu, 2002).

Protein: million reagent: To the test sample in a test tube, few drops of reagent was added and heated for two minutes, formation of red precipitate indicates the presence of protein.

2.6 Haematological Analysis

2.6.1 Determination of erythrocyte sedimentation rate

Method: Westergren [8]

Principle: When citrated blood in a vertically positioned Westergren pipette is left undisturbed, red cells aggregate, stacked rouleaux form and sediment through the plasma. The ESR is the rate at which this sedimentation occurs in one hour proportional to column length above the red cells, measured in mm.

Reagents:

i. Tri-Sodium citrate, 32 g/l
ii. 3.2% w/v anticoagulant

Procedure: The westergren stands were prepared on a horizontal non vibratory surface. 0.4ml of freshly prepared sodium citrate was introduced in each tube, this was followed by addition of 1.6ml of blood. The resulting solution was mixed and then aspirated into the Westergren's pipette until it attains the zero mark. It was then transferred to the stand vertically on a horizontal plane, in descending position for 60 mins. After 60 minutes, the descending rate was observed and recorded. This was done for all the samples.

2.7 Total WBC Count

Method: Improved Neubauer ruled chamber.

Principle: Whole blood is diluted 1 in 20 in an acid reagent which haemolyzes the red cells leaving the white cells to be counted.

Reagents:

i. Ammonia oxalate solution.

Procedure: White blood cell count was done manually with the use of counting chambers. 0.38ml of Turk's solution was aliqouted into a plain bottle, 20ul of whole blood collected with an EDTA bottle, was pipetted to each bottles, it was mixed gently for sedimenting. The counting chamber was covered with a cover slide and the edges sealed of distilled water. The solution in the plain bottle was then charged into the counting chamber at an angle of 45°, it was mounted on the microscope and focused using X40 magnification, the cells were accurately counted and the value was then multiplied by 50 and divided by 1000 to get the actual white blood cell count. This was done for all the samples.

2.8 White Blood Cell Differential Count

Method: Leishman stain (Leishman [9]).

Principle: Methanolic mixture-based of Methylene blue and eosin. The methanolic stock solution is undisturbed serving the intention of directly fixing the smear thereby eliminating a prefixing step.

Reagents:

i. Leishman's stain

Procedure: A thin film was made on a microscope slide for each sample it was allowed to air dry. It was stained with Leishman's stain for 2-3 minutes, after which, it was diluted with distilled water for another 2 minutes then allowed to air dry. A drop of oil immersion introduced and mounted on the microscope using X100 magnification to count the different differential cells (neutrophils, lymphocytes, monocytes, eosinophils and basophils). The cells were noted and documented. This was replicated for all collected samples.

2.9 Biochemical Assay

2.9.1 Nitric oxide

Method: Modified Griess reaction described by Bredt and Snyder [10].

Principle: The Griess reaction is a diazotation reaction of sulphanilamide and further condensation of the produced diazonium salt with naphthyl-ethylene diamine hydrochloride.

Procedure: Total Nitric Oxide content is measured after the sample is incubated with Nitrate Reductase and NADH. The reductase join with NADH reduces Nitrate to Nitrite. After 20 minute incubation at 25^{o}C, color reagents A & B are added and incubated at 25^{o}C for 5 minutes. The amount of nitrate in the sample is calculated by taking the measured nitrite concentration and subtracted from overall nitric oxide concentration for the sample.

2.9.2 C-reactive protein

Method: Enzyme immunoassay.

Principle: This method is anchored on solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the on the C-reactive protein (CRP) molecule. This mouse monoclonal anti-CRP antibody for solid phase immobilization (on the microtiter wells). A goat anti-CRP antibody present in antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is reacted simultaneously with both antibodies, resulting in the CRP molecules being sandwiched between enzyme-linked antibodies and solid phase. After a 30-45-minutes incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A tetramethylbenzidine (TMB) reagent is introduced and incubated for 20 minutes, then formation of blue colour. The colour formation is stopped by introduction of 1N HCl transforming the colour to yellow. The amount of CRP equals the test sample colour.

Procedure: Serum was diluted 100 fold befor use. The coated wells needed in the holder was secured. 10 microliter of CRP standards was dispensed, before diluting specimens and control into the appropriate wells. 100 microliters of CRP enzyme conjugate reagent was then dispensed. Thoroughly mix for 30 seconds. Incubate at room

temperature (18-25°C) for 30-45 minutes. The incubation mixture taken by flicking plate contents into a waste contains. Rinsing and flicking the microtiter wells repeatedly in penta-fold. 100µl of TMB were dispense into solution of each well and were gently mixed for five seconds. Incubation was done at 25^{o}C for 20mins, then halting with 100 ul of stop-solution. It was then gently mixed for 20 seconds before reading at 450nm within 15 minutes.

2.9.3 Interleukin-6

Method: Enzyme immunoassay

Principle: Enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The small titer plate provided in the test kit has monoclonal antibody just for IL-6.

Procedure: All samples and reagents were stored at 25^{O}C before starting. All reagents, standards, and samples were prepared as directed by the manufacturer's manual. Excess microplate strips were extracted from plate frame, and then re-introduced pouch foil containing the desiccant pack, and reseal. 100ml std. was poured into samples and control per well. The plate sealer provided was covered and incubated at 25^{o}C for 120 minutes. The cover was removed and liquid discarded into a waste receptacle. The plate was inverted on the bench top onto a paper towel and then taped gently blot any remaining liquid. 100ul of the prepared 1x biotinylated anti-human IL-6 anti body introduced in each well, which was then cover with wash buffer. The process was then repeated for each well before adding 90µl of color developing reagent to all well. In all the well, the sealer provided was covered and stored in the dark for 30mins at 25^{0}C for change in colour measurement at 450 nm.

2.10 Data Analysis

Laboratory data were analyzed with statistical packages for Social sciences (SPSS version 20). Values were reported as mean plus-minus standard error of mean employing analysis of variance (ANOVA) and least significant difference (LSD) for multiple comparison.

3. RESULTS

Table 1 showed significant elevation ($p \leq 0.05$) in paw size of animals in group 3, 4 and 5 after one hour when compared with group 2, a significant increase ($p \leq 0.05$) in paw size in group 4 after two hours when compared with group 2, a significant reduction ($p \leq 0.05$) in paw size in group 5 after two hours when compared with group 4, a non-significant increase ($p \geq 0.05$) in paw size in group 3 after two hours when compared with group 2. Table 1 also revealed a non-significant increase ($p \geq 0.05$) in paw size in group 3 and 4 after three hours when compared with group 2, a non-significant decrease ($p \geq 0.05$) in paw size in group 5 after three hours when compared with group 2. Table 1 further showed a non-significant decrease ($p \geq 0.05$) in paw size in groups 3 and 4 after four hours when compared with group 2, a non-significant increase ($p \geq 0.05$) in paw size in group 5 after four hours compared with that of group 3 and 4.

Table 2 shows a significant decrease ($p \geq 0.05$) in interleukin-6 concentration in group 3 when compared with group 2, a non-significant increase ($p \geq 0.05$) in interleukin-6 concentration in group 4, a non-significant decrease ($p \geq 0.05$) in interleukin-6 concentration in group 5 when compared with group 2.

Table 2 also show significant reduction ($p \leq 0.05$) in C-reactive protein concentration in groups 3, 4 and 5 when compared with group 2, a non-significant decrease ($p \geq 0.05$) in C-reactive protein concentration in groups 3 and 4 when compared with group 5.

Table 2 indicates a significant decrease ($p \leq 0.05$) in nitric oxide levels in groups 3 and 5, a non-significant decrease ($p \geq 0.05$) in nitric oxide in group 4 when compared with group 2, a non-significant decrease ($p \geq 0.05$) in nitric oxide concentration in group 3 and non-significant increase ($p \geq 0.05$) in group 4 when compared with group 5.

Chart 2. Phytochemical Analysis of *Alchornea cordifolia* (urbobo)

Alkaloid	Positive
Phenolic_compound	Positive
Tannins	Positive
Flavonoid	Negative
Saponnin	Positive
Quinine	Positive
Coumarin	Negative
Protein	Negative
Cardiac glycoside	Negative
Steroid	Negative

Table 1. Effect of oral administration of aqueous leave extract of *Alchornea cordifolia* on egg albumin induced paw oedema in wistar rats

Group	Treatment	Initial paw size (mm)	Paw (mm) size at different hours			
			1 hour	2 hours	3 hours	4 hours
1	Control (0.5 ml distilled water)	3.98±0.02	3.98±0.02[a,b]	3.98±0.02[a]	3.98±0.02[a]	3.98±0.02[a]
2	Control (-ve) 0.2% egg albumin	3.98±0.02	6.11±0.80[a,b]	6.43±0.95[a]	6.82±1.07[a]	7.24±1.22[a]
3	0.2% egg albumin + 400mg/kg *Alchornea cordifolia*	4.60±0.08	8.39±0.14[a]	7.33±0.16	7.22±0.20	5.82±0.22
4	0.2% egg albumin + 800mg/kg *Alchornea cordifolia*	4.68±0.10	8.55±0.08[a]	8.29±0.09[a,b]	7.17±0.18	5.00±1.62
5	0.2% egg albumin + 50mg/kg Dichlofenac	4.70±0.15	8.23±0.59[a,b]	6.76±0.31[b]	5.99±0.12	7.55±0.18

Values are reported as mean ± standard error of mean (M±SEM) (n =4). Values with similar superscript letters indicate statistical significant differences (p≤ 0.05) down the column while those without superscripts show non-significant differences (p≥ 0.05) down the column when compared with the control and between groups

Table 2. Effect of oral administration of aqueous leave extract of *Alchornea cordifolia* on interleukin-6, C-reactive protein and nitric oxide concentration of egg albumin induced-paw oedema in wistar rats

Group	Treatment	Interleukin-6 (pg/ml)	C-reactive protein (mg/l)	Nitric Oxide (mg/ml)
1	Control (0.5 ml distilled water)	249.75±59.79	0.06±0.00[a]	0.085±0.00[a]
2	Control (-ve) 0.2% egg albumin	210.20±15.98[a,b]	3.92±1.73[a]	3.08±1.29[a,b]
3	0.2% egg albumin + 400 mg/kg *Alchornea cordifolia*	88.23±21.03[a,b]	0.08±0.00[a]	0.62±0.24[a,b]
4	0.2% egg albumin + 800 mg/kg *Alchornea cordifolia*	112.88±10.40	0.09±0.03[a]	1.30±0.41
5	0.2% egg albumin + 50 mg/kg Dichlofenac	131.18±30.80	0.47±0.24[a]	0.76±0.11[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =4). Values with similar superscript letters indicate statistical significant differences (p≤ 0.05) down the column while those without superscripts show non-significant differences (p≥ 0.05) down the column when compared with the control and between groups

3.1 Effect of Oral Administration of Aqueous Leave Extract of *Alchornea cordifolia* on Some Haematological Parameters of Egg Albumin Induced-paw Oedema in Wistar Rats

The ESR shown in Table 3 revealed a non-significant increase (p≥ 0.05) in group 3, 4 and 5 when compared with group 2, a non-significant decrease (p≥ 0.05) in WBC and neutrophils levels in group 3 and 4, a significant increase (p ≥ 0.05) in WBC levels in group 5 and non-significant decrease in neutrophils levels in group 5 when compared with group 2.

Table 3 revealed a significant increase (p≤ 0.05) in lymphocytes levels in group 3, a non-significant decrease (p ≥ 0.05) in lymphocytes levels in group 4 when compared with group 2, a significant decrease (p ≤ 0.05) in lymphocytes levels in group 5 when compared with group 3.

Table 3. Effect of oral administration of aqueous leave extract of *Alchornea cordifolia* on some haematological parameters of egg albumin induced paw-oedema in wistar rats

Group	Treatment	ESR (mm/hour)	WBC (X 10^9/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	Control (0.5 ml distilled water)	0.00±0.00[a]	9.00±0.24	40.00±1.22	48.50±0.61	5.00±0.41	1.50±0.20[a]	0.00±0.00[a]
2	Control (-ve) 0.2% egg albumin	21.00±3.00[a]	13.65±1.91	44.50±2.10	41.25±3.15[a]	8.50±1.19	4.50±1.04[a]	1.25±0.63[a]
3	0.2% egg albumin + 400 mg/kg *Alchornea cordifolia*	40.00±8.16	10.00±1.00	32.33±4.90	57.67±6.34[a,b]	7.00±1.87	3.00±0.71[b]	0.00±0.00[a,b]
4	0.2% egg albumin + 800 mg/kg *Alchornea cordifolia*	21.33±7.36	11.73±1.80	33.67±7.04	55.33±6.79	4.67±0.85[a]	6.33±0.62[b]	0.00±0.00[a,b]
5	0.2% egg albumin + 50 mg/kg Dichlofenac	39.50±10.21	14.20±2.88	40.25±2.32	42.75±4.19[b]	11.00±2.48[a]	4.50±0.65	1.50±0.29[b]

Values are reported as mean ± standard error of mean (M±SEM) (n =4). Values with similar superscript letters indicate statistical significant differences (p≤ 0.05) down the column while those without superscripts show non-significant differences (p≥ 0.05) down the column when compared with the control and between groups

Table 3 also indicates a non-significant decrease ($p \geq 0.05$) in monocytes levels in group 4, a non-significant increase ($p \geq 0.05$) in group 5 when compared with group 2, a significant decrease in monocytes levels in group 4 when compared with group 5.

Furthermore, Table 3 shows a non-significant decrease ($p \geq 0.05$) in eosinophils levels in group 3 and 5, a significant increase ($p \leq 0.05$) in group 4 when compared with group 3, a non-significant increase ($p \leq 0.05$) in eosinophils levels in group 4 when compared with group 5.

Table 3 also showed a significant decrease ($p \leq 0.05$) in basophils levels in group 3, a non-significant increase ($p \geq 0.05$) in group 5 when compared with group 2, a significant decrease ($p \leq 0.05$) in basophils levels in groups 3 and 4 when compared with the group 5.

4. DISCUSSION OF FINDINGS

Inflammation in most living organisms is mounted to protect the organism from harm (physical), chemical noxious stimuli or microbiological toxins, which according to Guzik et al. [11] is involved in multiple pathologies. Ideally, inflammatory response is set to inactivate or destroy invading organisms, remove irritants, which will then be followed by tissue repair. The inflammatory response consists of specific immunological and non-specific immune reactions. The primary processes in inflammation include increase in vascular permeability, release of lipid-derived autacoids, such as eicosanoids or platelet-activating factor (PAF); large peptides, such as interleukin-1; small peptides (for instant bradykinin); and amines, such as histamine or 5-hydroxytryptamine from injured tissues and migrating cells. As stated by Guzik et al. [11] that this constitute the chemical network of inflammatory response and result in clinical and pathological manifestations of inflammation. Drugs prescription to reduce inflammation in orthodox medicine exist in literature, while in complementary and alternative medicine, plants parts are utilized in management of inflammation. This study finding on the anti-inflammatory potential of aqueous leaf extract of *Alchornea cordifolia* at varying doses of 400 and 800 mg/kg b.w (Table 1) exhibit significant reduction ($p \leq 0.05$) in the level of inflammation (egg albumin-induced paw oedema) in the reference drug (dichlofenac) treated group after two hours of oral administration when compared with the 800mg/kg b.w extract treated group, indicating the potency of dichlofenac after two hours over the *Alchornea cordifolia* leaves extract at 800mg/kg b.w. However, after four hours of observing the level of egg albumin-induced oedema in the 400 and 800mg/kg b.w *Alchornea cordifolia* extract treated groups, a non-significant decrease ($p \geq 0.05$) was observed, implying time-dependent action of *Alchornea cordifolia* extract which agreed with Osadebe and Okoye [12] that reportedly observed the anti-inflammatory activities of *Alchornea cordifolia* three hours post-administration. This might be due to time dependent ability of *Alchornea cordifolia* to interfere with vascular permeability that normally will leading to influx of leukocytes, accompanied by granuloma appearance and posible tissue repair [13]. *Alchornea cordifolia* extract may have also interfere with arachidonic acid, adhesion molecules, cytokines, chemokines, and platelet-activating factor that usually cause release of other mediators trigering chemotaxis [14]. Findings from the study further revealed that as time elapse, the effect of the anti-inflammatory drug dichlofenac reduces as the study found a non-significant increase ($p \geq 0.05$) in paw odema in the dichlofenac treated group after four hours of post administration.

Further findings on the anti-inflammatory activities of aqueous leaves extract of *Alchornea cordifolia* as shown in Table 2 revealed a significant decrease ($p \leq 0.05$) in interleukin-6 concentration of the 400mg/kg extract treated group when compared with the control group which agrees with Osadebe and Okoye [12] findings that the anti-inflammatory activities of *Alchornea cordifolia* can be seen even at 400mg/kg b.w. Increased expression of interleukin is one the biomarkers of inflammation [15], the findings of this study it can be inferred that *Alchornea cordifolia* may interfere with the expression of interleukin-6 which acts through a membrane-bound interleukin-6 receptor (mIL-6R), which, together with a second receptor, glycoprotein, leads to the initiation of intracellular signalling [16].

C-reactive protein as reported by Sproston and Ashworth [17] is an acute inflammatory protein that increases up to a thousand fold at sites of inflammation. An elevated level of C-reactive protein, an acute phase protein, is one of many downstream indicators of inflammation. This study found that the aqueous leaves extract of *Alchornea cordifolia* at 400 and 800mg/kg b.w significantly decrease ($p \leq 0.05$) the C-reactive protein concentration adding further credence to

the anti-inflammatory activities *Alchornea cordifolia*. It was also found in this study that *Alchornea cordifolia* aqueous extract at 400 and 800mg/kg b.w exhibit a non-significant decrease ($p \geq 0.05$) in C-reactive protein concentration when compared with 50mg/kg dichlofenac implying that the extract is slightly better than dichlofenac in terms of the ability to reduce C-reactive protein synthesis. This finding is relevant to the pharmaceutical industry in its search for better anti-inflammatory drugs in management of disorders that leads to production of inflammation.

Alchornea cordifolia was found in this study to significantly decrease ($p \leq 0.05$) nitric oxide concentration in animals given 400 mg/kg of the plant. The decrease in nitric oxide concentration as found in this study further support the anti-inflammatory potential of *Alchornea cordifolia* aqueous leave extract as Ying and Hofseth [18] reported that nitric oxide and ROS exert multiple modulating effects on inflammation and play a key role in the regulation of immune responses. According to Ying and Hofseth [18], nitric oxide and reactive oxygen species affect virtually every step of the development of inflammation. Low concentrations of nitric oxide produced by constitutive and neuronal nitric oxide synthases inhibit adhesion molecule expression, cytokine and chemokine synthesis, leukocyte adhesion and transmigration [18]. However, increased dose (800mg/kg) of the extract was observed to produce non-significant decrease in the nitric oxide concentration which can be inferred that the extract may be more effective at lower concentration.

The ESR which is a non-specific haematological indicator of inflammation, as seen in this study (Table 3) was observed to reduce in the extract treated groups. WBC, neutrophils, monocytes, eosinophils and basophils levels were not different from the ESR. However, lymphocytes levels significantly increased in the 400 and 800mg/kg *Alchornea cordifolia* treated groups which agrees with Mohammed et al*.* [19] finding that *n*-butanol fraction from aqueous leaf extract of *Alchornea cordifolia* caused elevation of total white blood cell, lymphocyte count and normalization of other haematological parameters [20].

5. CONCLUSION

This research findings indicates that *Alchornea cordifolia* at 400 and 800 mg/kg b.w showed anti-inflammatory activities properties.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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