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Anti-inflammatory and Analgesic Effects of Aqueous and Methanol Leaf Extracts of *Chrysophyllum Albidum* in Male Wistar Rats with Acetic Acid Induced Inflammation and Pain

Emmanuel Afen Eneji[a], Nancy Amalachukwu Mbachu[b*], Sandra Chioma Ugwu-Ejezie[c], Fidelis Ebele Ejezie[a]

[a]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus, Nigeria*
[b]*Department of Human Biochemistry, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria*
[c]*Department of Hematology and Immunology, University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu Campus, Nigeria*

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Abstract

Chrysophyllum albidum (African star apple) fruits and fresh leaves have been used as food widely. The mature leaves, bark, seeds and roots are used as folk medicine for the treatment and management of various ailments, hence the anti-inflammatory and analgesic properties of *Chrysophyllum albidum*on inflammation and pain were investigated. Thirty five male wistar rats weighing 150.41±13.27g were randomly placed in seven (I-VII) groups. Group I (normal control), group II (diclofenac), group III(positive control), groups IV and V received 200mg and 400mg/Kg bodyweight of methanolic extract; groups VI and VII received 200mg and 400mg/Kg bodyweight of aqueous extract, respectively. 0.1ml of 2.5% of acetic acid was injected into the hind paw of rats to induce inflammation and pain, and then treated for 14 days. Severity of pain was quantified using Likert scale. Standard biochemical and phytochemical parameters were determined. Statistical differences in the mean between groups was compared using one way analysis of variance at $p<0.05$. The extract had inhibition of edema at 92%, 95% and 92%, 93% at doses of 200mg/kg, 400mg/kg bodyweights of methanolic and aqueous extracts, respectively. The yield from quantitative analysis ranged from Cardiac glycosides>Saponins>flavonoids>alkaloids>phenols; 42.6% vs. 38.1% for methanolic vs. aqueous extracts, respectively. Animals treated with both extracts had significant increase in weights and white blood cells compared to the standard and normal control group ($p<0.05$). These suggest that the extract had ameliorative, preventive and curative potency on inflammation which may be attributed to the presence of glycosides, saponins and other phytoconstituents.

1. Introduction

Pain is an unfriendly sensation, physical and emotional experience allied by means of definite or latent tissue impairment; an unlikable reaction regularly caused by strong or harmful stimuli **(Aasvang*et al.*, 2010)** or a bodily sensitivity connecting to human feeling **(Price, 1999)**, it may be nociceptive, inflammatory or pathological **(Amr and Yousef, 2010)**. Pain is a major characteristic of inflammation - a protective reaction of the bodies to potential harmful elements (infection or injury) by certain chemical substances, driven by the immune system. Inflammation is important, protective and necessary for the defense as well as preservation of health **(Montero-Melendez, 2018)** and may result to pain because the distension and enhanced tissue press on the nerve endings. The response to pain depends on the stage, gender, beliefs, earlier agony, understanding, besides concern. Analgesics are medications that ease pain without causing loss of awareness **(Buttgeiret *et al.*, 2013)**.

Many developing countries have incorporated natural medicines as sources of therapeutic agents, based on the ease of availability, affordability, accessibility, and most importantly the "supposed" relatively lower incidence of side effects accompanying the consumption of natural herbal medicines when compared to the conventional or synthetic drugs **(Ahmad *et al.*, 2002)**.

Africa is characterized by massive gift of plants that can and are used for medicinal purposes. The bioactive substances responsible for its medicinal properties are contained in the plants' roots, leaves, stem, fruits, flowers, seeds and bark **(Prashant, 2011)**.

Chrysophyllum albidum (African *caimito* or star apple)is a fruit tree of the sapodilla family, with natural occurrence in numerous eco-zones in the Federal Republic of Nigeria, Republic of Ugandaand Niger Republic **(Ibrahim *et al.*, 2017)**.The plant is used as food among people of different believes in Nigeria and promotes health, acting against oxidative stress and other related diseases such as diabetes, tumor and coronary heart illness **(Burits and Bucar, 2000)**.The calciferol contained in the leaves have regulating ability against carcinoma **(Fasogbon *et al.*, 2017)**.

*Corresponding author: na.mbachu@unizik.edu.ng
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Irritation can lead to progressive tissue destruction by the harmful aggravation and change the survival of the organism. Inflammations are habitually coupled to harmful side effects **(Serhan *et al.*, 2007)**. The situation causes pain, redness, immobility of the joint, swelling and heat, which may result to fatigue, restlessness, fever and other related problems if not treated **(Serhan *et al.*, 2007)** which affects the general immune system **(Afsar and Ahmed, 2011)**. Some cultures use *Chrysophyllum albidum* fruits and fresh leaves as food, while mature leaves, bark and roots are used as folk medicine. Although previous studies have shown the antiplatelet, hypoglycemic **(Adebayo *et al.*, 2010)** and antidiarrheal **(Adewusi *et al.*, 1997)** properties of the leaves, there is paucity of information on its analgesic and anti-inflammatory effect hence, this study therefore investigated the ameliorative, protective, and curative (anti-inflammatory and analgesic) effects of aqueous and methanol leaf extracts of *Chrysophyllum albidum* in male Wistar rats with acetic acid induced inflammation and pain.

2. Material and methods

2.1 Plant collection and identification

Healthy fresh leaves of *Chrysophyllum albidum* were collected from their natural habitat at Anambra-Imo River Basin Development Authority Agbala, Owerri North, Imo State, Nigeria. The plant leaf was identified and validated by Ozioko, A of the Department of Botany, University of Nigeria, Nsukka, with herbarium voucher specimen number, UNH NO 2519.

2.2 Plant extract preparation

The healthy fresh leaves of *Chrysophyllum albidum* were washed in fresh water and air dried in the laboratory at room temperature. The air dried leaves were milled into fine powder in an electric blender (**Mondal *et al.*, (2011)**.).

2.3 Aqueous extraction

Fine powder weighing 430g was soaked in 2 liters of distilled water for 24 hours. The solution was then filtered using a Whatman no.1 filter paper (150 mm), and evaporated in a water bath at 50°C to the concentrated extract of *Chrysophyllum albidum* weighing 164g **(Bhande and Wasu, 2016)**

2.4 Methanol extraction

The dried leaves were ground to powder, 430g of *C. albidum* powder was weighed and dissolved in 2L of 20% methanol for two days, the extract was sieved using a Whatman no.1 filter paper and a cotton wool; then concentrated at 50°C and 48°C by rotary evaporator and water bath to an extract of *Chrysophyllum albidum* weighing 183g.The ratio of the weight of the concentrated extract and the crude extract was calculated and expressed as the percentage yield of the extract (**Adebayo, 2006)**

2.5 Phytochemical analysis

Analyses of phytochemical constituents were carried out at the Department of Pure and Industrial Chemistry, University of Nigeria Nsukka as described by **Trease and Evans (1989)**. The phytochemical screening involved detection of each of the following secondary metabolites: alkaloids, tannins, saponins, flavonoids, phenols, cardiac glycosides, and anthraquinones.

2.6 Dose selection

Dose selection studies were carried out at the College of Medicine, University of Nigeria, Enugu Campus as described by **Akuodor *et al.* (2011) and Okwuosa *et al.* (2012)**. Crude extract residue was weighed and dissolved in distilled water for use on each day of experiment (10 g of crude extract was dissolved in 100 ml of normal saline to get the stock solution of extract for daily administration). Stock concentrations (mg/kg body weights) of 100, 500, 1000, and 1500 were administered.

2.7 Toxicity study

The lethal dose (LD_{50}) of the plant extract was determined following the method of **Lorke (1983).** The toxicity study was in two stages using 12 rats. Phase one: the rats were divided in 4 collections of 2 wistar rats each and treated with the methanol and aqueous leaf extracts of the plant at oral doses of 100 and 1000 mg/kg bodyweight. They were observed for 24 h for signs of harmfulness. In phase two, the remaining rats were divided into 4 groups of 1 rat each and were also treated with aqueous and methanol extracts at doses of 500 and 1500mg/kg bodyweight orally.

2.8 Experimental animals

Thirty-five (35) adult male wistar rats weighing about 150g were procured from the animal house of Anatomy Department, University of Nigeria, Enugu campus and housed in cages. The rats received standard livestock pellets and water *ad libitum* throughout the 14-days acclimatization. The animals were handled in accordance with the directives for animal research as detailed in the NIH Guidelines for the Care and Use of laboratory animals **(Public Health Service, 1996)**. All experimental methods were reviewed as approved by the Institutional Animal Ethical Committee.

2.8.1 Ethical clearance

Ethical clearance was obtained from the Research and Ethics Committee of the College of Medicine University of Nigeria Enugu Campus.

2.8.2 Experimental design

The thirty five (35) rats were randomly divided into 7 groups of 5 rats each. *C. albidum* extract and diclofenac were administered to the rats orally.

2.8.3 Analgesic test

The painkilling properties of the solvent extracts of *Chrysophyllum albidum* leaf was tested according to **Akuodor *et al.* (2011)**. The rats were placed in surveillance space and examined for one hour. The severity of pain response was taken for each rat, quantified using categorical variable of Likert scale, determined in the early and late phases.

GROUP	TREATMENT
Group 1: Normal control (food and water) only	Distilled water and food pellet only for 14 days.
Group 2: Diclofenac only + Acetic acid	Diclofenac was given daily for 14 days at a dose of 50 mg/kg body weight.
Group 3: Induced with acetic acid	Acetic acid (0.1ml) only
Group 4: Methanol extract only (low dose) + Acetic acid	Methanol extract at a dose of 200 mg/kg body weightfor 14 days.
Group 5: Methanol extract only (high dose) + Acetic acid	Methanol extract at a dose of 400 mg/kg body weight for 14 days.
Group 6: Aqueous extract only (low dose) + Acetic acid	Aqueous extract at a dose of 200 mg/kg body weight for 14 days.
Group 7: Aqueous extract only (high dose) + Acetic acid	Aqueous extract at a dose of 400 mg/kg body weight for 14 days

2.8.4 Anti-inflammatory test

The counter-inflammatory consequence of the solvents extract of *Chrysophyllum albidum*leaf was verified as recorded by **Umar *et al*. (2018)**. The potential effects of the leaf extracts were confirmed by acetic acid injected into hind paw of rats which induced inflammation and pain.

The counter-inflammatory action was checked using leaf extracts of two solvents, aqueous and methanol. Inflammation and pain were noticed when 2.5% of concentrated acetic acid of 0.1 ml was introduced to each wistar rats of the groups apart from normal group one hour after extracts and drug were administered. Rise in volume (mm) of edema was measured using vernier caliper. Percentage inhibition of edema was calculated using the formula:

$$\% \text{ inhibition of oedema} = \frac{\text{Ec} - \text{Et} \times 100}{\text{Et}}$$

Ec = edema of the control group; Et = edema of the test group

On the first and last day of the experiment after induction, blood samples were collected for evaluation of blood parameters and markers of inflammation which include; Erythrocyte sedimentation rates (ESR), C-reactive protein (CRP) and plasma viscosity (PV), hemoglobin level (Hb), white blood cell (WBC) and red blood cell (RBC)count

2.8.5 Hematological and biochemical analyses (full blood count, CRP and ESR)

Erythrocyte sedimentation rates (ESR), C-reactive protein (CRP) and plasma viscosity (PV) were used to measure increase in protein in the blood and indicate the presence of inflammation (Serhan*et al*., 2007)

2.8.6 Statistical analysis

Data analysis was done using SPSS (version 21.0), difference in mean across groups was determined using one way analysis of variance (ANOVA) at $p<0.05$. Results were expressed as mean ±standard deviation (SD). The change in magnitude of the analysed parameters over the controls were calculated and expressed as percentages.

3. Results

The phytochemical constituents of leaves extracts ranged from Cardiac glycosides> Saponins>flavonoids>alkaloids>phenols (Table 1); 42.6% vs. 38.1% for methanolic vs. aqueous extracts, respectively.

Table 1 Quantitative analysis of leaves constituents

Phytochemical Constituents	Quantitative analysis (mg/100g)
Alkaloids	6.41±0.30
Saponins	18.20±1.20
Phenols	2.02±0.19
Cardic glycosides	40.10±2.60
Flavonoids	15.32±0.12

The body weights of experimental rats were significantly increased in group I (normal control); reduced in groups II and III treated with diclofenac and positive control group that were not treated. Significant body weight increase was observed in groups IV, V and VI, VII rats treated with 200mg methanol, 400mg methanol, and 200mg aqueous, 400mg aqueous extract per bodyweight respectively.

Table 3 shows the effect of administration of aqueous and methanol extracts of *Chrysophyllum albidum* on the hematological indices using acetic acid to induce edema and pain. There was asignificant decrease in the level of packed cell volume, haemoglobin and red cell count which shows protective effect of extracts on haematological parameters (full blood count) of treated rats

Table 4 shows anti-inflammatory effect of extracts on treated rats. From the table, comparing result of standard drug group with normal control group, in ESR a day after inflammation was persuaded, showed no change in weight ($p<0.05$). Compared with positive control, there was an alteration in both normal control and standard drug group. There was no significant difference after the 14th day post treatment. There was a significant difference between the positive control, normal control group and standard drug group ($p < 0.05$).

The outcome of treatment with methanol and aqueous extracts of *C. albidum* using ESR as marker displayed dose reliance. Rats administered with High dose of methanol extract displayed high significant difference at $p<0.05$. There was no significant difference between ESR and CRP after 14th day of treatment.

Table 5 shows the ameliorative effect of extracts on treated rats. The treatment mode of extracts administration showed significant increase ($p<0.05$) when compared to the standard and the normal control group, signifying less potency.

The percentages of edema inhibited by the extracts are showed in table 6. Initial edema is the degree of edema on each group of rats after induction. Final edema showed the stage of edema after treatment.

Percentage inhibition exposed the effectiveness of the extracts on edema; it checked capacity of the extracts to control inflammation. Calculation for each dose was done using the formula: $\% \text{ inhibition of edema} = \frac{\text{Ec} - \text{Et} \times 100}{\text{Et}}$ Ec: edema of a control group; Et: edema of a test group.

Comparison of extracts with the standard drug (DCF) showed an increase in percentage reduction of edema though dose dependent. The extracts showed effectiveness in reduction of edema. In the positive control group where rats were not treated, the percentage inhibition of edema was the same as that of the standard drug.

Table 2 Effect of methanol and aqueous extract of C.albidum on the mean body weight of experimental rats (g)

Group	Initial weight	Final weight	Weight change	% change in weight
I (NC)	150.2±9.7	164.9±11.8	15	10↑
II (DCF) (50mg/bw)	151.0±6.0	138.3±10.7	-13	8.6↓
III (PC)	151.9±12.5	146.2±10.8	-6	3.9↓
IV (LDME) (200mg/bw)	149.1±28.5	150.8±24.8	2	1.3↑
V (HDME) (400mg/bw)	151.2±3.4	153.7±3.2	3	1.9↑
VI (LDAE) (200mg/bw)	148.6±20.4	152.1±21.1	3	2.0↑
VII (HDAE) (400mg/bw)	150.9±12.4	157.3±12.5	6	3.9↑

NC: Negative control, DCF: Diclofenac, PC: Positive control, LDME: Low dose of methanol Extract, HDME: high dose of methanol extract, LDAE: low dose of aqueous extract, HDAE: high dose of aqueous extract. (↑) - Weight gain, (↓) - Weight loss. bw: body weight

Table 3 The Profile of haematological indices of treated animals

Group	PCV (%)	WBC ($\times10^9L^{-1}$)	RBC ($\times10^9L^{-1}$)	Hb (gdL^{-1})
I NC	49.0 ± 2.65^{b}	6666.7 ± 231.0^{b}	245.0 ± 5.00^{b}	16.7 ± 0.91^{b}
II DCF (50mg/b.w)	23.3 ± 3.50^{a}	4000.0 ± 400.0^{a}	216.7 ± 15.3^{a}	14.7 ± 2.88^{a}
III PC	45.0 ± 1.00^{a}	5400.0 ± 229.2^{a}	236.7 ± 25.2^{a}	15.3 ± 0.37^{ab}
IV (LDME) (200mg/b.w)	51.7 ± 6.51^{b}	8533.3 ± 2023.2^{ab}	236.7 ± 15.3^{a}	17.6 ± 2.21^{b}
V (HDME) (400mg/b.w)	35.7 ± 6.14^{a}	$7333.3\pm1222.^{ab}$	235.0 ± 8.67^{a}	18.9 ± 2.76^{b}
VI (LDAE) (200mg/b.w)	39.0 ± 7.14^{a}	6266.7 ± 1205.5^{b}	246.7 ± 15.3^{b}	15.0 ± 2.70^{b}
VII (HDAE) (400mg/b.w)	38.1 ± 7.02^{a}	6352.3 ± 1112.4^{b}	234.6 ± 14.2^{a}	14.1 ± 2.41^{a}

PCV: Packed cell volume (%), WBC: white blood cell ($\times10^9L^{-1}$), RBC: red blood cell ($\times10^9L^{-1}$), Hb:hemoglobin (gdL^{-1}). b.w: body weight, NC: Negative control, DCF: Diclofenac, PC: Positive control, LDME: Low dose of methanol Extract, HDME: high dose of methanol extract, LDAE: low dose of aqueous extract, HDAE**:** high dose of aqueous extract.Values with similar superscript on numbers in the vertical line in a table are not different at $p<0.05$. Those with different letters are considerably different at $p<0.05$.

Table 4 Anti-inflammatory effect of extracts on treated rats

Group	CRP (mg/L) (day after induction)	ESR (mm/hr) (day after induction)	ESR (mm/hr) (after two weeks)	CRP (mg/L) (after two weeks	Edema (cm)
1(NC)	$1.05\pm.0.53^{bc}$	0.62 ± 0.88^{bc}	1.62 ± 0.85^{bc}	$1.06\pm.054^{bc}$	0.00±0.00
2 (DCF)	3.57 ± 0.697^{bc}	$0.60\pm.0.55^{a}$	2.40 ± 1.67^{bc}	1.54 ± 0.27^{bc}	$1.52\pm.58^{bc}$
3 (PC)	6.02 ± 1.210^{a}	1.20 ± 0.836^{bc}	2.80 ± 0.45^{a}	3.33 ± 0.36^{bc}	$3.06\pm.66^{bc}$
4 (LDME)	2.01 ± 0.86^{bc}	0.80 ± 0.08^{a}	2.60 ± 1.51^{bc}	$1.66\pm.428^{bc}$	0.92 ± 0.85^{a}
5 (HDME)	2.20 ± 0.64^{bc}	1.80 ± 0.84^{bc}	2.00 ± 0.70^{bc}	1.63 ± 0.43^{bc}	1.48 ± 1.13^{bc}
6 (LDAE)	3.09 ± 0.67^{bc}	1.40 ± 1.14^{a}	$2.40\pm.547^{bc}$	2.02 ± 0.67^{bc}	$1.06\pm.639^{bc}$
7 (HDAE)	2.73 ± 0.77^{bc}	1.20 ± 1.30^{a}	1.80 ± 0.83^{bc}	1.57 ± 0.36^{bc}	0.94 ± 0.43^{bc}

CRP: C-Reactive proteins (mg/L), ESR: Erythrocyte Sedimentation Rate (mm/hr), Edema (cm), NC: Negative control,DCF: Diclofenac,PC: Positive control, LDME: Low dose of methanol Extract, HDME: high dose of methanol extract, LDAE: low dose of aqueous extract, HDAE: high dose of aqueous extract.Values with the same superscripts on numbers in the vertical line are not significantly different at $P<0.05$, those with different letters are significantly different at $p<0.05$.

Table 5 Ameliorative effect of extracts on treated rats

Group	CRP (mg/L) (day after induction)	ESR (mm/hr) (day after induction	CRP (mg/L) (after two weeks)	ESR (mm/ hr)(after two weeks)	Edema (cm)
I (NC)	1.25±0.041^{bc}	0.62±0.39^{bc}	1.06±0.24^{bc}	1.62±0.38^{bc}	0.00±0.00^{bc}
II (DCF)	3.57±0.31^{bc}	0.60±0.25^{a}	1.54±0.12^{bc}	2.40±0.75^{bc}	1.52±0.26^{bc}
III (PC)	6.02±0.54^{a}	1.20±0.37^{bc}	3.33±0.16^{bc}	2.80±0.20^{a}	3.06±0.30^{a}
IV (LDME)	2.01±0.38^{bc}	0.80±0.37^{a}	1.66±0.19^{bc}	2.60±0.68^{bc}	0.92±0.37^{a}
V (HDME)	2.20±0.29^{bc}	1.80±0.37^{bc}	1.63±0.20^{bc}	2.00±0.32^{bc}	1.48±0.51^{bc}
VI (LDAE)	3.09±0.30^{bc}	1.40±0.51^{bc}	2.03±0.30^{bc}	2.40±0.25^{bc}	1.06±0.29^{bc}
VII (HDAE)	2.73±0.35^{bc}	1.20±0.58^{a}	1.57±0.16^{bc}	1.80±0.37^{bc}	0.94±0.19^{bc}

CRP: C-Reactive proteins (mg/L), ESR: Erythrocyte Sedimentation Rate (mm/hr), Edema (cm),PC: Positive control, NC: Negative control,DCF: Diclofenac, LDME: Low dose of methanol Extract, HDME: high dose of methanol extract, LDAE: low dose of aqueous extract, HDAE: high dose of aqueous extract.The same superscripts on values in the column are not significantly different at $p < 0.05$; values with different superscripts are considered different at $p < 0.05$.

Table 6 Rate of edema inhibition by extracts

Group	Initial edema (cm)	Final edema (cm)	Change in edema (cm)	% change in edema	% inhibition of edema
I (NC)	2.2	2.0	-0.2	-9	0
II (DCF)	7.6	7.3	-0.3	-3.9	33
III (PC)	15.3	15.0	-0.3	-2	33
IV (LDME)	4.6	2.1	-2.5	-54	92
V (HDME)	7.4	3.2	-4.2	-57	95
VI (LDAE)	5.3	2.5	-2.8	-53	92
VII (HDAE)	4.7	1.8	-2.9	-62	93

NC: Negative control, DCF: Diclofenac, PC: Positive control, LDME: Low dose of methanol Extract, HDME: high dose of methanol extract, LDAE: low dose of aqueous extract, HDAE: high dose of aqueous extract.

Table 7 The analgesic effect of extracts on treated rats

Group	A	B	C	D	E	F
I (NC)	5.00±0.00^{a}	0.18±0.08^{a}	5.00±0.00^{a}	0.48±0.39^{a}	0.10±0.07^{a}	0.18±0.13^{a}
II DCF	3.00±0.71^{a}	1.72±1.73^{bc}	2.74±1.31^{a}	3.80±2.28^{a}	15.00±4.30^{bc}	5.20±1.79^{a}
III (PC)	1.90±1.14^{bc}	2.36±0.65^{bc}	1.24±1.35^{bc}	4.40±1.82^{bc}	18.40±9.58^{bc}	9.00±5.43^{bc}
IV (LDME)	2.20±1.10^{bc}	0.98±1.24^{a}	1.58±1.31^{bc}	3.00±2.00^{a}	7.60±3.98^{a}	5.40±1.95^{a}
V (HDME)	1.84±1.42^{bc}	2.52±2.75^{bc}	3.30±1.38^{a}	3.80±1.30^{bc}	3.62±3.55^{a}	3.14±3.54^{a}
VI (LDAE)	1.16±0.81^{bc}	2.30±2.44^{bc}	1.62±1.77^{bc}	3.00±1.58^{a}	5.82±5.04^{a}	3.50±3.81^{a}
VII (HDAE)	3.28±1.76^{a}	0.92±1.18^{a}	2.00±1.00^{a}	2.60±2.07^{a}	6.00±6.56^{a}	2.38±4.27^{a}

NC: Negative control, DCF: Diclofenac, PC:Positive control; LDME: Low dose of methanol Extract, HDME: high dose of methanol extract, LDAE: low dose of aqueous extract, HDAE: high dose of aqueous extract.

Table 7 presented the analgesic effects of treated rats. Analgesic was tested based on the physical behaviour of the rats after injected with acetic acid. The analgesic effect was monitored and the severity of the pain was quantified using categorical variable of likert scale as 3-5 for positive response and 1-2 for negative response. It was observed that only group II and VII treated with diclofenac and high dose of aqueous extract (400mg/kg b.w) walked firmly on their paw injected hind. The elevation of the injected parts was not prominently felt in groups IV and VII. Groups II, V and VII clearly walked with their injected paw hinds lifted off the floor. It was also shown from the result that treated rats in group II, IV, VI and VII did not lick their injected hind. Observing the constrictions, only rats treated with diclofenac and untreated group constricted for the first 10 to 30 minutes while in second observation, no constriction from any group was felt apart from group that was not treated.

In the table 7, letters with same superscript on values within the vertical line are not considerably different at p less than 0.05;

letters with different superscript are significantly different at p less than 0.05.

4. Discussion

The percentage yield of solvents used for the extraction displayed Percentage yield difference between methanol and aqueous, the encountered considered percentage interest of photochemistry using suitable solvent of best interest for adequate yield in plants. This also helps in order to ensure therapeutic practices in old-style healing physical activity **(Umar *et al.*, 2018)**, **(Afolabi *et al.*, 2007)**. The safety nature of the extracts was seen in its inability to cause any harm to the wistar rats even at the highest dosages. This perhaps indicates that the extracts have high safety index. In our study, phytochemical analysis of leaf extracts revealed presence of alkaloids, flavonoids, saponins, phenols and cardiac glycosides. Its established significant difference in their quantities is in agreement with previous works carried out by **Okoli and Okere, (2010)**. Increased in weight of rats treated with both methanol and aqueous extracts regardless their doses are also in line with the result by **Ene *et al.* (2021)** on the nutritional proximate analysis of *Chrysophyllum albidum.* Reduction in weight of rats treated with diclofenac (DCF) may be due to the dose of the drug and its active metabolites. DCF is safe depending on the dose taken; high dose of the drug can easily result to toxicity of the system in both human and animal model **(Solomon & Uche, 2019)**.

The hypothesis that oral administration of the aqueous and methanol extracts of *Chrysophyllum albidum* might affect the hematological parameters in acetic acid induced edema and pain was also tested. It was noted that haematological parameters can also be used to explain blood relating functions of a plant extract or its products **(Adebayo *et al.*, 2010; Yakubu *et al.*, 2007)**.

Adebayo *et al.* (2005) observed that haemoglobin, red cells and pack cell volume are connected with the total amount of red cells since they can be separated by differentiation reaction. Little or no change caused by extracts on red cells, haemoglobin, and pack cells of wistar rats, this might mean that extracts exert significant reduction in the platelets showed reduction or disease stage in the blood cloth. **Adebayo *et al.* (2010)** Platelet accumulation plays essential character in the physiopathology of thrombotic diseases. Differences in effects may be associated to free radical scavenging activity or interference with configuration of active metabolite **(Okwuosa *et al.*, 2012)**.

In accessing the protective, curative and ameliorative effect of the extracts, diclofenac was equated with the ordinary regulator group it was detected that there was no most important alteration in a day after swollen has been persuaded, could be traced to ameliorative, curative and protective effect of the extracts since there was displayed of inflammation from the positive control group. The modifications revealed the existent of inflammation; the non-significant variance could be the defensive outcome of the extracts irrespective of their dosages.

Value of treating rats with methanol and aqueous extracts of *C. albidum* using ESR as marker displayed dose reliant. The result demonstrated the biological remedy activities of the constituents connected to the plant leaves **(Umar *et al.*, 2018)**.

Study has vividly stated that C*. albidum* has the characteristic attributes of partial and main analgesic properties, that is not dependent on the concentration of the dosage but the phytochemical property is the one that carry out the act **(Adebayo, 2010)**.

The effectiveness outweighed the standard during analgesic test. This may be because the extracts had already pre-inhibited the prostaglandin synthesis. Increased in constrictions after first period may be due to further metabolism (Catabolism) of the active ingredients in the extract. The treatment mode of extract administration shows significant increase, reduction in activities of anti-inflammatory property of some analgesic drugs may result to failure in performing it full function **(Umar *el al.*, 2018)**. This could also be as a result of entirely new complexes formed through reaction of the extracts with acetic acid.

Increment in percentage reduction of edema though dosage dependent shows that the extracts showed effective reduction of edema it may be because the acetic acid had previously activated the let go of arachidonic acid and other endogenous compounds to synthesize prostaglandin. Decrease in constriction during the latter 30 to 60 minutes may be that time for metabolism of the orally administered extracts was quite late for it to have exerted phospholipase inhibition.It can be noted that, with *C. albidum* leaf extract, the most effective treatment for managing inflammations and pain is pre-treatment.

Acetic acid induced constriction is useful tool in experimenting whether analgesic drugs are active or not **(Otterness and Bliven, 1985)**. The ultimate gold is to activate the release of some endogenous substances results in the production of what cause inflammation through their sources **(Davies *et al.*, 1984).** Where inflammation came from and what bring about them can be some time be controlled by nerve cells, from it end known as nerve ending mechanism. It is very interesting and important to note that the results obtained from this work revealed that the plant contained biological active property and constitute agents which are connected with analgesic properties.

5. Conclusion

The aqueous and methanol leaf extracts of *C. albidum* have preventive, ameliorative curative effect against inflammation and pain caused by injury to the body as seen by decreased CRP and ESR during treatment (pre-treatment) and after treatment. The results obtained in this work provide evidence that *C. albidum* extracts contain some bioactive compounds responsible for this action. These findings confirmed the effectiveness and bioactivity of the leaf extracts of *C. albidum* against acetic acid induced inflammation and pain. Administration of leaf extracts controlled the CRP and ESR levels as positive control was used to control the degree of edema as well as the activity of leaf extracts of *C. albidum* on PVC, WBC, RBC, Hb and on the physical activity of the treated rats.

It is evidenced from the findings in this study that the natural vegetation around us has solution to some health challenges and the extents to which we discover it have great implications to handling these challenges. Therefore, adequate and extensive research on pharmacodynamics, kinetics, proper standardization and clinical trials is necessary to exploit their therapeutic uses in combating various diseases.

Conflict of Interest

The authors report no conflicts of interest. The authors are responsible for the content of the paper.

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