Anti-arthritic potential of aqueous leaf extract of *Combretum platypterum* (Welw) Hutch & Dalziel (Combretaceae) on rats

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Submitted:23rd Jul 2022 ; Accepted:25th Sept., 2022 ; Published online: 31st Oct., 2022
DOI: : https://doi.org/10.54117/jcbr.v2i5.4
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Abstract

Combretum platypterum is used in the treatment of malaria, swellings, lumps, conjunctivitis, backache, fever, helminthiasis and diarrhea in ethnomedicine. This study evaluates the anti-arthritis activity of aqueous leaf extract *of C. platypterum. C. platypterum* leaf sample was pulverized and the powdered sample was extracted using cold maceration, which was freeze dried, and graded doses of 50, 100, 200 and 400 mg/kg of the extract were administered for the anti-arthritis property using an established formaldehyde and Complete Freund's adjuvant induced arthritis models in rats. The result showed that the extract had a significant reduction in the diameter of formaldehyde induced inflammation at day 10 (0.97, 0.97, 0.67 and 0.8 mm) and Complete Freund's adjuvant at day 28 (1.98, 1.79, 1.77, 1.43 mm) when compared with arthritis control for days 10 and 28 (2.95 and 4.06 mm) ($p < 0.05$). Percentage inhibition of the leaf extract in formaldehyde at day 10 (67.12, 67.12, 77.29, and 71.86 %) and Complete Freund's adjuvant induced arthritis at day 28 (51.23, 55.91, 56.40 and 64.78 %) showed a significant increase in the inhibitory effect of *C. platypterum* in dose dependent order. The ankle weight, erythrocyte sedimentary rate (ESR) and malondialdehyde level had a significant ($p < 0.05$) decrease in graded doses on complete freund's adjuvant induced arthritis rats when compared with arthritis control *C. platypterum* leaf extract elicited a significant ($p < 0.05$) increase in endogenous superoxide dismutase on complete freund's adjuvant induced arthritis when compared with arthritis control. This study led it credence to *C. platypterum* leaf extract scientifically validated for its anti-arthritis property

Keywords: Arthritis, *Combretum platypterum*, aqueous, antioxidant

Introduction

Using plants as a source of relief from illness is as old as mankind, with recorded practices going back at least 4000 years ago (Akunyili 2003; Aigbokhan 2014). Herbal medicines and their derivatives have been incorporated into traditional medicine since the beginning of recorded history. But only in recent times that the broader use of medicinal plants is gaining acceptance in the more expansive international domain (Kamboji 2000). The reported advantages of herbal medicines are effectiveness, safety, fewer side effects, affordability and acceptability. Over the past

decade, herbal medicine has become a topic of global importance, impacting both world health and international trade. Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population. This is true in developing countries, where herbal medicine has a long and uninterrupted history of use (Mukeshwar *et al.* 2011). It has been proved that plants use to treat and manage illness may seem slower; the results are sometimes better in chronic diseases. Orthodox drugs act faster because they are targeted at a particular disease. However, their side effect may limit their use in chronic diseases. Arthritis is a chronic disease, which needs long term treatment. Plants that would offer a better treatment and has less or no side effect, including *Combretum platypterum* are better use in treating arthritis.

Combretum platypterum (Welw) Hutch and Dalziel belongs to the family *Combretaceae*. It is distributed from Guinea to Democratic Republic of Congo, Southern Sudan and Angola (Dalziel 1973; Bredenkamp 2000). *C. platypterum* is abundant in rain forests, secondary forests and Savanna regions, occasionally in swampy localities, from sea-level up to 450 m above sea level. *C. platypterum* is a scandent shrub or forest liana, up to 10 m long, stem up to 10 cm in diameter, older stems with many lenticels, and young stem short hair, glabrous and blackish when older, at the base sometimes with recurved spines. *C. platypterum* is used to treat malaria, swellings, lumps, conjunctivitis, backache, fever, helminthiasis, diarrhea, and used as a tonic and to stop post-partum bleeding in ethnomedicine (Bongers *et al.* 2005; Idu *et al.* 2016). Despite its traditional uses in the management of arthritis, no pharmacological studies have been carried out on this species in the treatment of arthritis. This study aimed at evaluating the anti-arthritis activity of aqueous leaf extract of *C. platypterum*.

Materials and Methods

Collection of Plant material

Fresh leaves (3.4 kg) of *Combretum platypterum* was obtained from Igbanke, Orhionmwon Local Government Area, Edo State, Nigeria in the month of July 2018. The plant was properly identified and authenticate by Dr. H. Akinnibosun in the Department of Plant and Biotechnology Faculty of life Sciences, University of Benin City, Edo State, Nigeria. The plant was deposited in the university of Benin herbarium with a number voucher UBH_c063.

Preparation of Plant extract

The dried leaves were washed with clean water and air dried for two weeks. The leaves were powdered using impact mill (Tigmax Petrol Gx160-5.5hp). Powdered leaves weighed (200 g) was macerated with distilled water (5L), the mixture was intermittently shaken and stirred at 6 h intervals for 24 hours. The extract was filtered, and the filtrate concentrated over a water bath and crucibles. The concentrated extract was stored in a universal bottle well labeled and refrigerated at 4 ^{O}C (Owolabi *et al.* 2008).

Experimental Animals

Albino rats (150-250 g) were gotten from a commercial farm in Benin City and housed in the animal facility of Department of Biochemistry, University of Benin, Benin City, Edo state, Nigeria. The animals were acclimatized for 14 days and kept under standard laboratory conditions with 12-hlight/dark cycle. They were fed with standard rodent pellet (Afrimash/ Afrimash Nigeria limited, Akobo, Ibadan) and water *ad libitum*. The animals were handled according to standard protocols for Laboratory animals (National Institute of Health USA: Public Health Service policy on humane care and use of Laboratory Animals 2002). The

animals were handled according to the standard protocols of the ethical committee of Life Sciences, University of Benin, with the ethical number LS20945.

Formaldehyde induced arthritis

Arthritis was induced by injecting 0.1 ml of 1 % of formaldehyde into the hind paw of albino rat using the model described by Fatima and Fatima (2016). Thirty-five (35) Wistar rats weighing 150-250 mg/kg were randomly divided into 7 groups (n=5). Group 1 received the vehicle (Distilled water) (1 ml/kg) without arthritis (normal control). Group 2 received the vehicle (Distilled water) with arthritis. Group 3 received the standard drug; dexamethasone (2 mg/kg). Group 4, 5, 6 and 7 received the extract (50, 100, 200 and 400 mg/kg). Before administration of the standard and test drug, the rat was induced arthritis by injecting 0.1 ml of formaldehyde into the hind paw of the animal. At the third day, formaldehyde was re-injected again into the hind paw followed by treatment with the test agent. The thickness of the paw was measured at days 3, 4, 6, 8, 9 and 10.

Complete Freund's adjuvant (CFA) induced arthritis in rats

Complete freund's adjuvant (Fatima and Fatima 2016). is a water-in-oil emulsion composed of inactivated and dried mycobacteria usually *Mycobacterium tuberculosis*. Thirty-five (35) Wistar rats weighing 150-250 g were randomly divided into 7 groups (n=5). Group 1 received the vehicle (distilled water), Group 2 received the vehicle (distilled water) with arthritis. Group 3 was administered with Methotrexate (standard drug) (0.7 mg/kg). Group 4, 5, 6 and 7 received the extract (50, 100, 200 and 400 mg/kg). Animals of all groups were induced arthritis by a single intra-dermal injection of 0.1 ml of complete freund's adjuvant (CFA) containing 1 mg/ml dry heat killed *Mycobacterium tuberculosis* in sterile paraffin oil into a foot pad of the left hind paw of rats. Extract and standard drugs were administered orally from day 9^{th} - day 30^{th}. The paw thickness was measured before inducing of arthritis and every 4 days of the experimental period using the Vernier caliper. The arthritic leg was measured 2 cm from the ankle down and was cut off and weighed for both the arthritic and non-arthritic.

Determination of erythrocyte sedimentation rate

Animal in the group of complete freund-induced arthritis (CFA) were anesthetized using chloroform. The animals were dissected and blood was collected from the abdominal Aorta, 1 ml of blood was transferred into a disposable ESR tube and other blood into the plain container. Blood in the disposable ESR tube was allowed to stand for 1hr and the result was taken (JAPSON disposable ESR Tubes/PSW-ESRPD01).

Determination of Superoxide Dismutase (SOD)

The assay is based on the reaction proposed by Idu *et al.* (2016). The test tube contains 2.5 ml of carbonate buffer measured with 0.2 ml of tissue homogenate was added and labeled. The test tubes had 0.2 ml of distilled water was also measured into reference test tube. 0.3 ml of 0.3 Mm of epinephrine solution was added to each of the test tubes and the reference test tube. They were mixed well and read at a 420 nm absorbance every 30 to 120 sec with UV-visible spectrophotometer (Model T80+UV is Spectrometer, PG Instruments Ltd) distilled water was used to zero the machine.

Determination of Malondialdehyde (MOD)

Malondialdehyde will be estimated by the method by Idu *et al.* (2016). A volume (1 ml) of the tissue homogenate (0.5 ml) were added to (1:1 v/v) TA-TBA, HCL reagent

thiobarbiturtic acid 0.375 % w/v, 15 % TAC w/vin 0.25 HCl and mixed. Solutions were heated at 15 min in a boiling water bath, cooled and centrifuged at 1000 rmp for 10 min. the absorbance of the supernatant was measured against a reference blank at 535 mm. Malondialdehyde concentration of the sample was calculated using extinction for efficient of $1.5\times10^{5}M^{-1}cm^{-1}$.

Histopathological analysis

The testes and penis were fixed in neutral buffered formalin. Affixed organs were utterly dehydrated with 99.9 % ethanol along with 70 % ethanol, and 96 % ethanol and washed using distilled water. 4 µm sections were prepared, and stained in hematoxylin-eosin dye. Stained tissues were optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) viewed at x 400 magnification

In-vitro anti-inflammatory effect of Combretum platypterum extract

Inhibition of Albumin Denaturation

Egg albumin (0.2 ml) (from hens' egg) was added into 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentration of extracts. Distilled water served a control. Then the mixture was as:

$$\text{Percent inhibition} = \frac{\text{Abs Control} - \text{Abs treated}}{\text{Abs Control}} \times 100$$

Membrane Stabilization Property and Inhibition of Albumin Denaturation were estimated using method describe by Mohamad *et al.* (2011).

Membrane Stabilization Property

Preparation of Red Blood Cells (RBCs) Suspension: Fresh human blood (10 ml) was collected and transferred to the centrifuged tubes containing an equal volume of Elsevier solution. The tubes were centrifuged at 3000 rpm for 10 min and were further washed three times with normal saline. 10 % v/v of the washed blood were made with normal saline.

Heat Induced Hemolysis

Test extract (1ml) at various concentration and 1ml of 10 % RBCs suspension. Instead of drug, saline was use as the control test tube and aspirin taken as a standard drug. All the tubes containing reaction mixture were incubated in a water bath at 56 ^{o}C for 30 min and centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The percentage of membrane stabilization activity was calculated as;

incubated at 37 ^{0} C for 15 mins. Heat for another 70 ^{o}C for 5 mins and cool. Read their absorbance after cooling at 660 nm by using the spectrophotometer. Aspirin (standard drug) was used as the reference drug. The percentage inhibition of protein denaturation was calculated

$$\text{Percentage inhibition} = \frac{\text{Abs Control} - \text{Abs treatment}}{\text{Abs Control}} \times 100$$

Statistical Analysis

Data were expressed as the mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical analysis was performed

using GraphPad Prism Version 6.01. $p < 0.05$ was considered significant

Result

The result shown from the report of graded doses of (50, 100, 200 and 400 mg/kg) of *C. platypterum* aqueous leaf extract and standard drug (dexamethasone) had a significant ($p < 0.05$) reduction in the diameter thickness of the inflamed arthritis in dose dependent manner when compared with untreated control (formaldehyde-induced arthritis without treatment) (Table 1).

The anti-arthritis report of graded doses of (50, 100, 200 and 400 mg/kg) of *C. platypterum* aqueous leaf extract experimented using complete Freund's adjuvant-induced anti-arthritic model as shown in when compared with the control. Table 2.

The Percentage inhibition of graded doses (50, 100, 200 and 400 mg/kg) of the treatment arthritis groups showed that the effect of the leaf aqueous extract was stimulated in dose dependent manner to inhibit the level of inflammation when compared with the control as shown in Table 1 and 2. The standard drug (0.7 mg/kg methotrexate) however, produced a better anti-arthritis effect when compared with the graded doses of the extract.

Table 1: Anti-arthritic effect of 10 days daily oral administration of *Combretum platypterum* aqueous leaf extract on Formaldehyde induced arthritis in rats.

Groups	**Dose**	**Diameter of inflammation (mm)**						
	(mg/kg)	**Day3**	**Day 4**	**Day5**	**Day 8**	**Day 9**	**Day**
	10	**% inhibition**						
Control	0.5 ml/kg	1.48±0.10	1.27±0.06*	1.49±0.29*	0.84±0.33*	0.99±0.18*	0.85±0.22*	71.19
Arthr. control		2.39±0.12*	2.40±0.37	2.87±0.28	2.96±0.40	3.51±0.40	2.95±0.21	-
Dexamethasone	2	2.71±0.22*	1.35±0.13*	1.48±0.18*	0.64±0.14*	1.55±0.33*	1.16±0.23*	60.68
C. platyterum	50	3.80±0.39*	2.80±0.27	2.43±0.42	2.20±0.33	1.55±0.24*	0.97±0.21*	67.12
C. platyterum	100	2.33±0.23*	2.33±0.29	1.95±0.47	1.53±0.21*	1.13±0.21*	0.97±0.28*	67.12
C. platyterum	200	2.39±0.23*	1.98±0.05	1.74±0.07	1.21±0.67*	0.87±0.25*	0.67±0.48*	77.29
C. platyterum	400	2.63±0.14*	2.18±0.26	2.15±0.12	1.00±0.11*	1.00±0.12#	0.83±0.12*	71.86

The aqueous leaf extract of *Combretum platyterum* significantly reduced (*$p < 0.05$) diameter of inflammation of arthritis compared to arthritis control. Values are represented as Mean ± SEM, n = 5 per group. Arthr. Control (Negative control).

Table 2: Anti-arthritic effect of 21 days daily oral administration of *Combretum platypterum* aqueous leaf extract of complete freund adjuvant induced arthritis in rats.

Groups	**Dose**	**Diameter of inflammation (mm)**						
	(mg/kg)	**Day9**	**Day12**	**Day16**	**Day20**	**Day24**	**Day28**	**% inhibition**
Control	0.5 ml/kg	0.54±0.05	0.68±0.12*	0.56±0.12*	0.22±0.79*	0.34±0.13*	0.33±0.12*	91.87
Arthr control		2.81±0.19*	4.27±0.16	3.48±0.46	3.30±6.31	3.02±0.40	4.06±0.68	-
Dexamethasone	2	2.74±0.41*	1.91±0.32*	0.90±0.13*	0.39±1.22*	0.83±0.17*	1.29±0.49*	68.23
C. platyterum	50	2.85±0.41*	3.02±0.54*	3.36±1.01	2.36±0.38	1.98±0.36*	1.98±0.35*	51.23
C. platyterum	100	2.15±0.19*	1.64±0.16*	1.65±0.07*	1.35±1.63*	1.75±0.12*	1.79±0.18*	55.91
C. platyterum	200	2.20±0.46*	2.19±0.37*	1.60±0.32*	1.17±2.35*	1.97±0.28*	1.77±0.37*	56.40
C. platyterum	400	2.07±0.05*	1.50±0.22*	1.35±0.17*	1.15±1.81*	1.56±0.22*	1.43±0.24*	64.78

Arthritis control: Arthritis control. The entire animals were made arthritis (*p < 0.05) compared to non-arthritis control. The aqueous leaves extract of *Combretum platyterum* significantly reduced (*p <0 .05) diameter of inflammation of arthritis compared to arthritis control. Values are represented as Mean ± SEM, n = 5 per group. Arthr. Control (Negative control).

Figure 1 showed the weight of the inflamed joint on the arthritic animals elicited a significant reduction across graded doses across graded doses (50, 100, 200 and 400 kg) of the leaf extract and methotrexate when compared with the arthritis control (p < 0.05).

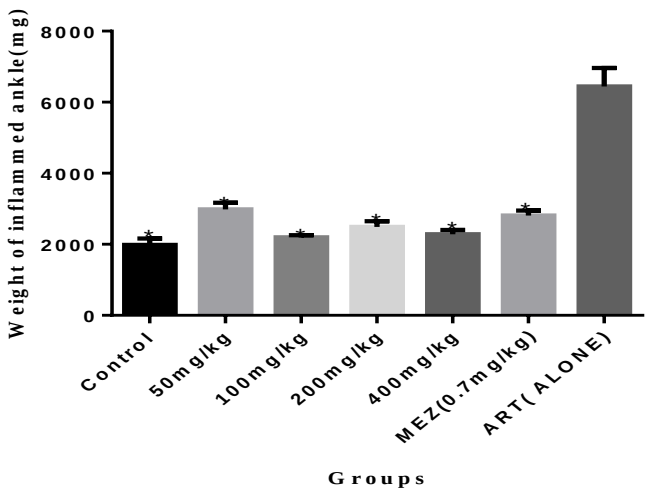

Figure 1: The effect of 21 days daily oral administration of aqueous leaf extract of *Combretum platypterum* on inflamed ankle joint of rats on complete freund adjuvant induced arthritis. The extract and standard (methatrozate 0.7mg/kg) reduced (*$p < 0.05$) the weight of inflamed joint on the arthritis animals compared to arthritis control. MEZ: methatrozate, ART (ALONE): arthritis control. Values are represented as Mean ± SEM, n = 5 per group.

The results from the erythrocyte sedimentation rate elicited a significant decrease across the graded doses (50, 100, 200 and 400 mg/kg) of the extract when compared with the untreated control $p > 0.05$, the therapeutic effect of the extract specifically at reduced dose. The number and size of red blood cell is associated with erythrocyte sedimentary rate results was more effective at 100, 200 and 400 mg/kg of the extract as shown in Figure 2.

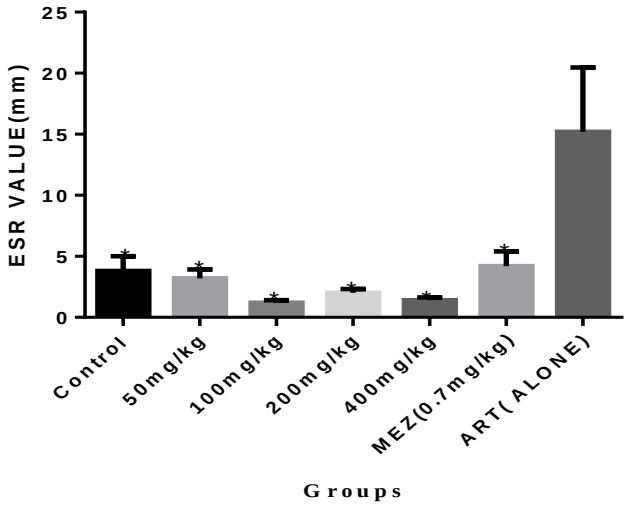

Figure 2: The effect of 28 days daily oral administration of aqueous leaf extract of *Combretum platypterum* on erythrocyte sedimentary rate on complete freund adjuvant induced arthritis on rats. The extract standard (methatrozate 0.7 mg/kg) reduced (*p < 0.05) the level of erythrocyte sedimentary rate on the arthritis animals compared to arthritis control. MEZ: methatrozate, ART (ALONE): arthritis control. Values are represented as Mean ± SEM, n = 5 per group

The level of endogenous superoxide dismutase enzyme activity in the blood of rat in complete freund adjuvant induced arthritis on rats with a significant ($p < 0.05$) increase in graded doses of (50, 100, 200 and 400 mg/kg) *C. platypterum* extract, elicited a potential effect to scavenge free radicals as shown in Figure 3.

The graded doses (50, 100, 200 and 400 mg/kg) of the extract displayed a significant ($p < 0.05$) decreased in the level of sera malondialdehyde in rat on complete freund adjuvant induced arthritis when compared with untreated control (Figure 4).

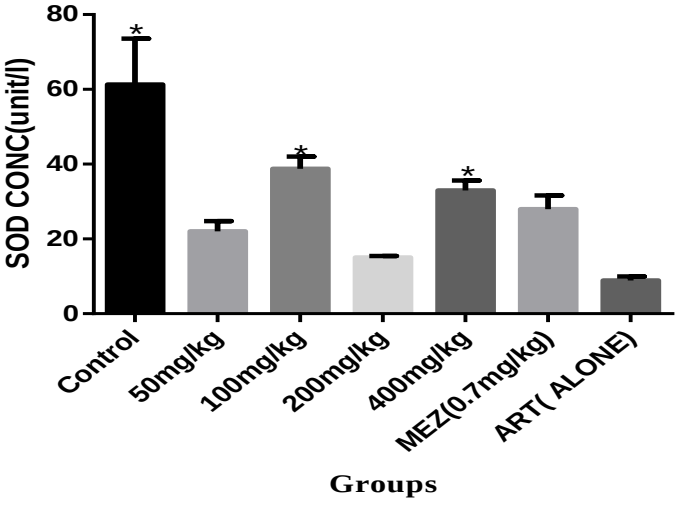

Figure 3: The effect of 21 days daily oral administration of aqueous leaf extract of *Combretum platypterum* on superoxide dismutase enzyme activity on complete freund adjuvant induced arthritis on rats. The extract 100 and 400 mg/kg increase (*p < 0.05) the level of superoxide dismutase enzyme activity on the arthritis animals compared to arthritis control. MEZ: methytrozate, ART (ALONE): arthritis control. Values are represented as Mean ± SEM, n = 5 per group

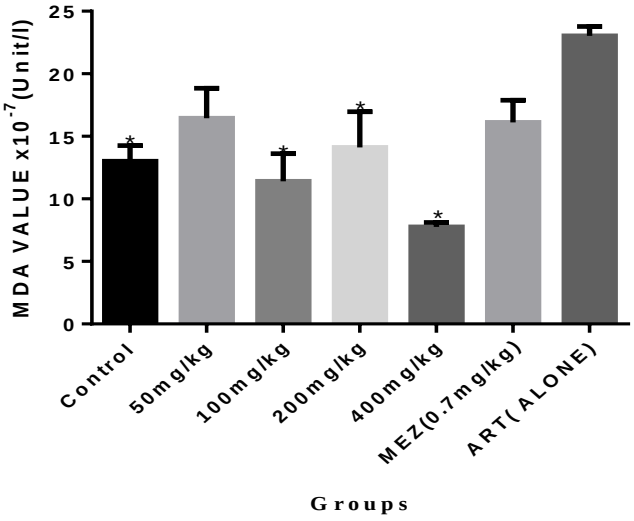

Figure 4: The effect of 21 days daily oral administration of aqueous leaf extract of *Combretum platypterum* on malondialdehyde enzyme activity on complete freund adjuvant induced arthritis on rats. The extract at 100, 200 and 400 mg/kg reduced (*$p < 0.05$) the level of malondialdehyde in the arthritis animals compared to arthritis control. MEZ: methytrozate, ART (ALONE): arthritis control. Values are represented as Mean ± SEM, n = 5 per group

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The histopathological study showed that the knee of the normal fibro-collagenous stroma in the animals' knee treated with graded doses (50, 100, 200 and 400) of *C. platypterum* extract and standard drug had a better architectural structure with absent arthritis where compared with arthritis control as shown in Plate 1.

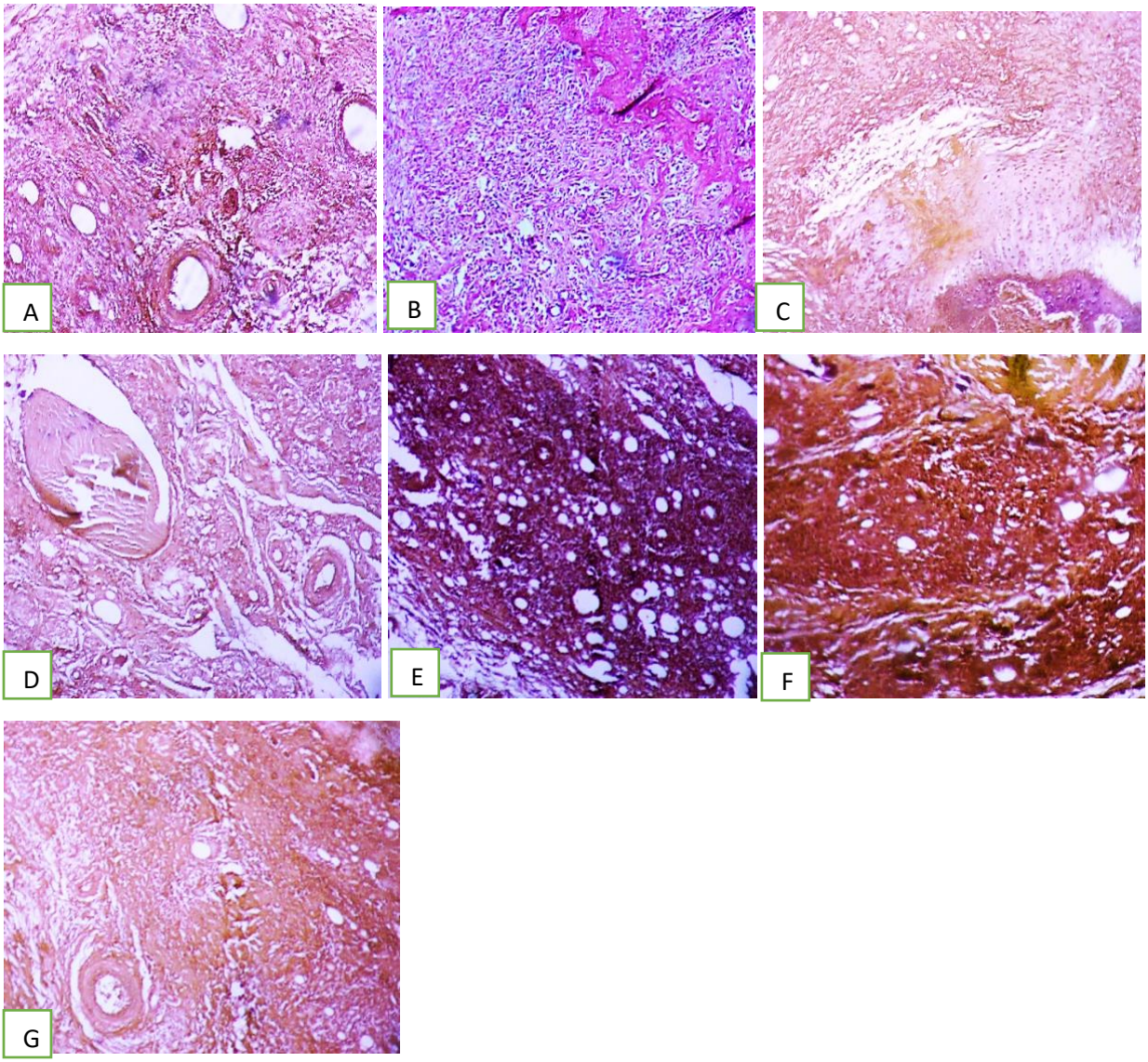

Plate 1: Effect of *Combretum platypterum* aqueous leaf extract on the rats knees

A. Normal control: Histological slide of the knee showing normal fibro-collagenous stroma
B. Arthritic control: Histological slide of the knee joint showing destruction of bony plate A (control arthritic knee).
C. Standard drug: Histological slide of the knee joint showing Normal joint.
D. 50 mg/kg leaf extract: Histological slide of the knee joint showing normal joint.
E. 100 mg/kg leaf extract: Histological slide of the knee joint showing normal joint.
F. 200 mg/kg leaf extract: Histological slide of the knee joint showing normal joint
G. 400 mg/kg leaf extract: Histological slide of the knee joint showing normal fibro-collagenous stroma H/E X100.

Figure 5 showed a significant increase in the percentage inhibition of the *in-vitro* anti-inflammatory property in protein denaturing effect at graded concentration specifically at

higher dose when compared with untreated control and standard drug (aspirin). While Figure 6 showed stabilizing erythrocyte membrane effect of *C. platypterum* leaf aqueous extract, had no significant difference when compared with the negative control and standard drug.

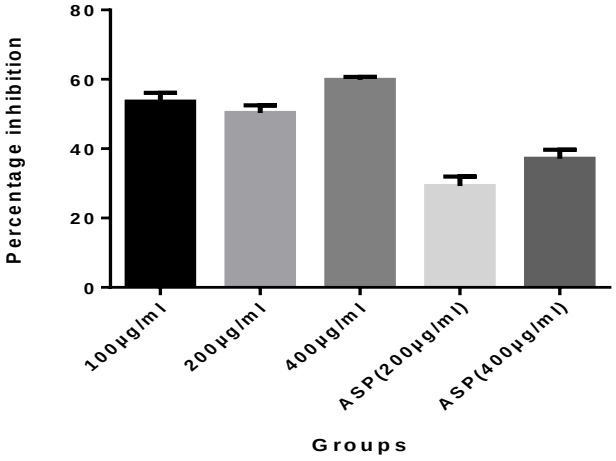

Figure 5: Effect of *in-vitro* anti-inflammatory effect of aqueous leaf extract of *Combretum platypterum* against protein denaturation. The extract at all dose level inhibit heat induced albumin denaturation compared to the effect of aspirin ($p > 0.05$), n=3.

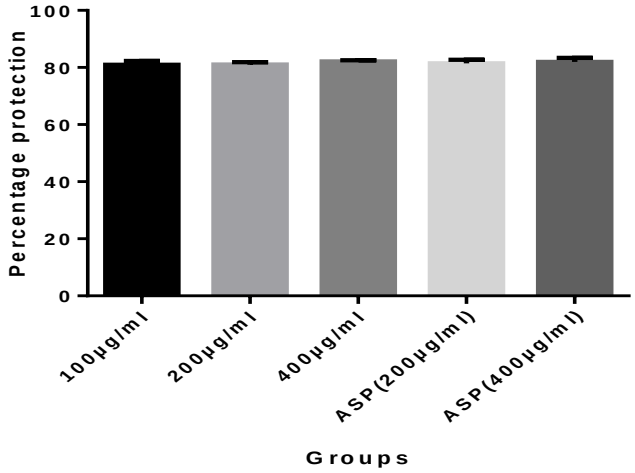

Figure 6: Red blood cell stabilizing effect of aqueous leaf extract of *Combretum platypterum*. Leaf extract at all dose level stabilize erythrocyte membrane compared to the effect of aspirin ($p > 0.05$),

Discussion

Arthritis is a systemic chronic autoimmune disease associated with multiple inflammatory mediators that lead to joint damage, synovial inflammation and cartilage and bone damage. There are several forms of arthritis but the most common are rheumatoid arthritis and osteoarthritis. The main treatment of arthritis is to target to stop disease development, reduce arthritis reaction and bone destruction, protect the joints and muscle role. Treatment is giving the patient education, treat early and combination therapy (William and Shiel 2016). Drug therapy include analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), Corticosteroids, immunosuppressive agents, immune and biological agents and botanicals (Roubenoff *et al.* 1997). This study showed the result of aqueous leaves extract of *Combretum platypterum* on formaldehyde-induced and complete freund's adjuvant induced anti-arthritic. Formaldehyde and Complete Freund's adjuvant are among the oldest experimental animal models used to study anti-arthritis drugs. Complete freund's adjuvant animal model mimics rheumatoid arthritis and osteoarthritis in human (Campbell *et al.* 2000; Walker *et al.* 2002). The aqueous leaves extract of *C. platypterum* reduced the thickness of the diameter of inflammation of arthritis when compared with arthritis control in formaldehyde-induced arthritis, thereby inhibit arthritis. The standard drug (dexamethasone) produced anti-arthritic effect by reducing the diameter of inflammation. The study of Fatima and Fatima (2016) worked on the pharmacological screening for anti-arthritic activity of *Moringa oleifera* had a similar effect as of the present study. Formaldehyde, the most used model for assessing anti-arthritic activity, develop edema in the rat paw after injection of formaldehyde is because of the release of histamines, serotonins and prostaglandins (Walker *et al.* 2002). The percentage inhibition of arthritis shows that the effect of leaf is comparable to the standard drug dexamethasone. Dexamethasone reduces diameter of inflammation by inhibiting inflammatory mediators such as histamine, serotonin, and the prostaglandins, responsible for inflammation (Benzie 2003). Hence, triggered anti-arthritic effect with a significant reduction in the level of arthritis due to the chemical mediators responsible for the occurrence of arthritis.

In complete freund's adjuvant induced anti-arthritis, the aqueous leaf extract of *C. platypterum* reduced the diameter of inflammation of arthritis when compared to arthritis control. Percentage inhibition of arthritis showed that the effect of the leaves extract was dose dependent and at dose 400 mg/kg the leaf is comparable to the effect of standard drug, methotrexate. Standard drug (methotrexate) but, produced a similar anti-arthritis effect to the leaf extract. The determination of paw swelling is simple, sensitive and quick procedure for testing inflammation and assessing of therapeutic effects of drugs. This concurred with the report of Peng (2014). The significant suppression of the swelling of the paws may be because of the suppression of inflammatory mediator released because of induction of freund's adjuvant. The leaf extract and standard (methatrozate 0.7 mg/kg) reduced the weight of the inflamed joint on the arthritis animals compared to arthritis control. The leaf extract and standard (methatrozate 0.7 mg/kg) elicited a significant reduction in the level of erythrocyte sedimentary rate when compared to the arthritis control. Erythrocyte sedimentary rate is a measure of inflammation and allergy. Erythrocyte sedimentary rate is an index of suspension stability of red blood cells in plasma. The

number and size of red blood cell is associated with erythrocyte sedimentary rate. It also involved in the speed up formation of endogenous proteins including plasma proteins such as fibrinogen, α and β globulins. Erythrocyte sedimentary rate was elevated during the inflammation, stress and cell necrosis (Campbell *et al.* 2000; Del Rio 2013). In this study, treatment with *C. platypterum* at graded doses (50, 100, 200 and 400 mg/kg) aid in normalizing the hematological parameters and rheumatoid factor when compared to untreated control. The leaf extract of *C. platypterum* exhibited a significant increase in endogenous superoxide dismutase enzyme activity of the blood in complete freund adjuvant induced arthritis on rats. Superoxide dismutase (SODs) is an endogenous antioxidant that catalyzes the breakdown of the superoxide anion into oxygen and hydrogen peroxide. This is in agreement with the report of Zelko *et al.* (2002) Superoxide dismutase mutigene family: A comparison of the Cu/Zn–SOD (SOD1), Mn–SOD (SOD2), and EC–SOD (SOD3) gene structures, evolution, and expression. It is present in almost all aerobic cells and in extracellular fluid and is one of the body primary internal antioxidant defenses. It plays a critical role in reducing inflammation and reduces pain associated with arthritis conditions (Takeya *et al*. 2006). Antioxidant is a molecule capable of preventing or inhibiting oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions produce free radicals. These radicals start chain reactions that damage cell. Antioxidants end these reactions by removing free radical intermediates and inhibit other oxidation reactions. Antioxidants also scavenge the activities of reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (Benzie 2003; Halliwell 2012; Bjelakovic 2013; Del Rio 2013; Dysken 2014; Idu *et al.* 2016;). Reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide are involve in Oxidative stress are involve in several diseases including diabetes mellitus, cancer, atherosclerosis, malaria, chronic fatigue, inflammation, asthma, osteoarthritis, rheumatoid arthritis and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease and ageing (Christian 2000; Hitchon and El-Gabala 2004; Davi *et al.* 2005; Lee 2006; Nunomura *et al.* 2006; Chaitanya *et al.* 2010)

The leaf extract of *C. platypterum* also displayed a significant reduction in malondialdehyde of the blood in complete freund adjuvant induced arthritis in rats (Figure 4). Malondialdehyde is a byproduct of lipid metabolism in the body. This is in line with the work of Hashimoto *et al.* (2003). It is one of many reactive electrophile species that result to toxic stress in cells and form covalent protein adducts, resulting to advance lipoxidation end products (Walker *et al.* 2002). The level of malondialdehyde plasma, serum and synovial fluid of arthritis patients are high in rheumatoid arthritis. Malondialdehyde play a vital role in pathogenesis of rheumatoid arthritis (Mohamad *et al.* 2011). It increases in arthritis and mediate cellular injury and tissue damage in rheumatoid arthritis (Thiele *et al.* 2015). The histological slide of the knee shows normal fibro collagenous stroma of the knee in the animals treated with *C. platypterum* leaf aqueous extract, control (distilled water) and standard drug. The histological slide of the knee of rats treated with distilled water and induced with arthritis, showed a destruction framework of the bony plate. Aqueous leaves of *C. platypterum* and standard drug (methotrexate, 0.7 mg/kg) protect the knee of rats from being destroyed by the arthritis. The

present study adheres to the study of Thiele *et al.* (2015) that worked on malondialdehyde-acetaldehyde adducts and anti-malondialdehyde-acetaldehyde antibodies in rheumatoid arthritis. Increase of *superoxide dismutase* and reduction of malondialdehyde by *C. platypterum* leaf extract correspond to the study by Idu *et al*. (2016) with a significant increase in endogenous antioxidant, which suggested that its mechanism of action depend on antioxidant potentials. This study led it credence to *C. platypterum* leaf extract scientifically validated with anti-arthritis activity, increases the level of endogenous antioxidant and reduces the level of malondialdehyde.

Conclusion

Combretum platypterum leaves have anti-arthritis activity, increases level of endogenous antioxidant and reduces the level of malondialdehyde, hence, suggest that the mechanism of action depend on its antioxidant potentials. This study validated the scientific uses of *C. platypterum* leaf extract in the management of anti-arthritis.

Disclosure statement

No competing interest

Funding

No funding was received, all founded by the authors.

Acknowledgements

Our earnest gratitude goes to Mrs. Queen Okoro and Dr. Kelvin Odega of the Department of Morbid Anatomy, University of Benin Teaching Hospital for the slide's preparation and interpretation. Dr Agu in the Department of Medical Biochemistry, university of Benin.

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