EVALUATION OF THE ANTI-ARTHRITIC ACTIVITY OF THE HYDROETHANOLIC LEAF EXTRACT OF *ALCHORNEA CORDIFOLIA* IN RATS

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Abstract

Background: Different decoctions of *Alchornea cordifolia* leaves are used by Yoruba herbalists (Southwest Nigeria) for the local treatment of ulcers, rheumatic pains, febrile convulsions, and for enhancing physical performance.

Materials and methods: In this study, the anti-arthritic effect of 100 – 400 mg/kg/day of the hydroethanolic leaf extract of *Alchornea cordifolia* (HEAC) was investigated in Complete Freund's Adjuvant (CFA)-induced arthritic rats as a way of evaluating its efficacy in the local management of arthritis. In addition, the effects of HEAC on liver and renal function parameters as well as its effect on the antioxidant enzyme system were investigated. Arthritis was induced using 0.1 ml of 10 mg/ml of Complete Freund's Adjuvant (CFA) following 1 h oral pretreatment and 8th day post-arthritic induction with 100, 200 and 400 mg/kg/day of HEAC and 3 mg/kg/day of celecoxib as the reference drug. The anti-arthritic activity of HEAC was assessed based on the ability of HEAC to alter the paw edema diameter, body weight, full blood count, renal and liver function markers, glycoprotein, lysosomal enzymes and possible antioxidant potential in the arthritic rats.

Results: Oral pretreatment with 100, 200, and 400 mg/kg/day of HEAC produced significant ($p<0.001$, $p<0.05$ and $p<0.01$) reductions in the paw edema diameter in a non-dose dependent fashion in ACF-induced arthritic rats with the 100 mg/kg/day of HEAC producing the most significant anti-arthritic effect. Similarly, HEAC increased hepatic GSH levels, CAT and SOD activities suggesting possible antioxidant mechanism for its anti-arthritic effect.

Conclusion: Overall, results of this study lend credence to the folkloric use of water decoction of *Alchornea cordifolia* leaves against rheumatoid arthritis. However, further pharmacological investigations would be required at isolating and determining the active anti-arthritic molecule(s) in HEAC in the nearest future.

Key words: Complete Freund's Adjuvant, Arthritis, Hydroethanolic leaf extract, *Alchornea cordifolia*, Rats

List of Abbreviations: AAAL - arthritis-attributable activity limitation**;** ALB – albumin**;** ALP – alkaline phosphatase**;** ALT – alanine aminotransferase**;** AST – aspartate aminotransferase ; BIL – bilirubin**;** CAT – catalase**;** CELE – celecoxib**;** CFA – Complete Freund's Adjuvant**;** CHOL – cholesterol**;** GSH – reduced glutathione**;** H_2SO_4 – tetraoxosulphate (VI) acid**;** HCl – hydrochloric acid**;** HEAC – hydroethanolic leaf extract of *Alchornea cordifolia***;** ID_{50} – median inhibitory concentration**;** MCH – mean cell hemoglobin**;** MCHC – mean cell hemoglobin concentration**;** MCV – mean cell volume**;** MDA – malonialdehyde**;** NaOH – sodium hydroxide**;** PCV – packed cell volume**;** PLT – platelet count**;** *p.o.* – per oral**;** RBC – red blood cells**;** SOD – superoxide dismutase**;** *subc*. – subcutaneous route**;** TG – triglyceride**;** TP – total protein**;** WBC – white blood cell count/leukocyte count

Introduction

Rheumatoid arthritis remains the most common auto-immune inflammatory disorder, affecting 1% of the adult population worldwide, and is usually associated with progressive joint destruction (Firestein, 2003). In the United States, a Centre for Disease Control (CDC), survey based on data from 2007 – 2009 revealed that 22.2% (49.9 million) of adults aged ≥ 18 years had self-reported doctor-diagnosed arthritis, and 9.4% (21.1 million or 42.4% of those with arthritis) had arthritis-attributable-to-activity-limitation (AAAL) and with an aging population this figure is expected to rise (Woolf and Pfleger, 2003; Centers for Disease Control and Prevention, 2010). In Nigeria, the true incidence of rheumatoid arthritis remains unknown but the crude incidence of RA in a Nigerian urban city has been estimated to be 12.3% with a female-to-male ratio of 2.4:1 and mean age of 46.9 years (Adelowo et al., 2010) although an earlier study of two rural townships of southern Nigeria reported an incidence rate of 0.0% despite the fact that 5.5% of individuals tested were positive for rheumatoid factor (Silman et al., 1993). However, till date, the aetiology of rheumatoid arthritis remains controversial and debatable but there is a consensus that its etiopathogenesis could be triggered by the interplay of both genetic and environmental factors (Ollier et al., 2001). In the treatment of the disease, non-steroidal drugs, steroidal agents and immuno-suppressants are usually used but these drugs are notorious for some unwanted effects such as gastrointestinal disorders, immunodeficiency and humoral disturbances (Ong et al., 2007). Therefore, efforts are currently channelled towards discovering and developing therapeutic agents which are more effective but of lesser toxicity profile, particularly, with prolonged use. Studies showed that in the United States, 60 - 90% patients suffering from arthritis, particularly rheumatoid arthritis, are on one form of Complementary and Alternative Medicine or the other, predominantly herbal therapies either alone and/or with conventional treatment (Rao et al., 1999). In Nigeria, the true statistics on the use of alternative and/or complementary remedy in the local management of rheumatoid arthritis remain unknown but these figures are expected to be higher bearing in mind that over 80% the population is reliant partly or wholly on one form of complementary and alternative medicine or the other for the treatment of their ailments. In addition, the World Health Organization (WHO) estimates that nearly 80% of the world population depend partly or wholly on traditional medicine, especially medicinal plants, for their primary health care needs (World Health Organization, 1996).

Alchornea cordifolia (Schumach. and Thonn.) Müll. Arg. (family: Euphorbiaceae) is a shrub widely distributed along the coastal regions of West Africa where decoctions made from its pulverized leaves are used for the local treatment of rheumatic pains, fever, wounds and diarrhea (Ogungbamila and Samuelsson, 1999; Tona *et al*., 2000). The plant is commonly known as the Christmas bush. It is known by other names such as dove wood (English), Arbre de djeman (French), Bugi-bugi, bunce, pô d'arco (Portuguese). Its local names include ewe eepa, esinsin, and eepa (in Yoruba, Southwest Nigeria) (Burkill, 1997; Adewunmi et al., 2001). The rural populace of Southwest Nigeria also employs the decoction of the leaves in the management of febrile convulsions in children (Ogungbamila and Samuelsson, 1990). Previous studies have established the spasmolytic (Ogungbamila and Samuelsson, 1990), anti-inflammatory (Manga et al., 2004; Mavar-Manga et al., 2008), anti-microbial (Okeke et al., 1999; Ebi, 2001), anti-diarrhea (Tona et al., 2000, Agbor et al., 2004), and analgesic properties of the extract of *Alchornea cordifolia* (Osadebe and Okoye, 2003). Other studies have reported the hepato-protective and anti-oxidant effects of *Alchornea cordifolia* (Olaleye et al., 2006, Olaleye and Rocha, 2008), as well as its anti-anaemic effect which was mediated via increased circulating hemoglobin and iron levels (Kapnang-Jepang, 1997).The ethanol extracts of the leaf and fruit showed significant trypanocidal, anti-helminthic and amoebicidal activities (Tona et al., 2000; Adewunmi et al., 2001). Phytochemical studies of the leaf extract also revealed the presence of flavonoid, alkaloids, saponins, phenols and tannins (Ogungbamila and Samuelsson, 1999; Tona et al., 2000). In addition, the leaf extract of *Alchornea cordifolia* is reported to contain alchornine, alchorneinone, gentisnic acid and yohimbine (Ogungbamila and Samuelsson, 1999).

Decoctions of *Alchornea cordifolia* leaves remain highly valued by Yoruba herbalists (Southwest Nigeria) and it is reputedly used in the local management of arthritis. Despite its ancestral use, however, there are no pharmacological data to either confirm or refute the therapeutic potential of this plant in the management of arthritis. In view of the above, the present study was designed to evaluate the possible anti-arthritic activity and possible mechanism(s) of action of 100, 200 and 400 mg/kg/day of the hydro-ethanolic leaf extract of *Alchornea cordifolia* against CFA-induced arthritic rats. The chosen doses of *Alchornea cordifolia* extract used in this study were based on results of preliminary study conducted before the main experiment (unpublished).

Materials and methods
Collection and identification of *Alchornea cordifolia* leaves

Fresh leaves of *Alchornea cordifolia* were collected from the deciduous forest of Abafadu Village, Ikire, Osun State, Nigeria. Botanical identification and authenticated was done by Mr. T.K. Odewo of the Herbarium, Department of Botany, University of Lagos, Akoka , Lagos State, Nigeria. A voucher specimen with number LUH 4846 was deposited in the herbarium of same department for reference purpose.

Preparation of hydroethanolic leaf extract of *Alchornea cordifolia*

The freshly harvested leaves of *Alchornea cordifolia* plant were gently but thoroughly washed under a running tap water and completely air-dried for one week under shade. The dried leaves were then pulverized into fine powder using laboratory hammer mill. 400 g of the powder was soaked in 10 L of 75% hydroethanolic solution (consisting of 7.5 L of "absolute" ethanol and 2.5 l of distilled water) for five days after which the preparation was filtered using the Whatmann's filter paper size – 9 cm. The filtrate was evaporated in a rotary evaporator attached to a thermochiller (Buchi 700®, Recirculation chiller) at a temperature of 40 °C. The residue was freeze-dried to obtain a deep-brown residue of the crude extract. This procedure was conducted for three more times and the residue were pooled into an air- and water-tight container and stored in the refrigerator at 4 ºC until required for experimentation.

Experimental Animals

Forty-eight healthy young Wistar Albino female rats weighing between 156 – 171 g and 30 male Swiss Albino mice weighing between 15 – 20 g were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Idi-Araba, Lagos State, Nigeria, and used for the study after institutional ethical approval has been obtained. The animals were allowed three weeks of acclimatization and were allowed free access to standard chow and drinking water. The animals were maintained under standard environmental conditions (23 - 25°C, 12 h/12 h light/dark cycle) and had free access to standard rodent pellet diet (Livestock Feed Plc, Lagos, Nigeria) and water. The animals were acclimatized in the laboratory conditions for a week before the commencement of the study. The experimental procedures adopted in this study were in strict compliance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (1985).

Experimental induction of arthritis and oral treatment of rats with HEAC

One week before the commencement of the experiment, the rats were randomly divided into 8 groups of 6 rats per group. Arthritis was induced by intradermal injection of 0.1 ml of Complete Freund's adjuvant (CFA) (10 mg/kg of heated-killed *Mycobacterium tuberculosis* in 1 ml paraffin oil) into the right hind paw (Ahmad et al., 2006). The treatment was given for 28 and 20 days as pretreatment and post treatment, respectively. Arthritis in rats was assessed by means of physical and biochemical measurements. The joint diameter and body weight was measured on day 0 (before injecting FCA) and every 4 days for 28 days using cotton thread method as described by Bamgbose and Noamesi (1981), in both control and treated rats. The experimental rats were randomly divided into eight groups of six rats per group and treated as follows:
Group I: 10 ml/kg of 0.9% normal saline, *p.o.* + 0.1 ml 0.9% normal saline, *subc*.
 Group II: 10 ml/kg of 0.9% normal saline, *p.o.* + 0.1 ml CFA, *subc*.
Groups III – V: oral pretreatments with 100, 200, and 400 mg/kg/day of HEAC, *p.o.* + 0.1 ml CFA, *subc*., given 1 h after HEAC pretreatment
Groups VI – VII: 200 and 400 mg/kg HEAC, *p.o.* (given 8 days post-CFA treatment) + 0.1 ml CFA, *subc*.
Group VIII: 3 mg/kg/day celecoxib, *p.o.* + 0.1 ml CFA

Blood collection and bioassays

On the 28th day post-arthritis induction, the rats were anaesthetized by intra-peritoneal administration of combination of 25% urethane (Sigma Chemical Co., U.S.A.) and 1% Chloralose (Sigma Chemical Co., U.S.A.) at a dose of 1.2 mg/kg body weight. Blood samples for hematological and biochemical assays were obtained through ocular puncture and collected into EDTA-treated sample bottles and plain bottles,

respectively. Thereafter, rats were sacrificed by cervical decapitation, a ventral longitudinal abdominal incision made and the liver and spleen were identified and immediately dissected out. These dissected organs were separately rinsed and homogenized in ice-cold 0.01M, Tris HCl buffer, pH 7.4 to give a 10% homogenate. Liver samples were taken for determination of *in vivo* antioxidants (reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT)), nitrite, and malondialdehyde (MDA).

Assay of lysosomal enzymes

Acid phosphatase was assayed using the method of King (1965) by colorimetric method, using disodium phenyl phosphate as the substrate. The enzyme activity was expressed as µmol phenol liberated/min/mg/protein.

Estimation of protein-bound carbohydrate

For glycoprotein analysis, 20% of the liver or spleen samples were defatted in hexane placed in a test tube, to which 1 ml of 2N HCl was added, and the tubes were sealed. Hydrolysis was completed by keeping the sealed tubes at 100 °C for 16 - 18 h. After hydrolysis, the contents were neutralized with NaOH and made up to a known volume, and aliquots were used for glycoprotein estimation. The tissue hexose level was determined by the method of Neibes (1972). The neutralized sample was mixed with orcinol-H_2SO_4 reagent, heated at 80 °C, cooled and left in the dark for 25 min. Sialic acid was determined by the method of Aminoff (1961) with modifications by Neibes (1972). The neutralized sample was mixed with 0.25 M periodate (in 0.1N H_2SO_4) and the reaction was inhibited after 30 min by arsenite solution. Then, thiobarbituric acid was added and the contents were heated. The pink color that developed on cooling was measured at 540 nm.

Estimation of serum liver and renal functions

Serum albumin, total protein (TP), total cholesterol (TC), triglycerides (TG), glucose, uric acid, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and total bilirubin as liver function parameters and serum urea and creatinine (as renal function parameters) were assayed for using Roche and Cobas commercial test kits and Roche/Hitachi 904 Automated Analyzer.

Antioxidant markers

Liver and splenic reduced glutathione (GSH) (Beutler and Kelley, 1963), superoxide dismutase (SOD) (Beauchamp and Fridovich, 1971), and catalase (CAT) activities (Goth, 1991) were assayed using standard procedures.

Hematological analysis

The full hematological parameters evaluated include red blood cell (RBC) count, hemoglobin (Hb), platelet count (PLT), total and differential white blood cell (WBC) count, neutrophil, % neutrophil, mean cell hemoglobin concentration (MCHC), mean red cell volume (MCV), and mean cell hemoglobin (MCH), and packed cell volume (PCV) were assayed for using Roche and Cobas commercial test kits and Roche/Hitachi 904 Automated Analyzer.

Statistical Analysis

Results were expressed as mean ± SD and statistical analysis was performed using Turkey's Multiple Comparison and Bonferroni post tests using GraphPad Prism 5 (GraphPad Software Inc., California, U.S.A.). Significant values were considered at $p<0.05$, $p<0.01$ and $p<0.001$.

Results
% Yield of HEAC

Hydroethanolic extraction of *Alchornea cordifolia* leaves yielded 11.06% (w/w) of the original plant material extracted. The crude extract obtained after complete dryness was a deep-brown, sweet-smelling solid residue.

Effect of HAEC on paw edema in CFA-induced arthritis in rats

Subcutaneous injection of CFA into the right hind paw produced an increase in the right paw diameter which was maximum on day 4 (0.92 ± 0.15 cm) but insignificantly decreased ($p>0.05$) to 0.80 ± 0.28 cm on day 28 (Table 1). Oral pretreatment with 100 mg/kg/day of HEAC initially produced an insignificant ($p>0.05$) increase in paw diameter, 1.18 ± 0.04 cm on day 4 but became significantly ($p<0.001$) reduced to 0.34 ± 0.08 cm on day 28. With oral pretreatment with 200 mg/kg/day of HEAC, there was a significant ($p<0.05$) decrease in the paw edema diameter on day 4 from 0.70 ± 0.09 cm to 0.41 ± 0.00 cm on day 28 while oral pretreatment with 400 mg/kg/day of HEAC caused a significant (p<0.01) reduction in the paw edema diameter from 1.20 ± 0.16 cm on day 4 to 0.36 ± 0.02 cm on day 28 (Table 1). However, the maximum significant ($p<0.001$) reduction in the paw edema diameter was produced by 100 mg/kg/day of HEAC and the degree of reductions in the paw edema diameter at this oral dose was comparable to that induced by 3 mg/kg/day of celecoxib (Table 1).

In the post-arthritic model, oral treatment with 200 mg/kg/day of HEAC was associated with a significant ($p<0.05$) decrease in the paw circumference from 1.15 ± 0.03 cm on day 8 to 0.37 ± 0.07 cm on day 28 (Table 1). Similarly, oral treatment with 400 mg/kg/day of HEAC caused a significant ($p<0.05$) decrease from 1.00 ± 0.14cm on day 8 to 0.38 ± 0.03 cm on day 28 and this significant reduction in paw edema was similar to that caused by 3 mg/kg/day of celecoxib (from 1.10 ± 0.04 cm on day 8 to 0.40 ± 0.04 cm on day 28) (Table 1).

Effect of 100 – 400 mg/kg/day HEAC on body weight changes in CFA-arthritic rats

Repeated daily subcutaneous injection of 0.1 ml of CFA was associated with non-significant ($p>0.05$) time dependent increase in the weight changes in CFA-induced arthritic rats over the 28-days treatment period (Table 2). Oral pre-treatment with 100 mg/kg/day of HEAC caused significant ($p<0.01$, $p<0.01$, $p<0.001$) time dependent increase in the body weight changes on days 20, 24 and 28, respectively, while similar significant ($p<0.05$, $p<0.01$, $p<0.01$) body weight changes were also recorded for 400 mg/kg/day of HEAC (Table 2). However, oral pre-treatment with 200 mg/kg/day of HEAC caused no significant alterations in body weight changes in the treated rats (Table 2).

In the post-arthritic rats, oral treatment with 400 mg/kg/day of HEAC caused significant ($p<0.05$, $p<0.01$) time related increase in the body weight changes on days 24 and 28 of treatment, an effect that was similar to that caused by oral treatment with 3 mg/kg/day of celecoxib (Table 2). Again, there were no significant alterations in the body weight changes at 200 mg/kg/day of HEAC over the same treatment period (Table 2).

Table 1: Effect of 100-400 mg/kg oral treatment of *Alchornea cordifolia* hydroethanolic leaf extract on paw edema in CFA-induced arthritic rats

Treatment	Day 4 (cm)	Day 8 (cm)	Day 12 (cm)	Day16 (cm)	Day 20 (cm)	Day 24 (cm)	Day 28 (cm)
10 ml/kg DW	0.92 ± 0.15	0.53 ± 0.03	0.80 ± 0.10	0.50 ± 0.06	0.50 ± 0.06	0.50 ± 0.09	0.80 ± 0.28
100 mg/kg HEAC (Pre)	1.18 ± 0.04	0.66 ± 0.02	0.56 ± 0.14	0.40 ± 0.09	0.38 ± 0.10	0.36 ± 0.09	0.34 ± 0.08[c]
200 mg/kg HEAC (Pre)	0.70 ± 0.09	0.48 ± 0.09	0.58 ± 0.15	0.45 ± 0.03	0.45 ± 0.03	0.45 ± 0.03	2.1 ± 0.00[a]
400 mg/kg HEAC (Pre)	1.20 ± 0.16	0.52 ± 0.05	0.52 ± 0.05	0.42 ± 0.02	0.40 ± 0.00	0.40 ± 0.00	0.36 ± 0.02[b]
200 mg/kg HEAC (Post)	1.15 ± 0.03	0.60 ± 0.04	0.60 ± 0.04	0.58 ± 0.05	0.48 ± 0.09	0.40 ± 0.06	0.37 ± 0.07[b]
400 mg/kg HEAC (Post)	1.00 ± 0.14	0.48 ± 0.05	0.45 ± 0.03[a]	0.40 ± 0.00	0.40 ± 0.00	0.38 ± 0.03	0.38 ± 0.03[b]
3 mg/kg CELE	1.10 ± 0.04	0.55 ± 0.06	0.45 ± 0.03[a]	0.43 ± 0.05	0.43 ± 0.05	0.43 ± 0.05	0.40 ± 0.04[b]

[a], [b] and [c] represent significant decreases at $p<0.05$, $p<0.01$ and $p<0.001$ on day 28, respectively, when compared to day 4 values

Table 2: Effect of 100-400 mg/kg of *Alchornea cordifolia* hydroethanolic leaf extract on body weight changes (g) of CFA-induced arthritic rats

Treatment	4th day ΔWt	8th day ΔWt	12th day ΔWt	16th day ΔWt	20th day ΔWt	24th day ΔWt	28th day ΔWt
Control	7.67 ± 3.37	12.70 ± 4.42	17.00 ± 4.50	21.30 ± 4.66	26.30 ± 8.95	38.30 ± 8.71	36.80 ± 8.34
100 mg/kg HEAC (Pre)	8.05 ± 1.80	8.26 ± 1.80	11.80 ± 8.85	51.00 ± 15.80[a]	75.40 ± 17.30[c]	84.60 ± 15.20[c]	96.00 ± 15.50[c]
200 mg/kg HEAC (Pre)	6.75 ± 0.85	7.50 ± 1.70	20.80 ± 6.17	33.80 ± 9.78	35.50 ± 10.70	41.30 ± 12.20	51.30 ± 13.30[a]
400 mg/kg HEAC (Pre)	14.00 ± 4.34	23.40 ± 3.97	30.80 ± 4.57	44.60 ± 7.46[a]	64.40 ± 6.74[b]	74.60 ± 5.18[c]	85.40 ± 5.56[c]
200 mg/kg HEAC (Post)	8.80 ± 1.16	11.20 ± 3.93	13.60 ± 7.78	18.60 ± 11.80	32.20 ± 12.50	40.20 ± 13.30	47.00 ± 14.00
400 mg/kg HEAC (Post)	8.60 ± 1.44	21.20 ± 5.88	34.60 ± 10.20[a]	42.20 ± 12.00[a]	53.80 ± 15.40[b]	61.40 ± 15.20[a]	70.80 ± 15.40[b]
3 mg/kg CELE	8.67 ± 1.12	23.30 ± 2.85	33.70 ± 4.98[a]	56.20 ± 7.35[b]	51.80 ± 12.60[b]	60.80 ± 12.90[a]	68.20±12.90[b]

[a], [b] and [c] represent significant increases at p<0.05, p<0.01 and p<0.001 when compared to control value on 12th, 16th, 20th, 24th and 28th day

Table 3: Effect of 100-400 mg/kg of hydroethanolic leaf extract of *Alchornea cordifolia* on the full blood count of CFA-induced arthritic rats

Treatment (mg/kg)	WBC ($10^3/\mu l$)	RBC ($10^6/\mu l$)	Hb (g/dl)	PCV (%)	PLT ($10^4/\mu l$)	MCV(fl)	MCH (pg)	MCHC (g/dl)	% neutrophil
Untreated control	10.90 ± 2.58	7.32 ± 0.51	13.60 ± 1.03	41.00 ± 3.84	716.00 ± 25.40	55.90 ± 2.18	18.50 ± 0.26	33.20 ± 0.85	30.20 ± 4.81
Arthritic control	11.10 ± 1.05	7.21 ± 0.22	13.70 ± 0.26	41.40 ± 0.59	741.00 ± 98.10	57.70 ± 1.41	19.00 ± 0.24	33.00 ± 0.39	30.40 ± 2.32
100 mg/kg HEAC (Pre)	15.90 ± 2.37[a]	7.87 ± 0.35	15.10 ± 0.45	45.90 ± 1.63	548.00 ± 69.20[d]	58.50 ± 1.15	19.20 ± 0.46	32.80 ± 0.20	30.40 ± 3.36
200 mg/kg HEAC (Pre)	14.50 ± 2.88[a]	7.59 ± 0.38	14.30 ± 0.61	45.10 ± 1.65	864.00 ± 59.60[c]	196.00 ± 36.00[c]	18.90 ± 0.25	31.70 ± 0.36	31.20 ± 3.32
400 mg/kg HEAC (Pre)	14.40 ± 3.60[a]	7.46 ± 0.26	14.40 ± 0.46	46.40 ± 1.42	676.00 ± 82.50	62.30 ± 0.92	19.30 ± 0.18	31.70 ± 0.81	34.30 ± 3.52
200 mg/kg HEAC (Post)	16.30 ± 3.16[a]	7.72 ± 0.42	14.20 ± 1.00	44.70 ± 2.34	646.00 ± 69.60	58.00 ± 0.49	18.30 ± 0.35	31.50 ± 0.86	29.70 ± 6.23
400 mg/kg HEAC (Post)	9.88 ± 1.62	7.64 ± 0.35	13.90 ± 0.58	41.50 ± 1.42	663.00 ± 33.00	54.50 ± 1.26	18.20 ± 0.41	33.50 ± 0.30	32.80 ± 2.17
3 mg/kg CELE	12.50 ± 0.77	7.72 ± 0.50	14.50 ± 1.01	45.10 ± 3.17	621.00 ± 47.60	58.50 ± 1.56	18.80 ± 0.50	32.10 ± 0.09	30.70 ± 4.86

Table 4: Effect of oral pretreatment with 100-400 mg/kg HEAC on liver function parameters of CFA-induced arthritic rats

Treatment	AST (U/l)	BIL (mg/dl)	CREA (mg/dl)	ALT (U/l)	UREA (mg/dl)	ALB (mg/dl)	TP (mg/dl)	CHOL (mg/dl)	TG	ALP
Untreated control	320.00 ± 51.40	5.33 ± 0.14	72.20 ± 8.76	59.50 ± 12.00	9.35 ± 1.27	40.00 ± 2.81	81.40 ± 1.33	2.51 ± 0.17	0.97±0.11	173.00±34.40
Control arthritic	390.00 ± 39.50[a]	4.45 ± 0.21	55.50 ± 2.27[d]	64.80 ± 1.91	9.40 ± 0.45	41.10 ± 1.06	76.60 ± 1.94	2.11 ± 0.13	0.80±0.20	215.00±32.70[a]
100 mg/kg HEAC (Pre)	304.00 ± 19.50	4.35 ± 0.21	54.60 ± 1.65[d]	67.30 ± 7.40	8.58 ± 0.59	42.70 ± 0.81	77.20 ± 2.09	2.47 ± 0.14	0.95±0.11	209.00±59.10
200 mg/kg HEAC (Pre)	397.00 ± 28.40[a]	4.85 ± 0.07	55.70 ± 2.16[d]	59.90 ± 1.66	10.00 ± 0.53	41.70 ± 1.55	76.60 ± 1.45	2.37 ± 0.24	0.61±0.07	256.00±42.00[b]
400 mg/kg HEAC (Pre)	319.00 ± 21.30	4.83 ± 0.21	55.50 ± 2.71[d]	57.10 ± 4.74	8.20 ± 0.78	43.90 ± 2.41	76.80 ± 2.72	2.37 ± 0.07	0.74±0.13	332.00±46.20[c]
200 mg/kg HEAC (Post)	339.00 ± 8.28	4.43 ± 0.19	51.40 ± 3.37[d]	64.00 ± 3.80	8.90 ± 0.65	40.30 ± 1.66	70.40 ± 1.44	2.54 ± 0.36	0.63±0.03	259.00±44.50[b]
400 mg/kg HEAC (Post)	306.00 ± 25.50	4.25 ± 0.13	50.90 ± 1.13[d]	55.60 ± 5.87	8.30 ± 0.65	42.00 ± 2.40	75.00 ± 3.13	2.08 ± 0.17	0.95±0.09	258.00±48.50[b]
3mg/kg CELE	325.00 ± 26.40	4.73 ± 0.43	51.50 ± 5.03[d]	57.90 ± 4.24	7.58 ± 0.55	39.90 ± 2.82	73.70 ± 3.67	2.43 ± 0.10	0.77±0.17	292.00±20.70[c]

[a], [b] and [c] represent significant increases at p<0.05, p<0.01 and p<0.001, respectively, when compared to untreated control group values and [d] represents a significant decrease at p<0.05 when compared to untreated control group

Table 5: Effect of HEAC on liver antioxidant system, lysosomal enzymes and glycoprotein in CFA-induced arthritic rats

Treatment	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (ng/mg protein)	Nitrite	Hexose sugar	Sialic	Acid phosphatase (U/l)	Prostatic phosphatase (U/l)
Untreated control	1.38 ± 0.20	1.89 ± 0.16	13.27 ± 2.67	0.02 ± 0.00	8.62 ± 1.24	522.90 ± 10.17	199.70 ± 2.58	67.20 ± 11.77	**60.50±13.43**
Arthritic control	1.07 ± 0.22[d]	1.09 ± 0.10[d]	12.34 ± 0.92	0.09 ± 0.00[c]	11.17 ± 0.77	676.40 ± 7.49	378.70 ± 5.92[c]	134.10 ± 10.96[c]	**94.71±2.36[a]**
100 mg/kg HEAC (Pre)	1.19 ± 0.15[a]	2.10 ± 0.52[a]	13.00 ± 1.15	0.02 ± 0.00[d]	7.09 ± 0.50	558.90 ± 16.93	338.80 ± 4.30[b]	76.02 ± 18.68[e]	**53.93±10.61[d]**
200 mg/kg HEAC (Pre)	1.59 ± 0.15[a]	2.14 ± 0.50[a]	14.39 ± 2.03	0.02 ± 0.00[d]	10.53 ± 1.68	576.50 ± 53.33	199.70 ± 22.52[e]	79.29 ± 2.12[e]	**41.34±1.04[e]**
400 mg/kg HEAC (Pre)	1.36 ± 0.19[a]	4.00 ± 0.20[b]	15.39 ± 2.03	0.03 ± 0.01[d]	7.98 ± 0.67	462.00 ± 14.91[e]	81.78 ± 10.11[f]	63.05±10.22[e]	**68.34±3.38[d]**
200 mg/kg HEAC (Post)	1.48 ± 0.19[a]	1.61 ± 0.15	17.03 ± 1.00	0.06 ± 0.02	10.32 ± 1.19	855.00 ± 71.66[b]	318.80 ± 39.54[b, d]	74.23±1.80[e]	**63.36±0.24[d]**
400 mg/kg HEAC (Post)	2.48 ± 0.46[c]	3.03 ± 0.60[b]	15.36 ± 2.85	0.04 ± 0.00[d]	10.76 ± 0.07	515.50 ± 61.77[d]	250.50 ± 18.68[a, d]	75.28±14.61[e]	**79.05±1.08[d]**
3 mg/kg CELE	1.66 ± 0.27[a]	1.54 ± 0.35	8.72 ± 0.63	0.02 ± 0.002[d]	10.78 ± 0.29	536.80 ± 7.86	235.60 ± 13.97[a, d]	42.96±7.19[f]	**21.75±1.06[f]**

[a], [b] and [c] represent significant increases at *p*<0.05, *p*<0.01 and *p*<0.001, respectively, when compared to untreated control. [d], [e] and [f] represent significant decreases at p<0.05, p<0.01 and p<0.001 when compared to arthritic control values

Table 6: Effect of 100 – 400 mg/kg of HEAC on splenic antioxidant and glycoprotein in CFA-induced arthritic rats

Treatment	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (ng/mg protein)	Nitrite	Hexose sugar	Sialic
Untreated control	0.56 ± 0.06	4.74 ± 1.45	16.51±3.54	0.02±0.006	2.72±0.20	473.50±28.47	119.60±10.46
Arthritic control	0.29 ± 0.08[d]	1.80 ± 0.41[d]	18.44±4.81	0.08±0.001[a]	4.48±1.70[a]	528.70±98.70	248.60±17.85[b]
100 mg/kg HEAC (Pre)	0.23 ± 0.03[d]	1.43 ± 0.28[d]	15.12±1.52	0.06±0.004	2.66±0.44[d]	567.40±6.25	90.11±9.50[e]
200 mg/kg HEAC (Pre)	0.27 ± 0.06[d]	2.04 ± 0.28[a]	19.38±0.81	0.03±0.005[d]	2.85±0.45[d]	378.50±20.21[d]	79.26±2.82[e]
400 mg/kg HEAC (Pre)	7.34 ± 1.53[b]	1.55 ± 0.09[d]	16.82±0.32	0.08±0.02[a]	2.66±0.28[d]	439.50±16.13[d]	93.55±0.78[d]
200 mg/kg HEAC (Post)	2.91 ± 0.97[a]	2.25 ± 0.27[a]	21.19±4.32	0.10±0.03	2.52±0.18[d]	509.20±20.09	67.11±9.56[f]
400 mg/kg HEAC (Post)	11.51±0.97[c]	1.76 ± 0.35[d]	18.09±3.90	0.06±0.01	1.54±0.00[e]	259.00±23.20[e]	70.97±15.38[f]
3mg/kg CELE	6.78±1.05[b]	1.18 ± 0.16[d]	13.46±1.31	0.06±0.01	3.05±0.04[a]	538.10±21.74	147.00±10.72[a]

[a], [b] and [c] represent a significant increase at *p*<0.05, *p*<0.01 and *p*<0.001 when compared to untreated control group while [d], [e] and [f] represent significant decreases at *p*<0.05, *p*<0.01 and *p*<0.001 when compared to untreated arthritic rats.

Effect of 100 – 400 mg/kg/day of HEAC on full blood counts in ACF-arthritic rats

In the CFA-arthritic rats pretreated with 100 mg/kg/day of HEAC, there was a significant ($p<0.05$) increase in the WBC and concomitant significant ($p<0.001$) decrease in PLT (Table 3). Similarly, 200 mg/kg/day of HEAC caused significant ($p<0.001$) increases in PLT and MCV while 400 mg/kg/day of HEAC caused no significant alterations in full blood count (Table 3). In the post-arthritic model, oral treatment with 200 mg/kg/day of HEAC caused a significant (p<0.05) increase in WBC and caused no significant alterations in other measured parameters of full blood count (Table 3).

Effect of 100 – 400 mg/kg/day of HEAC on liver function parameters in CFA-treated rats

Repeated daily subcutaneous injection of 0.1 ml of CFA caused significant ($p<0.05$) increases in the serum AST and ALP when compared to that of untreated control group (Table 4). Oral pretreatment with 200 mg/kg/day of HEAC caused significant ($p<0.05$ and $p<0.001$) increases in the serum AST and ALP levels, respectively, while oral pretreatment at 400 mg/kg/day of HEAC caused a significant ($p<0.001$) increase in the serum ALP (Table 4). In the post-arthritic model, oral treatment with 200 and 400 mg/kg/day of HEAC, and 3 mg/kg/day of celecoxib caused significant ($p<0.001$) in the serum ALP levels only while having no significant alterations in the serum levels of other measured liver function parameters such as TP, ALB, BIL, ALT, CHOL and TG (Table 4). On the renal function parameters, oral treatment with 100 – 400 mg/kg/day of HEAC caused non-significant ($p>0.05$) reductions in the serum urea and creatinine in both pre- and post-arthritic models (Table 4).

Effect of 100 – 400 mg/kg/day of HEAC on hepatic tissue oxidative stress markers, hepatic lysosomal enzymes and glycoproteins in CFA-arthritic rats

Subcutaneous injection of 0.1 ml of CFA caused non-significant ($p>0.05$), reductions in the hepatic tissue SOD, CAT and GSH levels. Although repeated subcutaneous injection of CFA caused significant increases in hepatic sialic ($p<0.001$), and prostatic phosphate ($p<0.05$) levels but caused non-significant ($p>0.05$), increases in the hepatic tissue levels of MDA, nitrite, hexose sugar and acid phosphatase levels (Table 5). However, 100 mg/kg/day of HEAC caused a significant ($p<0.01$) increase in the sialic level but equally caused a significant (p<0.001) decrease in prostatic phosphatase levels when compared to untreated control values (Table 5). 200 mg/kg/day of HEAC also caused significant ($p<0.001$) decreases in the sialic and prostatic phosphatase levels (Table 5). In the same vein, 400 mg/kg/day of HEAC caused significant ($p<0.001$) increases in the hepatic SOD activity while causing significant ($p<0.05$, $p<0.001$) decreases in the hexose sugar and sialic levels when compared to values obtained for untreated arthritic group (Table 5).

In the post-arthritic model, oral treatment with 400 mg/kg/day of HEAC caused significant ($p<0.05$) increases in the hepatic GSH level and SOD activity and significant ($p<0.01$, $p<0.001$) decreases in the hepatic hexose sugar, sialic, and prostatic phosphatase levels when compared to untreated arthritic group values (Table 5).

Effect of 100 – 400 mg/kg/day of HEAC on splenic tissue oxidative stress markers and glycoproteins in CFA-arthritic rats

In CFA-treated rats, there were significant ($p<0.05$) decreases in the splenic GSH level and SOD activity and concomitant significant ($p<0.05$, $p<0.001$) increases in MDA, nitrite and sialic levels when compared to the arthritic group values (Table 6). Oral treatment at 400 mg/kg/day of HEAC caused significant ($p<0.05$, $p<0.001$) increase in the splenic GSH level and MDA activity but significant ($p<0.05$, $p<0.001$) decreases in the splenic SOD activity and sialic levels when compared to untreated arthritic group (Table 6). In the post-arthritic rats, 200 and 400 mg/kg/day of HEAC caused significant ($p<0.01$, $p<0.001$) dose-dependent increases in the splenic GSH levels and significant ($p<0.001$) non-dose dependent decreases in the sialic levels when compared to the untreated arthritic group (Table 6).

Discussion

Complete Freund's Adjuvant-induced arthritis remains one of the oldest experimental animal models used to mimick human rheumatoid arthritis and it is typically and extensively used in the study of suspected agents with anti-arthritic potentials (Roubenoff et al., 1997). Other experimental animal models of rheumatoid arthritis include rat collagen-induced arthritis model (Kaklamarius, 1992; Stasiuk et al., 1996; Vierboom et al., 2005), mouse collagen-induced arthritis (Boissier et al., 1987; Campbell et al., 2000; Brand et al., 2007) and mouse collagen antibody-induced arthritis models (Brand, 2005). Inoculation of animals with CFA (which was prepared by suspending heat-killed *Mycobacterium butyricum* in liquid paraffin at a dose of 10 mg/ml *Mycobacterium butyricum* in paraffin oil), is known to produce a profound systemic inflammation resulting in severe joint swelling and remodeling (Omoto et al., 2005) and this is usually characterized by mechanical allodynia, thermal hyperalgesia and pain on joint movement (joint hyperalgesia) (Tatsuo et al., 1994; Jasmin et al., 1998; Bertorelli et al., 1999; Nagakura et al., 2003).

In the present study, experimental arthritis was reliably established with repeated and daily subcutaneous plantar injection of 0.1 ml of CFA over a period of 28 days which was characterized by plantar edema formation which maximized on day 4 of the treatment and subsequently subsided for the remaining part of the study. The peak inflammation on day 4 induced by CFA is primarily due to edema formation and cellular influx (Kleinau et al., 1994). Thus, our results were similar to those reported by Rajkapoor et al. (2007) and Sumanth et al. (2012). Oral pre-arthritic treatment with 100-400 mg/kg/day of HEAC and post-arthritic oral treatment with 200 and 400 mg/kg of HEAC in arthritic rats profoundly decreased paw edema formation in the CFA-induced arthritic rats, with 100 mg/kg of HEAC producing the most profound effect. The observation that 100 mg/kg HEAC produced anti-edematous effect that is similar to that of the standard anti-inflammatory drug, celecoxib strongly suggests the possible anti-inflammatory effect of the extract. Previous studies have reported that the aqueous and methanol leaf extracts of *Alchornea cordifolia* produced marked anti-inflammatory effect following oral and topical administration, respectively (Osadebe and Okoye, 2003; Mavar-Manga et al., 2004). Osadebe and Okoye (2003) have shown that the terpenoids and tannin containing fraction of the extract produced marked anti-inflammatory effect that is devoid of ulcerogenic adverse effect. It was evidenced in this study that both pre-treatment and post-treatment of rats with *A. cordifolia* significantly inhibited CFA-induced arthritis as well as inhibition of inflammatory markers. This was substantiated by the efficacy of celecoxib (selective cyclooxygenase II inhibitor (COX-II)) in the present study. The anti-arthritic effect of *A. cordifolia* was found to be comparable to that of celecoxib.

Another significant finding of the present study is the effect of CFA treatment on body weight changes of treated rats. While CFA-treated rats were observed to have reduced weight gain pattern over the treatment period which is in concordance with that previously reported by Roubenoff

et al. (1997), pretreatment with 100-400 mg/kg of HEAC caused significant weight gain changes in the treated rats reversing the cachexic effect of CFA treatment with 100 mg/kg of the extract still causing the most significant weight gain. It is well documented in the literature that weight loss and cachexia are common features in rheumatoid arthritis and these are thought to be due to increased cytokine production which increases resting metabolic rate and protein breakdown as well as poor appetite (Yoshizaki et al., 1998; Rall and Roubenoff, 2004). Due to failure in adequately meeting the nutritional needs especially the increased calorie and protein needs, weight loss usually ensue (Gartlehner et al., 2006; Rall and Roubenoff, 2004). The fact that oral treatment with HEAC improved the weight gain pattern in the treated arthritic rats as observed in this study suggest that this could be due to improvement in the arthritic state of the rats. However, this remains an assertion until validated.

On the hematological profile, CFA-induced arthritis in rats is known to be associated with leukocytosis with relative lymphocytosis (Sumanth et al., 2012). In the present study, induction with CFA is associated with elevation in the total leukocyte count which again is in strong compliance with that previously reported by Sumanth et al. (2012) although in this study, differential lymphocyte was not done. Similarly, elevations in the total leukocyte count, PCV, Hb and MCV by oral pretreatment with 100-400 mg/kg of HEAC are suggestive of the possible anti-anemic effect of HEAC, although a study by Kapnang-Jepang (1997) has previously reported the anti-anemic effect of this plant.

On the liver enzyme markers, repeated subcutaneous CFA injection caused significant elevations in the serum AST and ALP while other measured parameters of hepatic injury were not significantly altered. AST and ALP are considered non-specific markers of acute hepatocellular injury and their elevations could be found in hepatobiliary disease (Kantharia and Woolf, 1987; Aida, 1993). This hypothesis may not be plausible in this study since other markers of hepatocellular injury such as alanine aminotransferase, total protein, total bilirubin, total cholesterol and triglyceride were not significantly altered in the arthritic rats. However, the profound elevations in the serum AST and ALP in the CFA-treated rats could also have arisen from extra-hepatic sources such as acute myocardial infarction and/or bone diseases and remodeling (Kantharia and Woolf, 1987; Aida, 1993). Thus, the profound elevation in the serum ALP in the arthritic rats strongly points to the establishment of an active bone lesion such as arthritis in the CFA-treated rats which is in complete agreement with results of other studies (Price and Sammons, 1974; Rosalki et al., 1982; Cimmino and Accardo, 1990; Cimmino et al., 1990). Further elevations in the serum levels of AST and ALP caused by oral treatment with 100-400 mg/kg of HEAC in both pre- and post-arthritic rat models could possibly have arisen from worsening of the chemically induced arthritis or remodeling phase of bone healing. However, the former is unlikely since oral treatment with HEAC has elicited anti-edematous effect in the CFA-induced arthritic rats. The non-hepatic toxicity of the extract was further corroborated by the non-significant elevations in the hepatic GSH levels and CAT activities but significant elevations in the SOD activities particularly at the oral dose of 400 mg/kg of the extract. Literature has shown that reductions in the GSH, SOD and CAT are strong and reliable markers of oxidative stress and increases in their levels are indicative of the antioxidative property (Siacic et al., 1997; Djordjevic et al., 2004; Anuradha and Krishnamoorthy, 2011). Thus, the increase in the levels of the oxidative stress markers by oral treatment with the extract is indicative of the inherent oxidative property of the extract. Also, antioxidant effects of some medicinal plants have been attributed to their rich abundance in flavonoids, phenols and alkaloids and previous reports on the phytochemical analysis of the leaf extract have shown *Alchornea cordifolia* leaves to contain flavonoid, alkaloids, saponins, phenols and tannins (Ogungbamila and Samuelsson, 1999; Tona et al., 2000). Therefore, the antioxidant effect of HEAC as seen in this study could be to the presence of the phytochemicals either singly or in combination with one another.

Conclusion

Overall, the result of this study shows the anti-arthritic effect of 100-400 mg/kg of HEAC which was possibly mediated via anti-edematous and anti-oxidative mechanisms. Further studies in the area of isolation and characterization of the active principle(s) responsible for the anti-arthritic effect of HEAC would be required in the nearest future.

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