Hematological and lipid profile evaluation of a hexane fraction of Costus afer leaves in arthritic rats

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Hematological and lipid profile evaluation of a hexane fraction of Costus afer leaves in arthritic rats

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Abstract

Context: Costus afer Ker Gawl. (Costaceae) is an ethnomedical plant used as therapy against inflammatory disorders.

Objective: The objective of this study was to evaluate the hematological and lipid profile analysis of hexane fraction of C. afer leaves (CAHLF) in arthritic rats.

Materials and methods: Male albino rats were randomly distributed into seven groups of six rats each. Rats were induced with arthritis using formaldehyde and Complete Freund's Adjuvant (CFA) for 7 and 21 d, respectively. The animals were administered orally with 50, 100, and 250 mg/kg CAHLF, 10 mg/kg diclofenac and prednisolone, 0.9% NaCl (control), and 0.9% NaCl (normal). At the end of treatment periods, blood samples were withdrawn and subjected to hematological and biochemical analysis using auto-analyzer and spectrophotometric methods.

Results: Hematological analysis revealed that in formaldehyde- and CFA-induced arthritic rat models, 250 mg/kg CAHLF-treated groups had significantly reduced (p < 0.05) hematocrit counts (HC) (30.98 ± 1.59% and 33.55 ± 1.10%), white blood cell counts (WBC) (5.50 ± 0.35 and 4.15 ± 0.82 × 10^9/L), and platelet counts (PC) (401.50 ± 48.94 and 246.33 ± 5.54 × 10^9/L) compared with control HC (46.90 ± 1.92 and 41.88 ± 2.19%), WBC (11.09 ± 0.26 and 7.37 ± 0.34 × 10^9/L), and PC (783.67 ± 59.51 and 593.83 ± 36.3 × 10^9/L). Furthermore, blood analysis showed that CAHLF-treated groups had reduced total cholesterol, low-density lipoprotein cholesterol, and triglycerides while they had an elevated high-density lipoprotein compared with the control group.

Discussion and conclusion: Findings from this study indicated that CAHLF could possess immunomodulatory and hypolipidemic properties in arthritic rats. CAHLF could be considered as a source of biopharmaceutical agents in anti-arthritis drug discovery process.

Keywords

Bioactive compounds, ethno-medicine, immunomodulation

History

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Introduction

Rheumatoid arthritis (RA) is a debilitating autoimmune disease characterized by destruction of synovial fluids causing pain, swelling, and stiffness in polyarticular joints leading to disability and premature mortality (Aletaha et al., 2010). It is a chronic inflammatory disease that affects over 21 million people worldwide and most of them are women (Chopra & Abdel-Nasser, 2008). RA is one of the leading causes of chronic morbidity in the developed world, and mostly affecting the work force population throughout the world (Mody, 2009). In developing countries, especially in tropical Africa, the use of traditional approach in the treatment of disease is still in practice (Akinyemi et al., 2006). Medicinal plant extracts are rich in phytoconstituents that have been used by man as prophylactic or therapeutic agent in the treatment of several inflammatory-related diseases (Balekar et al., 2013; Sofowora, 1993). Hence, the search for an alternative source of anti-inflammatory drugs by several interest groups including research institutes, pharmaceutical companies, and academia (Musa et al., 2012). This is because of the undesirable side effects experienced by arthritis patients after the use of either synthetic steroidal or non-steroidal anti-inflammatory drugs. In addition, the WHO had reported that these drugs are often associated with drug-induced toxic effects or secondary adverse effects on long-term use (Sarwar et al., 2011; Stig, 2006).

Costus afer Ker Gawl. (Costaceae, formerly Zingiberaceae) is one of the 150 species of tall, perennial, and rhizomatous creeping herbs. It grows in moist or shady farm lands and river banks (Aweke, 2007). It can be found in shady forest and riverbanks of Senegal, South Africa, Guinea, Nigeria, Ghana, and Cameroon (Iwu, 1993). It is commonly
called ginger lily or bush cane and in Nigeria, it is called by different names such as ‘‘ireke omode’’ in Yoruba-Western Region, while it is called ‘‘okpeta’’ in Igbo-Eastern Region, ‘‘kakizawa’’ in Hausa-Northern region, ‘‘mbritem’’ in the Efik-Southern region, and Anglophone speaking part of Cameroon calls it ‘‘monkey sugar cane’’ (Iwu, 1993; Nyananyo, 2006). All parts of C. afer (flower, fruit, stem, leaves, and rhizomes) have been used traditionally in the treatment of inflammatory-related ailments, especially rheumatoid arthritis, hepatic disorders, stomach disturbance, cough, malaria, and eye defects (Aweke, 2007; Burkill, 2000; Soladoye & Oyesika, 2008). Previous studies have shown that C. afer extracts may possess properties of hypoglycemic (Momoh et al., 2011), antioxidant (Anyasor et al., 2010), anti-inflammatory (Soladoye & Oyesika, 2008), anti-arthritis (Anyasor et al., 2014a,b), and some studies have shown that it may not contain antibiotics (Asekun & Adeniyi, 2003).

The majority of the chemical compounds identified in C. afer plant are from the rhizome, which have been reported to contain saponins aferosides A–C, dioxacin and paryphyllin C, and it also contain flavonoid glycoside kaempferol 3-O-α-L-rhamnopyranoside (Lin et al., 1996). Sesquidulavulacte, β-carophyllene, and Z- E-farnesol have also been identified in the essence oil of C. afer leaves (Asekun & Adeniyi, 2003). We have recently isolated the bioactive compounds in the leaf and stem fractions using gas chromatographic/mass spectrometric methods (Anyasor et al., 2014a,b). Therefore, this study was designed to evaluate the hypolipidemic and immunomodulatory activities of hexane fraction of C. afer leaves in arthritis rats with the objective to provide a scientific rationale to probe into its anti-inflammatory mechanism of action.

Materials and methods

Plant material

The plant C. afer was obtained from a farm land at Irolu in Ikenne Local Government Area, Ogun State, Nigeria on 30th May 2013. It was identified and authenticated by Professor E.B. Esan, a botanist in the Department of Biosciences and Biotechnology, Babcock University. A voucher sample with number FHI-108001 was deposited at Forestry Herbarium Babcock University. The plant material (CAHLF). Treatment lasted for 7 d.

Plant processing, extraction, and solvent partitioning

The leaves were plucked from the stem and air-dried under room temperature (28 ± 2°C). The air-dried leaves were pulverized using a mechanical grinder. Powdered leaf samples (300 g) were extracted using 1800 mL of 70% methanol with intermittent shaking for 48 h. The extract was filtered using Whatman No. 1 filter paper and the filtrate was subsequently concentrated using rotary evaporator at 30°C (Buchi Rotavapor RE, Buchi, Switzerland). The concentrates were reconstituted with distilled water in a ratio of 1:2 (concentrate:distilled water) and partitioned using hexane solvent. The hexane fraction obtained was concentrated in a rotary evaporator at 30°C. The fraction obtained was kept in the refrigerator at 4°C until further use.

Animals

Male albino rats (Wistar strain) weighing 180 ± 10.15 g were purchased from an inbred colony at the Preclinical Animal House, Physiology Department, University of Ibadan, Ibadan, Nigeria. The animals were acclimatized for 2 weeks at Babcock University Animal House. Animals were maintained and cared for following the National Institute of Health (NIH) animal care guidelines and approval was given by the department animal ethics committee.

Acute toxicity study

The acute toxicity study for the hexane fraction of C. afer leaves was performed using the median lethal dose of fraction in albino rats following the Organization for Economic Cooperation and Development (OECD) guideline 425 (OECD, 2001). A male Wistar albino rat was administered 2000 mg/kg p.o. after fasting overnight. The animal was observed for 24 h for any clinical signs of toxicity such as change in fur color, accelerated breathing, or death. The animal survived without any observable change. Subsequently, five male albino rats were selected by random sampling technique and subjected to the same protocol for 72 h and they all survived. The 2000 mg/kg fraction was considered safe and doses of 50–250 mg/kg body weight (bw) were adopted for further studies.

Induction of arthritis protocol

Model I: Formaldehyde-induced arthritis in rat (acute inflammation study)

Formaldehyde-induced arthritis in rats was carried out according to the method described by Gupta et al. (2010). Formaldehyde (2% v/v) solution, 0.02 mL, was injected in rats on the 1st and 3rd day into the left hind paw just beneath the plantar aponeurosis to induce arthritis. This study involved seven groups of six rats each, namely the normal group (0.2 mL of 0.9% NaCl), the control group induced with arthritis using formaldehyde and orally administered with 0.2 mL 0.9% NaCl, 10 mg/kg diclofenac sodium (2-[(2,6-dichlorophenyl) amino] benzene acetic acid sodium salt) (non-steroidal anti-inflammatory drug [NSAID]), and 10 mg/kg prednisolone [(pregna-1,4-diene-3,20-dione,11,17-dihydroxy-21-(phosphonoxy)] (glucocorticoid), respectively, as reference drugs, treated groups, and three test groups orally administered with 50, 100, and 250 mg/kg hexane fraction of C. afer leaves (CAHLF). Treatment lasted for 7 d.

Model II: Complete Freund’s adjuvant-induced arthritis in rats (chronic inflammation study)

Arthritis was induced in rats by injecting 0.1 mL of Complete Freund’s Adjuvant (CFA) emulsion into the sub-plantar surface of right hind paw and monitored for 21 d according to the method described by Sheetal et al. (2012). The animals were divided into seven groups of six rats each namely first group (normal) was orally administered with 1 mL 0.9% NaCl as placebo, the second group was (control) was induced with arthritis and received orally, 1 mL 0.9% NaCl, the third group and fourth groups received Diclofenac sodium and...
prednisolone, respectively, while the fifth, sixth, and seventh groups received 50, 100, and 250 mg/kg CAHLF, respectively. After 30 min of injection of CFA into their sub-plantar region of left hind paw at day ‘‘0’’, saline or fractions were orally administered to the animals once daily (0 d) and continued till the 21st day.

**Biochemical assays**

Twenty-four hours after the end of treatment periods in formaldehyde or CFA-induced arthritis; the animals were euthanized using cervical dislocation and subsequently sacrificed. Blood samples were collected through cardiac puncture using 5 mL hypodermic syringes into ethylenediaminetetraacetic acid (EDTA) and heparinized bottles to prevent the blood samples from clotting.

**Hematological analysis**

Blood samples obtain using EDTA bottles were subjected to hematological analysis to determine hematocrit, platelet, white blood cell (WBC), lymphocyte, neutrophil, basophil, eosinophil, and monocyte counts using an autoanalyzer (Swelab Alfa 3-Part Hematology Analyzer, Boule Medicals, Spånga, Sweden) at Babcock University Teaching Hospital Medical Laboratory.

**Lipid profile analysis**

Lipid profile analysis was performed using a spectrophotometric method with TECO diagnostic kits (TECO Diagnostic, Anaheim, CA) on blood samples obtained using heparinized bottles. The following parameters were analyzed: plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. The low-density lipoprotein (LDL) cholesterol and very low-density (VLDL) cholesterol were calculated using formula described by Friedewald (1972) as stated below:

- LDL cholesterol (mg/dl) = Total cholesterol - \( \frac{(Triglycerides + HDL \text{ cholesterol})}{5} \)
- VLDL cholesterol (mg/dl) = \( \frac{Triglycerides}{2.2} \)

**Statistical analysis**

Statistical analysis was carried out the aid of SPSS for Windows; SPSS Inc., Chicago, IL, Standard version 17.0. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by post hoc analysis using the least significant difference test. p Values at least <0.05 were considered to indicate statistical significance. Data are expressed as mean ± S.E.M.

**Results**

The data in Figures 1–3 indicated that the 50, 100, and 250 mg/kg CAHLF-treated formaldehyde-induced arthritic rats had significantly reduced (\( p < 0.05 \)) hematocrit counts (HC) (37.61 ± 0.85%, 35.23 ± 1.90%, and 30.98 ± 1.59%), white blood cell counts (WBC) (9.29 ± 0.34 \( \times 10^9/L \), 5.66 ± 0.53 \( \times 10^9/L \), and 5.50 ± 0.35 \( \times 10^9/L \)), lymphocytes counts (LC) (7.58 ± 0.31 \( \times 10^9/L \), 4.43 ± 0.52 \( \times 10^9/L \), and 4.34 ± 0.30 \( \times 10^9/L \)), neutrophils counts (NC) (0.58 ± 0.09 \( \times 10^9/L \), 0.58 ± 0.14 \( \times 10^9/L \), and 0.58 ± 0.05 \( \times 10^9/L \)), basophils counts (BEMC) (1.12 ± 0.09 \( \times 10^9/L \), 0.63 ± 0.07 \( \times 10^9/L \), and 0.57 ± 0.06 \( \times 10^9/L \)), and platelet counts (PC) (780.00 ± 37.42 \( \times 10^9/L \), 323.33 ± 52.65 \( \times 10^9/L \) and 401.50 ± 48.94 \( \times 10^9/L \)) compared with HC (32.80 ± 1.59%), WBC (11.09 ± 0.26 \( \times 10^9/L \),

Figure 1. Effects of varying doses of hexane fraction of C. afer leaf on hematocrit and white blood cell count in rats treated with formaldehyde and CFA for 7 and 21 d, respectively. Superscripts indicate significantly different at \( p < 0.05 \), superscript c is significantly different from a at \( p < 0.01 \). Superscript j is significantly different from g at \( p < 0.01 \). Superscript n is significantly different from k at \( p < 0.01 \).
LC (7.64 ± 0.27 × 10^9/L), NC (1.64 ± 0.12 × 10^9/L), BEMC (1.80 ± 0.05 × 10^9/L), and PC (783.67 ± 59.51 × 10^9/L) of the control group.

Furthermore, in CFA-induced arthritic rat model, the 50, 100, and 250 mg/kg CAHLF-treated groups had significantly reduced (p < 0.05) HC (37.05 ± 1.51%, 39.01 ± 3.82%, and 33.55 ± 1.10%), WBC (10.89 ± 1.49 × 10^9/L, 9.73 ± 0.48 × 10^9/L, and 7.37 ± 0.34 × 10^9/L), LC (7.94 ± 1.01 × 10^9/L, 7.70 ± 0.52 × 10^9/L, and 6.12 ± 0.32 × 10^9/L), NC (1.49 ± 0.49 × 10^9/L, 0.61 ± 0.14 × 10^9/L, and 0.24 ± 0.03 × 10^9/L), BEMC (1.47 ± 0.09 × 10^9/L, 1.42 ± 0.05 × 10^9/L, and 1.01 ± 0.06 × 10^8/L), and PC (473.67 ± 15.03 × 10^9/L, 548.00 ± 56.57 × 10^9/L, and 246.33 ± 5.54 × 10^9/L) compared with HC (12.82 ± 0.90%), WBC (15.58 ± 0.82 × 10^9/L), LC (12.82 ± 0.90 × 10^9/L), NC (0.91 ± 0.03 × 10^9/L) BEMC (1.85 ± 0.14 × 10^9/L), and PC (593.830 ± 36.3 × 10^9/L) of the control group.

Lipid profile analysis showed that the 50, 100, and 250 mg/kg CAHLF-treated groups had significantly decreased total cholesterol (152.83 ± 1.94 mg/dL,
146.33 ± 5.96 mg/dL, and 144.00 ± 2.28 mg/dL), LDL-cholesterol (74.67 ± 5.36 mg/dL, 59.93 ± 7.53 mg/dL, and 53.47 ± 4.53 mg/dL), VLDL-cholesterol (32.20 ± 5.00 mg/dL, 25.15 ± 3.83 mg/dL, and 25.83 ± 5.49 mg/dL), and triglycerides (70.83 ± 5.00 mg/dL, 55.33 ± 3.83 mg/dL, and 56.83 ± 5.49 mg/dL) compared with the LDL-cholesterol (105.30 ± 6.34 mg/dL), VLDL-cholesterol (61.06 ± 8.69 mg/dL), and triglyceride (134.33 ± 8.69 mg/dL) levels in the control group induced arthritis for 21 d. Furthermore, HDL-cholesterol levels were significantly (p < 0.05) elevated in 50, 100, and 250 mg/kg CAHLF-treated groups as 50.83 ± 4.26 mg/dL, 64.00 ± 4.56 mg/dL, and 79.17 ± 4.54 mg/dL, respectively, when compared with control (36.33 ± 2.42 mg/dL) (Figure 4).

Discussion

In this study, hematological analysis was performed on rats induced arthritis with either formaldehyde or CFA. Results indicated that 100 and 250 mg/kg CAHLF-treated groups had a significantly reduced hematocrit, white blood cell, lymphocytes, neutrophils, basophil, eosinophils, monocytes, and blood platelet counts when compared with the control group. This suggests a heightened inflammatory response in the control group induced with arthritis. The reductions in hematological and immunological cells in the CAHLF-treated groups suggested that CAHLF may have an immunomodulatory activity against the pro-inflammatory mediators. It has been previously reported that NSAID and steroidal anti-inflammatory drugs exhibit an immunomodulatory action against infiltrating cells at the sites of inflammation (Prempah & Mensah-Attipoe, 2008). A key aspect of inflammatory response is the cellular response due to the pivotal role played by leukocytes. As part of their defensive roles during inflammation, these cells release their lysosomal contents such as bactericidal enzymes and proteases exacerbating tissue damage (Chou, 1997; Okoli et al., 2008). Previous work done in inflammatory studies involving animal models had shown a clinical relevance in risk evaluation as the changes in hematological and immunological systems do have a higher predictive value when the data are translated from the animal studies to humans (Rhiouani et al., 2008).

Further study showed that the CAHLF-treated groups had a significantly (p < 0.05) decreased total cholesterol, LDL-cholesterol, VLDL-cholesterol, and triglycerides compared with the saline-treated control group induced arthritis for 21 d while HDL-cholesterol was found to be elevated in the treated groups. This suggests that the CAHLF may possess anti-inflammatory activity against lipid-related inflammatory diseases. Previous studied had associated inflammation with atherosclerosis (Gonzalez-Gay et al., 2005; Li & Glass, 2002). It has also been reported that patients with untreated rheumatoid arthritis and chronic systemic inflammation may have altered lipoprotein patterns that contribute to their higher risk of atherosclerosis (Choi & Seeger, 2005). A consistent pattern of reduced HDL-cholesterol levels has been observed in rheumatoid arthritis patients (Dursunoglu et al., 2005). A previous study had also shown that agents that can reduce serum total-cholesterol, LDL-cholesterol, VLDL-cholesterol, and triglycerides and also induce an elevation in HDL-cholesterol may confer significant protection against inflammatory-related diseases (Dursunoglu et al., 2005).

Conclusion

Findings from this study highlighted that hexane fraction of C. afer leaves expressed hypocholesterolemic and immunomodulatory properties in arthritic rats. This could explain the ethnomedical use of CAHLF as a therapeutic agent in the treatment of arthritis. CAHLF should be studied further to gain complete understanding of its anti-inflammatory mechanism of action and also it could be exploited in pharmaceutical drug development.

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Declaration of interest

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