Chloroform seed extract of *Buchholzia coriacea* (Capparaceae) ameliorates complete Freund's adjuvant–induced chronic inflammation in rat

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ABSTRACT

Background: *Buchholzia coriacea* seeds are used in traditional African medicine as remedies for various diseases including sinusitis, nasal congestion, ulcers, rheumatic pains, febrile convulsions, and dementia.

Objective: This study was carried out to evaluate the effect of chloroform seed extract of *Buchholzia coriacea* (CEBC) on complete Freund's adjuvant (CFA)-induced inflammation in rats.

Methods: CEBC (100, 200 or 400 mg/kg/day, p.o.) or celecoxib (30 mg/kg, p.o.; standard reference drug) were administered for 28 days after administration of CFA on day 1. The effects of CEBC on inflammation index, haematological and biochemical profiles as well as oxidative stress parameters were assayed.

Results: CEBC (100, 200 and 400 mg/kg) produced significant ($p<0.001$, $p<0.05$ and $p<0.01$ respectively) reduction in CFA-induced oedema in the rats' paw in a non-dose-dependent manner with highest effect observed at 200 mg/kg/day. The extract (200 or 400 mg/kg) also caused significant decrease ($p<0.001$ and $p<0.0001$, respectively) in serum AST levels when compared to CFA-vehicle treated. Furthermore, CEBC increased hepatic glutathione (GSH) levels, catalase (CAT) and superoxide dismutase (SOD) activities with a corresponding reduction in the MDA concentration when compared with CFA-vehicle treated.

Conclusion: Findings from this study showed that the chloroform extract of *B. coriacea* possesses anti-inflammatory effect possibly through inhibition of release of inflammatory mediators and enhancement of antioxidant defense system. This lends further credence to the folklore use of *B. coriacea* in the management of inflammatory conditions such as rheumatoid arthritis.

Key words: antioxidants; Complete Freund's Adjuvant (CFA); chronic inflammation; liver injury; creatinine

L'extrait de graines de chloroforme de *Buchholzia coriacea* (Capparaceae) améliore complètement l'inflammation chronique de Freund induite par l'adjuvant chez le rat

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RÉSUMÉ

Contexte: Les graines de *Buchholzia coriacea* sont utilisées dans la médecine traditionnelle africaine comme remèdes pour plusieurs maladies, notamment la sinusite, la congestion nasale, les ulcères, les douleurs rhumatismales, les convulsions fébriles et la démence.

Objectif: Cette étude a été réalisée pour évaluer l'effet de l'extrait de graines de chloroforme *Buchholzia coriacea* (CEBC) sur l'adjuvant complet de Freund (ACF) induite par inflammation chez les rats.

Méthodes: Le CEBC (100, 200 ou 400 mg/kg/jour, p.o.) ou le célécoxib (30 mg/kg, p.o.; médicament standard de référence) ont été administrés pendant 28 jours après l'administration de ACF au jour 1. Les effets de la CEBC sur l'indice d'inflammation, les profils hématologiques et biochimiques ainsi que les paramètres du stress oxydatif ont été analysés.

Résultats: La CEBC (100, 200 et 400 mg/kg) a entraîné une réduction significative ($p<0,001$, $p<0,05$ et $p<0,01$ respectivement) de l'œdème induit par l'ACF chez les rats de manière non dépendante de la dose, l'effet le plus élevé étant observé à 200 mg/kg/jour. L'extrait (200 ou 400 mg/kg) a également provoqué une diminution significative ($p<0,001$ et $p<0,0001$, respectivement) des taux d'AST sériques comparativement à ceux traités à l'ACF. En outre, CEBC a augmenté les taux de glutathion hépatique (GSH), de la catalase (CAT) et de la superoxyde dismutase (SOD), avec une réduction correspondante de la concentration de MDA par rapport au traitement par l'ACF.

Conclusion: Les résultats de cette étude ont montré que l'extrait de chloroforme de *B. coriacea* possède un effet anti-inflammatoire, probablement en inhibant la libération de médiateurs inflammatoires et en renforçant le système de défense antioxydant. Cela donne encore plus de crédibilité à l'utilisation folklorique par *B. coriacea* dans la prise en charge de maladies inflammatoires telles que la polyarthrite rhumatoïde.

Mots-clés: antioxydants; Adjuvant complet de Freund (ACF); inflammation chronique; lésion hépatique; la créatinine

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by progressive damage of synovial-lined joints and variable extra-articular manifestations. Tendon and bursal involvement are frequent and often clinically dominant in early disease. RA can affect any joint, but it is usually found in metacarpophalangeal, proximal interphalangeal and metatarsophalangeal joints, as well as in the wrists and knee. Articular and periarticular manifestations include joint swelling and tenderness to palpation, with morning stiffness and severe motion impairment in the involved joints. The clinical presentation of RA varies, but an insidious onset of pain with symmetric swelling of small joints is the most frequent finding. RA onset is acute or sub-acute in about 25% of patients, but its patterns of presentation also include palindromic onset, monoarticular presentation (both slow and acute forms), extra-articular synovitis (tenosynovitis, bursitis), polymyalgia-like onset, and general symptoms (malaise, fatigue, weight loss, fever).[1] Early, targeted treatment improves the outcome of rheumatoid arthritis, reducing disease-associated disability and mortality.[2]

Drugs currently used in the treatment of RA caused adverse effects such as; gastrointestinal ulceration, cardiovascular, renal, and hepato-cellular injuries following, non-steroidal anti-inflammatory drugs (NSAIDs) administration.[3-5] There is, therefore, a need to search for newer, safer, and more effective drugs for RA.
Buchholzia coriacea Engl. [family Capparaceae], English musk tree (Unwin 411) French kola pimenté, oignon de gorille (Gabon, Walker), an evergreen understorey tree of the lowland rain-forest, to 20 m high, occurring from Guinea to West Cameroons, and East Cameroon and Gabon.[6] In Liberia the seeds are used on skin-eruptions and internally for worms, oedema, and pains, while crushed up they are pasted over the stomach in Ivory Coast for a difficult childbirth.[6] The seeds have a hot spicy flavour. The seeds are covered in a purple aril which is chewed in Ivory Coast like the kola and has a sharp pungent taste like that of Capsicum frutescens (Solenaceae). In Southern Nigeria, the Benins boil and eat the fruit after storage for a few days.[6] Anti-ulcer, anti-inflammatory, antidepressant, immunomodulatory, antihelminthic and neuropharmacologic effect of *Buchholzia coriacea* have been reported.[7-11] Hence, this study was carried out to evaluate the effect of chloroform seed extract of *Buchholzia coriacea* in complete Freund's adjuvant – induced chronic inflammation in rats.

METHODS

Plant material

Buchholzia coriacea seeds were collected from Abeokuta, Ogun state, Nigeria. Botanical identification and authentication were carried out at the Department of Botany, University of Lagos, Akoka, Lagos State, Nigeria. A voucher specimen with number LUH 5831 was deposited in the herbarium of the same department for reference.

Extraction procedure

Buchholzia coriacea seeds were chopped into small pieces and air dried. The dried material (1600 g) was powdered and soaked in 1L ethanol and exhaustively extracted by maceration. The extract was concentrated under vacuum. The concentrated sample was further dried in the oven at 40°C. Twenty grams of ethanol crude extract was dissolved in 60 mL of distilled water and then partitioned with chloroform ($CHCl_3$). The chloroform fraction was concentrated under vacuum and further dried in the oven at 40°C, yielding 15 g of the chloroform extract.

Laboratory animals

Thirty-six healthy young male rats (140 – 165 g) and 25 Swiss mice (18 –21 g) were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Idi-Araba, Lagos State, Nigeria. The animals were maintained under standard environmental conditions (23 - 25°C, 12/12 h light/dark cycle) and had free access to standard rodent pellet diet (Livestock Feed Plc, Lagos, Nigeria) and water. The experimental procedures adopted in this study were in strict compliance with the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals.[12]

Acute toxicity study

Acute toxicity studies were carried out using Swiss mice as stated in the guidelines given by the Organization for Economic Co-operation and Development (OECD NO: 423). Five Swiss mice were fasted overnight. A single limit dose of 5000 mg/kg of the extracts was administered in a stepwise manner to all the five animals. Animals were observed for the period of one hour, and occasionally for three hours for severity of any toxic sign and mortality. If no mortality is observed at this dose, the extract is regarded as safe. The LD_{50} was thus determined and the animals were observed up to 14 days for delayed toxicity and mortality.

Treatment regimen

This is according to the method described by Adeneye *et al.*[13] Three days before the commencement of the experiment, the rats were randomly divided into 6

groups of 6 rats per group. Inflammation was induced by intra-dermal injection of 0.1 ml of Complete Freund's adjuvant (CFA) (10 mg/ml of heated-killed *Mycobacterium tuberculosis* in 1 ml paraffin oil) into the right hind paw [14] and was repeated after 3 days within 28 days of the experiment. The treatment with *B. coriacea* was done for 28 days as follows:

Group 1 (Vehicle): 10 ml/kg of 0.9% normal saline, *p.o.*+ 0.1 ml 0.9% normal saline, *sc*.
Group 2 (Vehicle + CFA): 10 ml/kg of 0.9% normal saline, *p.o.*+ 0.1 ml CFA, *sc*.
Group 3 (Celecoxib + CFA): 3 mg/kg/day celecoxib, *p.o.*+ 0.1 ml CFA, *sc*.
Groups 4-6: CEBC (100, 200, and 400 mg/kg/day, *p.o.,* respectively, + 0.1 ml CFA, *sc*.
Inflammation in rats was assessed by means of physical examination and biochemical measurements. The joint diameter and body weight was measured on day 0 (before injecting CFA) and every 4 days for 28 days using digital vernier caliper, in both the control and the treated rats.

Measurement of paw size and body weight
Inflammation on the right hind paw (paw edema) of each rat was assessed. This presents in the form of redness, increased heat, swelling, pain, and loss of function. The magnitude of the inflammation was measured using digital vernier calipers on days 4,8,12,16,20,24 and 28 after CFA injection.
Percentage inhibition of oedema was calculated using the formula: $(Vc-Vt/Vc) \times 100$
Where V_c is the paw diameter of control group (Vehicle + CFA),
V_t is the paw diameter of test groups
The body weight of each animal was measured on days 4, 8, 12, 16, 20, 24 and 28 post induction of inflammation.

Blood collection and bioassays
On the 28th day post-induction, blood samples were obtained via retro-orbital plexus and collected into EDTA-treated and plain bottles for haematological and biochemical assays respectively. Thereafter, rats were sacrificed by cervical dislocation, a ventral longitudinal abdominal incision made and the liver was identified and immediately dissected out. These dissected organs were rinsed separately and homogenized in ice-cold Tris HCl buffer (0.01M, pH 7.4) to give a 10% homogenate. Liver samples were taken for determination of enzymatic and non-enzymatic antioxidants (reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) as well as lipid peroxidation by measuring malondialdehyde (MDA).

Estimation of protein-bound carbonyl
For protein carbonylation analysis, 20% of the liver 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 Niebes.[15] 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 [16] with modifications by Niebes.[15]

Estimation of serum liver functions
Serum albumin, total protein (TP), total cholesterol (Chol), 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 reduced glutathione (GSH), [17] superoxide dismutase (SOD) [18] and catalase (CAT) [19] activities were assayed using standard procedures.

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

Statistical analysis
Results were expressed as mean ± SD and statistical analysis were carried out using one or two-way analysis of variance (ANOVA) followed by Dunnett's test for comparison test. GraphPad Prism 6 (GraphPad Software Inc., California; U.S.A.) was used for the analysis. Significant values were considered at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$.

RESULTS

Acute toxicity test

In acute toxicity studies, CEBC was found to be safe up to 5000 mg/kg. No mortality or toxic symptoms were observed during the entire duration of the study.

Anti-inflammatory activity

Effects of CEBC on paw oedema

Subcutaneous administration of 0.1 ml of CFA into the right hind paw of rats every three days within 28 days resulted in significant-time dependent increase in the paw thickness in CFA-treated rats. Oral treatment with 100 mg/kg/day of CEBC resulted in a time-dependent significant (p<0.001) decrease on day 4 and (p<0.0001) on days 8, 12, 16, 20, 24, and 28, of the paw thickness in the CFA induced arthritic rats. Similarly, Oral treatment with 200 mg/kg/day of CEBC resulted in a time-dependent significant (p<0.05, p<0.01) decrease on days 8 and 12 respectively, also (p<0.0001) on days 16 to 28 of the paw thickness in the CFA-treated rats. Similarly, oral treatment with 400 mg/kg/day of CEBC resulted in a time-dependent significant (p<0.05) decrease on days 4 and 12, and (p<0.0001) on days 20, 24, and 28. However, a maximum significant decrease (p<0.0001) was recorded in the group with oral treatment of 200 mg/kg/day on the 28th day of treatment. This effect was comparable to the effect recorded in the groups with oral treatment of 3 mg/kg/day of celecoxib (Table 1). Similarly, a maximum degree of inhibition of inflammation (% inhibition) (61. 13%) was recorded in the group with oral treatment with 200 mg/kg/day and is comparable to 3 mg/kg/day celecoxib treatment group (69.39%) (Table 1).

Table 1: Effect of CEBC on paw oedema

Treatment (mg/kg)	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28
Vehicle	0.07± 0.04^{d}	0.03± 0.08^{d}	0.07± 0.07^{d}	0.06± 0.11^{d}	0.12± 0.09^{d}	0.16± 0.04^{d}	0.18± 0.03^{d}
Vehicle + CFA	3.71± 0.06	3.79 ± 0.06	3.74± 0.08	3.81 ± 0.06	3.92 ± 0.07	4.15 ± 0.05	4.53 ± 0.10
Celecoxib +CFA	2.09± 0.09^{d}	2.06± 0.19^{d}	1.79± 0.17^{d}	1.49± 0.23^{d}	1.55± 0.25^{d}	1.63± 0.39d	1.39± 0.29^{d}
Inhibition (%)	43.78	45.71	52.21	61	60.34	60.63	69.39
BC 100 + CFA	2.54 ± 0.16^{c}	2.47 ± 0.16^{d}	2.28 ± 0.10^{d}	2.11 ± 0.13^{d}	1.99 ± 0.25^{d}	1.80 ± 0.27^{d}	1.85 ± 0.41^{d}
Inhibition (%)	31.61	34.9	39.14	44.69	49.09	56.53	59.15
BC 200 + CFA	3.44 ± 0.26	2.91 ± 0.21^{a}	2.70 ± 0.20^{b}	2.19 ± 0.12^{d}	1.19 ± 0.20^{d}	2.13 ± 0.37^{d}	1.76 ± 0.44^{d}
Inhibition (%)	7.1	23.34	27.82	42.59	49.91	48.67	61.13
BC 400 + CFA	2.81 ± 0.14^{a}	3.17 ± 0.30	2.86 ± 0.45^{a}	3.06 ± 0.33^{a}	2.35 ± 0.24^{d}	2.19± 0.24^{d}	2.21± 0.14^{d}
Inhibition (%)	24.15	16.15	23.63	19.85	40.09	47.19	51.31

Values presented as mean ± SD (n=6), ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001, and ^{d}p<0.0001 versus vehicle + CFA group

Effects of CEBC on body weight

CFA subcutaneous injection into the right hind paw of rats resulted in decreased body weight over 28 days period. Oral treatment with 100 mg/kg/day of CEBC resulted in a time dependent significant (p<0.05) increase in body weight on day 8 and between day 12 to day 28 (p<0.0001) in CFA-treated rats. Similarly, oral treatment with 200 mg/kg/day of CEBC resulted in a time dependent significant (p<0.001) increase in body weight on day 8 and between days 12 and 28 (p<0.0001) in CFA-treated rats. Also, oral treatment with 400 mg/kg/day of CEBC resulted in time dependent significant (p<0.0001) increase in body weight from day 12 to 28 in CFA-treated rats. While oral daily treatment with 3 mg/kg/day of celecoxib also resulted in significant (p<0.05) increase in body weight on day 8 and (p<0.0001) between days 12 and 28 in CFA-treated rats. However, the maximum increase in body weight was recorded in groups treated with 200mg/kg/day on day 28th, this which was comparable to body weight recorded in group treated with 3mg/kg/day of celecoxib as shown in Table 2.

Table 2: Effect of CEBC on body weight

Treatment (mg/kg)	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28
Vehicle	1.17±1.28 [b]	2.83±0.65 [d]	4.33±0.80 [d]	6.50±0.81[d]	11.67±1.33 [d]	13.80±1.96 [d]	16.75±1.89 [d]
Vehicle + CFA	-5.17±0.48	-7.17±1.30	-8.83±1.38	-11.0±1.26	-9.17±3.57	-16.00±1.21	-18.33±1.17
Celecoxib + CFA	-3.17±0.70	-1.67±0.80 [a]	-0.33±0.56 [d]	1.83±0.65 [d]	4.67±1.09 [d]	5.80±1.46 [d]	7.80±1.39 [d]
BC 100 + CFA	-2.33± 0.42	-1.67±0.76 [a]	0.83±1.01 [d]	1.67±1.71 [d]	2.17±1.45 [d]	3.00±1.06 [d]	6.00±1.29 [d]
BC 200 + CFA	-2.67±0.21	-1.00±0.37[b]	-0.17±0.87[d]	2.17±0.60[d]	3.60±1.03[d]	5.80±1.77[d]	7.80±1.69[d]
BC 400 + CFA	-3.67±0.56	-2.83±1.01	-0.67±0.99[d]	2.17±0.95[d]	4.50±1.61[d]	6.40±1.63[d]	7.20±2.54[d]

Values presented as mean ± SD (n=6), [a]p<0.05, [b]p<0.01, [c]p<0.001, and [d]p<0.0001 versus vehicle + CFA group

Table 3: Effect of CEBC on mobility

Treatment mg/kg	No of stairs climbed
Vehicle	1.72 ±0.46 [b]
Vehicle + CFA	0.60 ±0.32
Celecoxib +CFA	1.46 ±0. 34 [a]
BC 100 + CFA	0.50 ±0.12
BC 200 + CFA	0.75 ±0.22
BC 400 + CFA	1.16 ±0.22 [a]

Values presented as mean±SD (n=6), [a]p<0.05, [b]p<0.01, versus vehicle + CFA group

Effect of CEBC on mobility

In the CFA-treated rat, mobility was significantly (p<0.0001) impaired compared to vehicle treated group (Table 3). Oral administration of 400 mg/kg of CEBC caused a significant (p<0.001) restoration of mobility compared to vehicle + CFA group (Table 3).

Table 4: Effect of CEBC on haematological parameters in CFA-treated rats

Treatment (mg/kg)	WBC (10^9/µl)	RBC (10^{12}/µl)	Hb (g/dl)	PCV (%)	PLT (10^9/µl)	MCV(fl)	MCH (pg)	MCHC (g/dl)	Neutrophil (%)	LYMPH (%)	LYMPH#
Vehicle	11.78±3.44	9.46± 0.23	17.30± 1.4[a]	55.30± 2.76[a]	1099.00± 95.40	58.46± 1.45	18.23± 0.46	31.23± 0.41	49.23± 1.79	48.00± 5.90 [a]	5.36± 0.63
Vehicle + CFA	10.28±1.86	8.04± 0.30	14.76± 0.37	46.50± 1.71	927.00± 45.67	57.90± 1.14	18.34± 0.37	31.76± 0.43	51.42± 1.00	19.25± 10.27	3.42± 0.51
Celecoxib +CFA	11.78± 2.58	8.56± 0.29	15.60± 0.85	49.23± 2.42	1061.00 ± 37.20	56.93± 1.44	18.0± 0.49	31.70± 0.75	41.30± 9.45	46.30± 11.02	7.12± 1.34[b]
BC 100 + CFA	11.78±2.15	8.24 ±0.44	14.76± 0.61	46.82± 1.64	978.80± 75.76	55.94± 1.51	17.92± 0.41	32.14± 0.31	45.28± 3.04	30.32± 7.42	5.56± 0.50
BC 200 + CFA	12.92± 1.33[a]	8.39± 0.30	15.68± 0.72	48.62± 2.44	1115.00± 123.22[a]	57.92± 1.32	18.62± 0.46	32.24± 0.55	47.15± 4.15	39.90± 8.85[a]	6.38± 0.37[a]
BC 400 + CFA	11.78 ±1.81	8.46± 0.50	15.53± 1.23	48.16 ±3.81	1063.20± 70.56	56.90± 1.82	18.26± 0.49	32.2± 0.34	50.54± 3.88	29.25± 7.23	5.70± 0.50

Values presented as mean ± SD (n=6), [a]p<0.05, [b]p<0.01 versus vehicle + CFA group
[#]number

Effects of CEBC on full blood count

In the CFA-treated rats and administered with 200 mg/kg/day of CEBCs, there was a significant ($p<0.05$) increase in the WBC, LYMPH% and concomitant significant ($p<0.001$) increase in PLT (Table 4). Similarly, 400 mg/kg/day of CEBCs caused significant ($p<0.001$) increases in PLT and % neutrophil while 100 mg/kg/day of CEBCs caused no significant ($p>0.05$) alterations in full blood count (Table 4).

Effect of CEBC on the liver and renal function parameters

Serum AST was non-significantly ($p>0.05$) decreased in CFA treated rats, although serum ALP was significantly ($p<0.001$) increased when compared to vehicle-treated group (Table 5). Oral treatment with 200 mg/kg and 400 mg/kg of CEBC caused a significant ($p<0.01$ and $p<0.001$) decrease in the serum AST levels, respectively. Also, oral treatment with 100, 200 and 400 mg/kg/day of CEBC caused a further ($p<0.0001$) increase in serum ALP (Table 5). No significant alteration in the serum levels of other measured liver function parameters such as TP, ALB, BIL, ALT, CHOL and TG were recorded (Table 5). On the renal function parameters, oral treatment with 100, 200, and 400 mg/kg/day of B. coriacea show non-significant ($p>0.05$) alteration in serum urea and creatinine in the model (Table 5).

Table 5: Effect of CEBC on liver and renal function parameters in CFA-treated rats

Treatment mg/kg	AST (U/l)	ALT (U/l)	ALB (mg/dl)	CREA (mg/dl)	CHOL (mg/dl)	BIL (mg/dl)	UREA (mg/dl)	TG	TP (mg/dl)	ALP	HDLC	LDLC
Vehicle	222.14 ± 12.4	5.55 ±1.83	38.26 ± 2.46	66.22 ± 2.48	1.32 ±0.08	6.65 ±1.13	6.16 ±0.58	0.62 ±0.09	79.30 ±4.01	170.46 ±14.38[c]	1.65 ± 0.11	0.45 ±0.04
Vehicle + CFA	213.78 ± 18.35	8.86 ± 1.48	34.86 ± 1.45	59.84 ± 3.85	1.10 ± 0.12	9.15 ± 0.92	4.54 ± 0.37	0.77 ± 0.17	72.90 ± 1.38	120.90 ± 28.70	1.48 ± 0.16	0.30 ± 0.04
Celecoxib +CFA	203.90 ± 29.06	13.50 ± 1.20	35.30 ± 2.76	46.77 ± 3.21	0.92 ± 0.08	7.47 ± 2.33	5.03 ± 0.61	0.79 ± 0.15	67.16 ± 1.06	179.70 ± 0.01[c]	1.17 ± 0.09	0.19 ± 0.03
BC 100 + CFA	199.84 ± 20.47	14.75 ± 2.45	30.24 ± 1.46	42.40 ± 1.33	0.84 ± 0.03	5.70 ± 3.70	5.70 ± 0.40	0.92 ± 0.09	65.24 ± 2.37	211.76 ± 12.87[d]	0.92 ± 0.09	0.16 ± 0.02
BC 200 + CFA	170.78 ± 23.29[a]	24.53 ± 5.12	28.45 ±1.86	51.78 ±6.63	0.75 ±0.11	5.87 ±0.99	6.20 ±0.32	0.54 ±0.13	74.26 ±2.27	213.85 ±27.14[d]	1.02 ±0.13	0.17 ±0.02
BC 400 + CFA	169.94 ± 12.11[b]	27.68 ± 3.67	31.00 ± 1.72	38.59 ± 1.08	0.86 ± 0.04	4.32 ± 1.05	5.90 ± 0.52	0.70 ± 0.11	61.91 ± 1.44	227.32 ±18.39[d]	1.04 ±0.06	0.20 ±0.03

Values presented as mean ± SD (n=6), [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$, and [d]$p<0.0001$ versus vehicle + CFA group

Effect of CEBC on liver antioxidant system and glycoprotein

CFA treatment in rats resulted in non-significant ($p>0.05$) increase in the hepatic tissue SOD, CAT and reduction in GSH levels when compared to untreated group. Oral treatment with 400 mg/kg/day of CEBC caused significant ($p<0.0001$) increase in hepatic GSH levels as against significant ($p<0.0001$) decrease recorded in celecoxib treated group. Although repeated subcutaneous injection of CFA caused significant ($p<0.05$), increase in hepatic hexose sugar levels but caused non-significant ($p>0.05$), increases in the hepatic tissue levels of MDA levels (Table 6). However, 400 mg/kg/day of CEBC caused a significant ($p<0.01$) increase in GSH when compared to untreated control values (Table 6).

Table 6: Effect of CEBC on liver antioxidant system and glycoprotein in CFA-treated rats

Treatment (mg/kg)	GSH (µmol/ml protein)	SOD (µmol/ml protein)	CAT (µmol/ml protein)	MDA (µmol/ml protein)	Hexose sugar	Sialic acid
Vehicle	19.24 ± 8.80	60.76 ± 12.62	716.63 ± 1.86	2.75 ± 0.76	416.22 ± 5.83[a]	193.66 ± 53.96
Vehicle + CFA	10.62 ± 4.30	77.89 ± 3.85	730.32 ± 10.76	2.93± 0.77	562.15 ± 78.23	205.82 ± 31.30
Celecoxib + CFA	5.72 ± 1.57[d]	55.00 ± 5.01	733.56 ± 10.43	2.37 ± 0.45	548.75 ± 27.86	166.63 ± 32.31
BC 100 + CFA	9.95 ± 1.57	88.51± 7.04	719.65 ± 1.25	1.49 ± 0.43[c]	816.41 ± 57.60[b]	104.93 ± 30.18
BC 200 + CFA	9.75 ± 3.92	75.76 ± 5.39	717.21 ± 17.47	3.28 ± 0.91	641.65± 101.55[a]	230.59 ± 64.68
BC 400 + CFA	22.18 ± 9.81[b]	70.81 ± 7.01	735.88 ± 1.62	1.32 ± 0.22[c]	581.02 ± 19.40	93.22 ± 16.11[d]

Values presented as mean ± SD (n=6), [a]p<0.05, [b]p<0.01, [c]p<0.001, and [d]p<0.0001 versus vehicle + CFA group

DISCUSSION

In the present study, experimental inflammation which is an important symptom in rheumatoid arthritis was reliably established with subcutaneous injection of 0.1 ml of CFA characterized by plantar oedema formation which peaked 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 oedema formation and proinflammatotory cell infiltrations.[20] Thus, our results were similar to those reported previously.[21, 22] However, the pretreatment of rats with *B. coriacea* chloroform seeds extract prevented CFA-induced inflammation in rats, with peak effect at 200 mg/kg, similar to the effect of celecoxib, a selective cyclooxygenase 2 inhibitor, which suggests inhibition of synthesis of inflammatory mediators, this corroborated the report of Ezike *et al*.[10] This effect could be possibly due to the presence of terpenoids, and sterols compounds in the chloroform fraction.[8] *B. coriacea* seeds contain potent anti-hypercholesterolemic agent which may find clinical application in ameliorating hypercholesterolemia and its attendant complications.[23] In this study, the increase in leukocyte count induced by CFA was ameliorated by *B. coriacea* seed extract. Leukocytes play a key role in the autoimmune response that occurs during rheumatoid arthritis. The reduction in leucocytes count by *B. coriacea* seeds extract may be a key mechanism of its anti-inflammatory property.

A wide spectrum of hepatotoxic effects is described with anti-rheumatic drugs. Studies investigating genetic susceptibility to diclofenac hepatotoxicity have expanded our understanding of the potential drug-specific, class-specific and general factors involved in its pathogenesis, and methotrexate-associated liver disease demonstrates the interaction between drug, host and environmental factors that determine the likelihood and magnitude of liver disease. Infliximab therapy is associated with typical drug-induced autoimmune hepatitis.[24] NSAIDs are responsible for an important aliquot of transaminase elevation in the general population. Genetic susceptibility to diclofenac hepatotoxicity has promoted the knowledge about drug-specific, class-specific reactions. Some drugs (sulfasalazine, azathioprine, and leflunomide) may cause acute liver injury, whereas other compounds (methotrexate) may cause chronic liver damage as the result of the interaction among drugs, host and environmental factors. [25] Hence, the need for a hepatoprotective drug. In this study, *B. coriacea* seed extract significantly decreased serum AST levels but not serum ALP. NSAID administration to susceptible persons may cause decrease in renal plasma flow and glomerular filtration rate within hours. Such acute noxious renal effects are mediated by products of arachidonic acid metabolism. Precipitous decrements in glomerular filtration and renal ischemia, manifested by increased serum creatinine and urea nitrogen, are possible.[26] In this study, CFA induced renal injury was ameliorated by *B. coriacea* administration evidenced in renal integrity maintenance.

Oxidative damage induced by reactive oxygen species has been related to the pathophysiology of rheumatoid arthritis in several studies, although results have been inconsistent and contradictory.[27] In this study CFA injection caused significant increase in oxidative stress parameters such as MDA and decreased antioxidant enzymes activity which was attenuated by *B. coriacea* administration.

This study showed the potential of *B. coriacea* in the management of inflammatory conditions, however, further studies are needed to assess the effects of *B.*

coriacea on inflammatory cytokines and histological changes that occur in these conditions.

CONCLUSION

Findings from this study showed that *B. coriacea* seed extract possesses anti-inflammatory effect possibly through inhibition of inflammatory mediators release or enhancement of antioxidant enzymes activity. This plant could be a potential source of phytotherapeutic substances for the management of inflammatory conditions such as rheumatoid arthritis. These findings, therefore, support its use in the folkloric medicine.

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