

ANTIOXIDANT EFFECT OF *Buchholzia coriacea* ETHANOL LEAF-EXTRACT AND FRACTIONS ON FREUND'S ADJUVANT-INDUCED ARTHRITIS IN ALBINO RATS: A COMPARATIVE STUDY

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Abstract: Several studies have implicated reactive oxygen species in perpetuation of inflammation and subsequent destruction of joints in patients with arthritis. Antioxidant effects of *Buchholzia coriacea* ethanol leaf-extract, aqueous and ethyl acetate fractions on oxidative stress indices in rheumatoid arthritic female Wistar albino rats were examined. 180 rats were randomly divided into 12 groups. Fifteen rats were placed in each group. Rats without arthritis were placed in Group 1. Rheumatoid arthritis was induced in groups 2 to 12 by intradermal injection of 0.1 ml complete Freund's adjuvant into the left hind paws of rats. Group 2 (arthritic rats) did not receive any treatment but rather were given normal saline while group 3 (arthritic rats) received 5 mg/kg indomethacinTM (a standard drug). Rats in Groups 4 to 12 were administered the samples at doses of 200, 400 and 800 mg/kg body weight. Freund's adjuvant administration led to inflammation and oxidative stress which were marked by significant ($P < 0.05$) increase in paw sizes, oxidative stress markers and reduced body weight of the rats. Arthritic rats were treated with standard drug and samples (at varied doses) and this resulted to reversal of the trend of those parameters in a time and dose-dependent manner. Rats that received 800 mg/kg of the aqueous fraction displayed the best desirable result which was similar to the effect of indomethacin. Thus, *Buchholzia coriacea* ethanol extract and fractions may be useful in the management of oxidative stress which is very common among individuals with rheumatoid arthritis.

Key words: rheumatoid arthritis; *Buchholzia coriacea*; free radicals; oxidative stress; reactive oxygen species

Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder. In individuals with autoimmune disorders, their immune system attack joints and some other tissues (1). Patients with these disorders have antibodies in their blood that target their own body tissues, resulting to inflammation (2). RA is a systemic (body-wide) disease, involving many organs of the body (3). Symptoms of RA include morning stiffness, joint pain, limited range

of motion in the affected joints, fatigue, occasional redness, fever, firm bumps (nodules) under the skin and symptoms are always worse in the morning (4). RA is the most common rheumatic disease, affecting about 1 % of individuals world-wide (5). Male to female prevalence is 1:3 (6). Increased risk in family members of patients with RA may suggest that it can be hereditary (7). Increased risk among smokers and women may justify the role of environmental pollution and hormonal factors at perpetuating the inflammatory process and joint destruction (8).

The joint-damaging role of free radicals during inflammatory and other immunological response

is paramount. This is because free radicals can be deposited into joint cartilage, attacking its proteoglycan and hence inhibits its synthesis (9).

RA is diagnosed by measurement of acute phase reactants, full blood count, and auto-antibody such as rheumatoid factor and anti-cyclic citrullinated peptide assay (14).

The purpose of RA treatments is to inhibit disease severity, reduce symptoms and delay the onset of joint damage and other associated functional limitations (15).

Some RA medications include non-steroidal anti-inflammatory agents, steroids and anti-tumor necrosis factor therapy (2, 3). Though these drugs reduce pain but they are unable to repair damaged tissues. They are mainly used for managing the pain and slowing the progression of RA. Therefore, there is no known drug for curing RA completely (16).

About 80 % of world's population depends on use of various plant parts in the prevention and treatment of diseases (17, 18). The adverse reaction and toxicity associated with the use of anti-inflammatory drugs, in addition to the high cost of the drugs have expeditiously promoted the use of natural plant products or procedures to manage RA locally (19).

Buchholzia coriacea (*B. coriacea*) is in the family of *Capparidaceae* (20, 21). It is commonly called Wonderful kola, Musk tree, Cola pime, and Elephant cola. It has multiple medicinal values. It is useful in treatment of hypertension and also prevents premature aging. Its methanol seed extract has hypoglycemic, hypolipidemic, anti-lipid peroxidation and anti-ulcer effects (22, 23, 24, 25, 26, 27). It has anti-microbial, antihelmintic and antifungal properties (28, 29, 30, 31).

B. coriacea has been in use for quite some time by rural dwellers but there is scarce information on its antioxidant potentials. Therefore, this study was aimed at investigating the effect of *B. coriacea* ethanol leaf extract and fractions on oxidative stress markers in Freund's adjuvant-induced arthritis in albino rats

Material and methods

Materials

Chemicals and reagents used were of analytical standard. Freund's adjuvant was purchased from Sigma Aldrich Company, USA.

Biological materials: Biological materials used for this study were *B. coriacea* leaves and female Wistar albino rats.

Methods

Collection and Identification of Biological Materials: Leaves of *B. coriacea* were collected from Ngodo Village in Afikpo North Local Government Area of Ebonyi State, South-Eastern Nigeria. *B. coriacea* leaf was identified by a Taxonomist in Department of Applied Biology, Ebonyi State University, Abakaliki. Female albino rats (Wistar strain) weighing 121–146 g were obtained from the Department of Animal Science, University of Nigeria, Nsukka, Enugu. The rats were acclimatized for a period of two weeks in the Animal House of Divine Analytical Laboratory, Nsukka under standard laboratory conditions and fed with commercial rat feed and were allowed free access to clean water. The study was approved by the Departmental Institutional Ethical Committee of Biochemistry Department, Ebonyi State University Abakaliki, Nigeria with the Ethical approval number: EBSU/BCH/ET/ 19/010. The guidelines agree with world standard for care and use of laboratory animals in research.

Preparation of extract and fractions: *B. coriacea* leaves were washed and shade dried and later pulverized in a grinder and sifted to obtain powdered sample. Eight hundred grammes of the sample were soaked in 2000 ml of ethanol for 48 hours with intermittent rocking. Thereafter, it was filtered and filtrate was dried. The extracts were stored in airtight container. The dried crude ethanol leaf extract was fractionated in a glass column by eluting in succession with 500 ml water, 500 ml ethyl acetate to obtain aqueous and ethyl acetate fractions, respectively. The crude ethanol leaf extract, aqueous and ethyl acetate fractions were subsequently used for other analyses.

Acute Toxicity Study: Modified Lorke (32) method was used in acute toxicity study. The modification was in the use of albino rats instead of mice. This is because the animal model of this work is albino rats. Thirty-six (36) rats were used for the acute toxicity test. The rats were weighed and fasted overnight before the acute toxicity testing. They were assigned to two experimental groups A and B. Group A which had four rats and served as the normal control group was

administered normal saline. The B group received crude ethanol leaf-extracts of *B. coriacea*. Group B animals were further sub-divided into eight groups with each group having four rats. The sub-groups (B₁-B₈) were administered orally with *B. coriacea* ethanol leaf-extracts at 200, 400, 800, 1200, 1800, 2000, 3000 and 5000 mg/Kg body weight, respectively. All the experimental rats were allowed to have access to food and water and were observed for a 24 hours period for possible signs of toxicity and possible death.

Induction of arthritis in albino rats: Pearson method (33) was employed in induction of arthritis by intradermal injection of 0.1 ml Complete Freund's adjuvant (CFA) into the left hind paws of rats in groups 2 to 12, according to their body weights. The paw sizes of all rats were checked with the aid of vernier caliper and this was done before and after administration of the adjuvant. It was observed that by day 10, arthritis had completely set in.

Rats and experimental groups: Female albino rats (Wistar) were used in this study and a total of 180 rats were utilized. Rats were distributed into 12 groups comprising fifteen rats in each group. The study lasted for 31 days and route of administration of extract and fractions was via oral intubation. The rats were grouped as follows: Group 1 comprises of non-arthritic rats that were given normal saline (1 ml/kg) while Group 2 are arthritic rats that received normal saline (1 ml/kg). Arthritic rats treated with 5 mg/kg indomethacinTM (standard drug) were placed in Group 3 and this served as standard control. Rats in Groups 4-6 are arthritic rats treated with 200, 400 and 800 mg/kg body weight of *B. coriacea* ethanol leaf-extract, respectively. Arthritic rats administered with 200, 400 and 800 mg/kg body weight of aqueous fraction of the crude ethanol leaf extract of *B. coriacea* were placed in Groups 7-9, respectively. Groups 10-12 were arthritic rats treated with 200, 400 and 800 mg/kg body weight of ethyl acetate fraction of ethanol leaf extract of *B. coriacea*. Administration of drugs and samples was through oral intubation.

Determination of Body Weight and Paw Size: The changes in body weight and paw size were measured before and after adjuvant-induced arthritis with the aid of a weighing balance and vernier caliper, respectively. This was done daily. However, records of 10th, 17th, 24th and 31st day of study were used in data analysis.

Collection of Blood Samples for Biochemical Evaluation: Blood samples were humanely collected from three rats each from the groups by cardiac puncture via cervical dislocation on days 10, 17, 24, and 31 into plain sterile bottles. Thereafter, the blood samples were centrifuged at 3,000 rpm for 15 min and serum obtained for biochemical studies.

Determination of Oxidative stress indices: Malondialdehyde (MDA) was evaluated spectrophotometrically by measuring thiobarbituric acid reactive substance (TBARS) as outlined by Wallin *et al.* (34). Nitric oxide (NO) concentration was determined following the procedure described by Bories and Bories (35) based on the Griess reaction in which nitrite is reacted with sulfanilamide (diazotizing reagent) and N-(1-naphthyl) ethylenediamine (a coupling reagent) to produce an azo dye. NO being a labile compound with a brief half-life is rapidly converted to nitrite and nitrate, in oxygenated aqueous solutions. Nitrite levels were measured after the enzymatic reduction of nitrate to nitrite with nitrate reductase. Reduced glutathione (GSH) was assessed following the procedure outlined by Ellman (36). The mechanism was based on the fact that thiols react with Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB), cleaving the disulfide bond to give 2-nitro-5-thiobenzoate (TNB⁻), that in turn ionizes to the TNB²⁻ dianion in water at neutral and alkaline pH. The concentration of Tocopherol was determined according to the method described by Desai (37). The activity of superoxide dismutase (SOD) was determined as outlined by McCord and Fridovich (38) based on the production super-oxide radicals produced by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. Catalase was spectrophotometrically determined using the method explained by Sinha (39).

The activity of glutathione peroxidase was assayed as outlined by Paglia and Valentine (40) based on the fact that GPX catalyzed the oxidation of glutathione by cumene hydroperoxide. In presence of the glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione was immediately converted to the reduced form with concomitant oxidation of NADPH to NADP⁺.

Statistical analysis

All results were expressed as Mean \pm Standard deviation (SD) and data were subjected to one-way analyses of variance (ANOVA) with Duncan multiple range test for assessment of significant differences between means. A significance threshold of $P < 0.05$ was taken for the analyses. Data were analyzed using IBM statistical package for social sciences (IBM-SPSS), version 20 (IBM, Corp., Atlanta, GA). Value of ($P < 0.05$) was considered statistically significant.

Results

Acute toxicity study

The extracts were subjected to acute toxicity study in Wistar albino rats and the rats were monitored for 24 hours. No mortality was recorded and this revealed that the extracts were not toxic even at a high dose of 5,000 mg/kg. This formed the basis of our dose selection for the study.

Effect of Ethanol leaf-extract, Aqueous and Ethyl acetate Leaf Fractions of B. coriacea on Body weight and Paw Size of Adjuvant-induced Arthritic Rats

A significant ($P < 0.05$) increase in body weight of rats in the treated groups was observed relative to negative control as shown in Table 1. There were significant ($P < 0.05$) differences in body weight of rats treated with standard drug (indomethacin) relative to those treated with the extract, aqueous and ethyl acetate fractions. However, rats treated with ethyl acetate fraction had highest increase in body weight.

There was increase in paw size in the feet of rats injected with Freund's adjuvant. A significant ($P < 0.05$) reduction in paw size of rats treated with crude ethanol leaf-extract, aqueous and ethyl acetate leaf fractions of *B. coriacea* at 200, 400 and 800 mg/kg body weight, relative to normal control was observed. The effect was both dose and time-dependent, as shown in Table 2.

There were significant ($P < 0.05$) differences in paw size of rats in all the treated groups. Maximum reduction of paw size with aqueous and ethyl acetate fractions at 200 mg/kg and 800mg/kg on day 31, respectively, occurred, relative to normal control. This effect was similar to that of the standard drug.

Effect of Ethanol leaf-extract, Aqueous and Ethyl acetate leaf fractions of B. coriacea on Oxidative Stress Indices in Adjuvant-induced Arthritic Rats

The results showed that the levels of MDA and NO increased in RA-induced rats relative to normal control as shown Tables 3 and 4, respectively. Treatment with varied doses of the ethanol extract and fractions of the sample at 200, 400 and 800 mg/kg body weight significantly ($P < 0.05$) reduced the levels of MDA and NO in a time-dependent manner. This reduction was comparable to that of indomethacinTM. Treatment with aqueous fraction at doses 200 and 400 mg/kg on day 31 yielded the highest reduction in the level of NO, and this was better than the effect of the indomethacinTM. Activities of SOD, CAT and GPx were lowered in adjuvant-induced arthritic rats (Tables 5-7), respectively. Treatment with standard drug and varied doses of the ethanol extract, aqueous and ethyl acetate fractions at 200, 400 and 800 mg/kg body weight caused a significant ($P < 0.05$) increase in the activities of SOD, CAT and GPx, likewise an increase in the level of reduced GSH. The observed rise in the activity of SOD during treatment was time-dependent but there was no difference among the treated groups. Aqueous and ethyl acetate fractions at 800 mg/kg on day 31 yielded a significantly ($P < 0.05$) higher value in the activity of catalase and this effect was better than that of standard drug. Treatment with ethanol extract and aqueous fraction on days 17 and 24 yielded highest increase in the activity of GPx, when compared with indomethacinTM and ethyl acetate fraction. Treatment with aqueous fraction produced the highest increase in the level of GSH especially on day 31.

Table 1: Effect of Ethanol Leaf-extracts, Aqueous and Ethyl acetate Fractions of *B. coriacea* on Body Weight (g) of Adjuvant-induced Arthritic Rats

Groups	Day 10	Day 17	Day 24	Day 31
1	145.25 ± 9.53 ^a	146.33 ± 7.57 ^a	147.33 ± 0.58 ^a	147.00 ± 5.29 ^a
2	145.67 ± 8.72 ^a	140.33 ± 4.04 ^a	132.67 ± 5.13 ^e	131.75 ± 3.79 ^e
3	127.17 ± 3.86 ⁱ	131.00 ± 7.00 ^g	134.00 ± 3.46 ^c	135.00 ± 2.00 ^c
4	125.17 ± 3.33 ^k	127.00 ± 2.65 ⁱ	132.00 ± 2.65 ^e	136.33 ± 2.08 ^b
5	133.00 ± 3.46 ^d	136.67 ± 4.04 ^b	137.67 ± 1.53 ^b	138.67 ± 2.52 ^b
6	127.50 ± 2.28 ⁱ	131.67 ± 3.06 ^f	133.00 ± 3.00 ^d	136.00 ± 3.00 ^b
7	126.67 ± 2.57 ^k	130.67 ± 3.06 ^h	136.00 ± 2.65 ^b	132.33 ± 1.53 ^e
8	132.83 ± 4.09 ^e	135.67 ± 1.53 ^b	138.00 ± 2.65 ^b	139.67 ± 3.51 ^b
9	131.92 ± 2.39 ^f	137.00 ± 1.00 ^b	138.33 ± 1.53 ^b	142.00 ± 1.00 ^a
10	121.08 ± 2.94 ^m	122.67 ± 2.52 ^l	131.67 ± 2.08 ^f	134.33 ± 1.53 ^c
11	123.08 ± 2.75 ^l	127.67 ± 1.15 ⁱ	132.33 ± 4.93 ^e	135.67 ± 5.03 ^b
12	124.50 ± 2.47 ^l	127.33 ± 3.06 ⁱ	132.33 ± 3.21 ^e	136.00 ± 2.65 ^b

The degree of significance was set at $P < 0.05$. Means (on the same column and row) with different superscripts are significantly different at $P < 0.05$. This implies that comparison was done on both rows (point of sampling) and columns (among experimental groups). Values are mean ± standard deviation of 3 results obtained from 3 rats ($n=3$). BCE=*B. coriacea* Crude Ethanol extract, BCA=*B. coriacea* Aqueous fraction, BCZ=*B. coriacea* Ethyl acetate fraction. 1=normal control, 2= positive control, 3= standard control, 4=200mg/Kg BCE, 5=400mg/Kg BCE, 6=800mg/Kg BCE, 7=200mg/Kg BCA, 8=400mg/Kg BCA, 9=800mg/Kg BCA, 10=200mg/Kg BCZ, 11=400mg/Kg BCZ, 12=800mg/kg BCZ

Table 2: Effect of Ethanol Leaf-extract, Aqueous and Ethyl acetate Fractions of *B. coriacea* on Paw size (mm) of Adjuvant-induced Arthritic Rats

Groups	Before induction	Day 10	Day 17	Day 24	Day 31
1	2.14 ± 0.02 ^a	2.14 ± 0.01 ^a	2.14 ± 0.02 ^a	2.14 ± 0.01 ^a	2.14 ± 0.02 ^a
2	2.20 ± 0.03 ^a	5.39 ± 0.20 ^d	6.59 ± 0.22 ^a	7.33 ± 0.07 ^x	7.78 ± 0.04 ^z
3	2.14 ± 0.07 ^a	4.75 ± 0.55 ^e	3.89 ± 0.59 ^g	2.86 ± 0.32 ^{gh}	2.16 ± 0.22 ^a
4	2.16 ± 0.02 ^a	5.60 ± 0.20 ^{cd}	4.53 ± 0.42 ^{def}	3.48 ± 0.28 ^{efg}	3.01 ± 0.14 ^{bc}
5	2.14 ± 0.03 ^a	5.61 ± 0.30 ^{cd}	4.47 ± 0.32 ^{def}	4.00 ± 0.22 ^{bcd}	3.30 ± 0.35 ^b
6	2.21 ± 0.04 ^a	5.57 ± 0.33 ^{cd}	4.44 ± 0.27 ^{def}	3.44 ± 0.28 ^{fg}	3.00 ± 0.45 ^{bc}
7	2.16 ± 0.02 ^a	5.80 ± 0.20 ^{bc}	4.48 ± 0.38 ^{def}	3.36 ± 0.30 ^g	2.16 ± 0.34 ^a
8	2.15 ± 0.02 ^a	5.73 ± 0.24 ^{bc}	4.64 ± 0.23 ^{cdef}	4.13 ± 0.10 ^b	3.34 ± 0.28 ^b
9	2.16 ± 0.03 ^a	5.97 ± 0.40 ^{ab}	5.03 ± 0.67 ^b	3.66 ± 0.48 ^{efg}	3.25 ± 0.27 ^b
10	2.14 ± 0.03 ^a	5.68 ± 0.32 ^c	4.70 ± 0.26 ^{bcd}	3.73 ± 0.34 ^{def}	3.10 ± 0.23 ^{bc}
11	2.20 ± 0.04 ^a	5.68 ± 0.16 ^c	4.26 ± 0.42 ^f	3.80 ± 0.43 ^{cde}	3.08 ± 0.41 ^{bc}
12	2.16 ± 0.03 ^a	5.70 ± 0.40 ^{ac}	4.33 ± 0.55 ^{ef}	3.53 ± 0.40 ^{efg}	2.16 ± 0.44 ^a

The degree of significance was set at $P < 0.05$. Means (on the same column and row) with different superscripts are significantly different at $P < 0.05$. This implies that comparison was done on both rows (point of sampling) and columns (among experimental groups). Values are the mean ± standard deviation of 3 results obtained from 3 rats ($n=3$). BCE=*B. coriacea* Crude Ethanol extract, BCA=*B. coriacea* Aqueous leaf fraction, BCZ=*B. coriacea* Ethyl acetate leaf fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/Kg BCE, 5=400 mg/Kg BCE, 6=800 mg/Kg BCE, 7=200 mg/Kg BCA, 8=400 mg/Kg BCA, 9=800 mg/Kg BCA, 10=200 mg/Kg BCZ, 11=400 mg/Kg BCZ, 12=800 mg/Kg BCZ

Table 3: Effect of Ethanol leaf-extract, Aqueous and Ethyl acetate leaf fractions of *B. coriacea* on Malondialdehyde (MDA) level (Nmol/g) of Adjuvant-induced Arthritic Rats

Groups	Day 10	Day 17	Day 24	Day 31
1	6.60 ± 0.27 ^a	6.81 ± 0.19 ^a	6.61 ± 0.58 ^a	6.62 ± 0.42 ^a
2	6.62 ± 0.40 ^a	6.83 ± 0.55 ^a	7.70 ± 0.54 ^a	8.27 ± 0.52 ^a
3	6.68 ± 0.34 ^a	6.20 ± 0.66 ^b	5.68 ± 0.25 ^b	5.70 ± 0.25 ^c
4	6.11 ± 0.61 ^a	6.03 ± 0.83 ^b	5.84 ± 0.08 ^b	5.33 ± 0.30 ^k
5	6.29 ± 0.65 ^a	6.43 ± 0.41 ^a	5.74 ± 0.14 ^c	5.57 ± 0.47 ^f
6	6.35 ± 0.20 ^a	5.68 ± 0.24 ^h	5.70 ± 0.17 ^c	5.54 ± 0.23 ^g
7	6.50 ± 0.34 ^a	5.89 ± 0.16 ^b	5.83 ± 0.11 ^b	5.55 ± 0.17 ^f
8	6.82 ± 0.37 ^a	6.08 ± 0.28 ^b	5.92 ± 0.20 ^b	5.48 ± 0.08 ⁱ
9	6.93 ± 0.15 ^a	5.76 ± 0.13 ^c	5.80 ± 0.12 ^b	5.59 ± 0.10 ^f
10	6.62 ± 0.43 ^a	5.71 ± 0.13 ^h	5.80 ± 0.07 ^b	5.38 ± 0.23 ^j
11	6.76 ± 0.13 ^a	5.77 ± 0.03 ^c	5.74 ± 0.04 ^c	5.67 ± 0.27 ^d
12	6.66 ± 0.09 ^a	5.80 ± 0.29 ^b	5.71 ± 0.11 ^c	5.52 ± 0.21 ^h

The degree of significance was set at $P < 0.05$. Means (on the same column and row) with different superscripts are significantly different at $P < 0.05$. This implies that comparison was done on both rows (point of sampling) and columns (among experimental groups). Values are the mean \pm standard deviation of 3 results obtained from 3 rats ($n=3$). BCE=*B. coriacea* Crude Ethanol extract, BCA=*B. coriacea* Aqueous fraction, BCZ=*B. coriacea* Ethyl acetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ

Table 4: Effect of Ethanol leaf-extract, Aqueous and Ethyl acetate leaf fractions of *B. coriacea* on Nitric oxide (NO) level (Nmol/ml) of Adjuvant-induced Arthritic Rats

Groups	Day 10	Day 17	Day 24	Day 31
1	20.32 ± 1.02 ^a	19.78 ± 0.68 ^a	20.35 ± 0.07 ^a	19.21 ± 0.91 ^b
2	17.41 ± 0.95 ^e	18.23 ± 1.00 ^c	19.42 ± 0.15 ^b	22.01 ± 0.07 ^a
3	19.02 ± 2.10 ^b	18.65 ± 0.48 ^c	17.92 ± 1.13 ^d	17.91 ± 2.20 ^d
4	18.71 ± 0.48 ^c	18.38 ± 0.65 ^c	18.35 ± 0.45 ^c	16.81 ± 0.47 ^h
5	18.45 ± 1.54 ^c	18.31 ± 0.82 ^c	18.39 ± 0.46 ^c	16.63 ± 0.59 ⁱ
6	18.88 ± 0.46 ^d	18.19 ± 1.08 ^c	17.61 ± 0.43 ^c	16.45 ± 0.16 ^j
7	18.79 ± 0.27 ^b	18.66 ± 0.38 ^c	17.99 ± 0.53 ^d	16.34 ± 0.58 ^k
8	18.41 ± 0.55 ^c	18.31 ± 0.83 ^c	17.86 ± 0.70 ^d	16.95 ± 0.27 ^g
9	17.92 ± 0.36 ^d	17.72 ± 0.94 ^d	17.91 ± 0.87 ^d	17.86 ± 0.21 ^d
10	18.64 ± 0.38 ^c	18.30 ± 0.42 ^c	17.98 ± 0.67 ^d	16.72 ± 0.50 ^h
11	18.19 ± 1.70 ^c	18.28 ± 0.86 ^c	17.89 ± 1.59 ^d	17.05 ± 0.36 ^f
12	20.97 ± 0.35 ^a	18.34 ± 9.42 ^c	18.11 ± 1.18 ^c	18.14 ± 0.43 ^c

The degree of significance was set at $P < 0.05$. Means (on the same column and row) with different superscripts are significantly different at $P < 0.05$. This implies that comparison was done on both rows (point of sampling) and columns (among experimental groups). Values are the mean \pm standard deviation of 3 results obtained from 3 rats ($n=3$). BCE=*B. coriacea* Crude Ethanol extract, BCA=*B. coriacea* Aqueous fraction, BCZ=*B. coriacea* Ethyl acetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ

Table 5: Effect of Ethanol leaf-extract, Aqueous and Ethyl acetate leaf fractions of *B. coriacea* on SOD activity (u/mg) of Adjuvant-induced Arthritic Rats

Groups	Day 10	Day 17	Day 24	Day 31
1	23.10 ± 0.98 ^a	23.11 ± 2.08 ^a	23.11 ± 1.29 ^a	23.21 ± 1.91 ^a
2	22.25 ± 1.67 ^c	20.72 ± 0.57 ^d	19.50 ± 0.79 ^e	18.47 ± 1.22 ^c
3	22.23 ± 0.92 ^c	23.07 ± 0.56 ^a	24.56 ± 1.16 ^a	26.00 ± 0.48 ^a
4	21.91 ± 1.01 ^c	22.56 ± 1.04 ^c	22.80 ± 0.45 ^b	24.21 ± 0.70 ^a
5	20.91 ± 1.93 ^d	21.96 ± 0.77 ^c	21.41 ± 0.52 ^c	25.21 ± 0.43 ^a
6	22.13 ± 1.51 ^c	21.51 ± 0.18 ^c	21.64 ± 0.83 ^d	22.88 ± 0.73 ^b
7	22.16 ± 0.22 ^c	22.53 ± 1.41 ^c	23.42 ± 1.36 ^a	25.88 ± 0.72 ^a
8	21.14 ± 1.17 ^d	21.67 ± 0.75 ^c	22.30 ± 0.38 ^d	23.48 ± 0.23 ^a
9	21.88 ± 0.66 ^c	22.20 ± 1.09 ^c	24.21 ± 0.56 ^a	26.17 ± 0.32 ^a
10	21.44 ± 1.54 ^d	22.17 ± 0.14 ^c	24.28 ± 0.70 ^a	26.41 ± 1.09 ^a
11	20.91 ± 1.61 ^d	21.55 ± 0.89 ^d	23.41 ± 0.16 ^a	26.48 ± 0.42 ^a
12	20.47 ± 1.18 ^d	20.63 ± 0.09 ^d	23.03 ± 1.65 ^a	25.28 ± 0.26 ^a

The degree of significance was set at $P < 0.05$. Means (on the same column and row) with different superscripts are significantly different at $P < 0.05$. This implies that comparison was done on both rows (point of sampling) and columns (among experimental groups). Values are the mean ± standard deviation of 3 results obtained from 3 rats ($n=3$). BCE=*B. coriacea* Crude Ethanol extract, BCA=*B. coriacea* Aqueous fraction, BCZ=*B. coriacea* Ethyl acetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ

Table 6: Effect of Ethanol leaf-extract, Aqueous and Ethyl acetate leaf fractions of *B. coriacea* on Catalase activity (u/mg) of Adjuvant-induced Arthritic Rats

Groups	Day 10	Day 17	Day 24	Day 31
1	67.67 ± 3.02 ^a	67.84 ± 1.26 ^a	66.52 ± 2.03 ^a	66.63 ± 5.57 ^a
2	58.49 ± 2.73 ^c	55.79 ± 0.41 ^d	52.21 ± 4.34 ^f	45.62 ± 3.16 ^g
3	57.48 ± 2.46 ^d	59.21 ± 1.14 ^b	58.42 ± 2.66 ^e	61.60 ± 1.41 ^a
4	53.25 ± 2.77 ^f	54.19 ± 1.66 ^e	57.31 ± 0.80 ^d	59.84 ± 1.12 ^a
5	54.59 ± 3.25 ^d	55.63 ± 2.35 ^d	57.92 ± 4.32 ^d	59.66 ± 3.22 ^a
6	55.16 ± 2.51 ^d	56.04 ± 1.10 ^d	55.68 ± 1.25 ^d	60.44 ± 0.63 ^a
7	55.44 ± 2.20 ^d	57.57 ± 0.74 ^d	57.58 ± 2.16 ^d	57.99 ± 2.41 ^d
8	55.56 ± 3.13 ^d	57.52 ± 2.52 ^d	56.52 ± 1.50 ^d	58.21 ± 2.28 ^c
9	55.59 ± 0.54 ^d	55.32 ± 2.38 ^d	56.56 ± 4.36 ^d	62.14 ± 1.55 ^a
10	53.90 ± 3.64 ^e	55.95 ± 0.30 ^d	55.61 ± 3.06 ^d	59.65 ± 1.96 ^a
11	54.20 ± 2.74 ^e	54.61 ± 1.32 ^d	56.24 ± 0.97 ^d	59.35 ± 4.88 ^a
12	57.25 ± 2.84 ^d	57.30 ± 2.39 ^d	58.97 ± 2.54 ^c	59.81 ± 1.76 ^a

The degree of significance was set at $P < 0.05$. Means (on the same column and row) with different superscripts are significantly different at $P < 0.05$. This implies that comparison was done on both rows (point of sampling) and columns (among experimental groups). Values are the mean ± standard deviation of 3 results obtained from 3 rats ($n=3$). BCE=*B. coriacea* Crude Ethanol extract, BCA=*B. coriacea* Aqueous fraction, BCZ=*B. coriacea* Ethyl acetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ

Table 7: Effect of Ethanol leaf-extract, Aqueous and Ethyl acetate leaf fractions of *B. coriacea* on glutathione peroxidase (GPx) activity (u/l) of Adjuvant-induced Arthritic Rats

Groups	Day 10	Day 17	Day 24	Day 31
1	23.64 ± 1.18 ^b	24.37 ± 0.72 ^a	24.43 ± 2.66 ^a	23.89 ± 0.24 ^b
2	21.61 ± 2.16 ^b	20.76 ± 0.41 ^d	19.51 ± 2.70 ^e	18.21 ± 0.66 ^f
3	20.43 ± 0.69 ^c	21.38 ± 0.51 ^b	22.26 ± 2.47 ^b	22.31 ± 0.85 ^b
4	20.63 ± 0.42 ^c	21.42 ± 1.11 ^b	22.31 ± 1.04 ^b	21.58 ± 0.17 ^b
5	19.82 ± 1.23 ^e	20.71 ± 0.56 ^c	21.83 ± 1.02 ^b	21.55 ± 0.59 ^b
6	20.19 ± 0.21 ^d	20.63 ± 0.66 ^c	21.16 ± 0.38 ^b	22.05 ± 1.21 ^b
7	20.73 ± 1.02 ^c	21.07 ± 0.55 ^b	21.96 ± 0.62 ^b	22.21 ± 0.39 ^b
8	20.91 ± 0.64 ^b	21.08 ± 0.54 ^b	21.30 ± 0.52 ^b	22.09 ± 0.61 ^b
9	20.15 ± 0.98 ^d	21.23 ± 0.46 ^b	21.52 ± 1.42 ^b	22.84 ± 0.98 ^b
10	21.23 ± 0.78 ^b	20.51 ± 0.18 ^c	21.64 ± 1.35 ^b	21.64 ± 0.90 ^b
11	21.72 ± 1.67 ^b	20.76 ± 0.55 ^c	21.26 ± 0.13 ^b	22.70 ± 1.06 ^b
12	21.31 ± 0.62 ^b	21.04 ± 0.47 ^b	21.36 ± 0.80 ^b	22.50 ± 0.59 ^b

The degree of significance was set at $P < 0.05$. Means (on the same column and row) with different superscripts are significantly different at $P < 0.05$. This implies that comparison was done on both rows (point of sampling) and columns (among experimental groups). Values are the mean ± standard deviation of 3 results obtained from 3 rats ($n=3$). BCE=*B. coriacea* Crude Ethanol extract, BCA=*B. coriacea* Aqueous fraction, BCZ=*B. coriacea* Ethyl acetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ

Table 8: Effect of Ethanol leaf-extract, Aqueous and Ethyl acetate leaf fractions of *B. coriacea* on reduced glutathione (GSH) level (Umol/l) of Adjuvant-induced Arthritic Rats

Groups	Day 10	Day 17	Day 24	Day 31
1	26.45±2.37 ^a	26.82±0.59 ^a	26.760±0.03 ^a	25.99±0.08 ^a
2	22.88±0.71 ^d	19.18±0.62 ^h	16.36±0.18 ⁱ	10.17±1.43 ^d
3	18.57±0.86 ^b	21.99±0.39 ^f	22.03±0.41 ^e	22.38±0.76 ^c
4	17.09±0.24 ⁱ	20.42±0.50 ^g	23.80±0.59 ^c	24.82±1.21 ^a
5	15.60±0.25 ^j	19.31±0.33 ^h	22.94±0.58 ^d	25.54±0.33 ^a
6	15.91±0.61 ^j	20.12±1.54 ^g	22.34±1.80 ^d	25.62±0.70 ^a
7	15.26±0.55 ^e	22.53±0.34 ^d	23.54±0.40 ^c	24.51±0.57 ^a
8	16.27±0.97 ⁱ	22.72±0.32 ^d	22.85±0.14 ^d	25.16±1.46 ^a
9	16.27±0.20 ⁱ	21.77±0.89 ^f	24.02±0.38 ^b	25.12±0.88 ^a
10	15.46±0.66 ^j	23.22±1.48 ^c	25.14±0.15 ^a	25.48±0.53 ^a
11	15.22±0.43 ^j	22.40±1.63 ^d	22.95±0.73 ^d	23.70±1.11 ^b
12	15.29±0.90 ⁱ	21.91±1.03 ^f	23.60±0.70 ^c	24.36±0.65 ^a

The degree of significance was set at $P < 0.05$. Means (on the same column and row) with different superscripts are significantly different at $P < 0.05$. This implies that comparison was done on both rows (point of sampling) and columns (among experimental groups). Values are the mean ± standard deviation of 3 results obtained from 3 rats ($n=3$). BCE=*B. coriacea* Crude Ethanol extract, BCA=*B. coriacea* Aqueous fraction, BCZ=*B. coriacea* Ethyl acetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ

Discussion

In the first week following induction of arthritis, all adjuvant-induced arthritic rats showed a significant ($P < 0.05$) decrease in the body weight relative to rats in normal control group. A significant ($P < 0.05$) increase in the body weight of rats was observed in all the treated groups while progressive weight loss was observed in the untreated-arthritic group till the end of the study. Our present results are in agreement with previous studies that showed that Complete Freund's Adjuvant (CFA)-injected rats showed decreases in body weight relative to non-arthritic rats (41). Administration of CFA leads to increase in leptin level, anorexia and weight loss (42). In this study, we speculate that the cause of the decrease in body weight on injection of CFA might be due to increase in leptin level in the arthritic rats. Leptin is a hormone secreted by fat cells and is known for suppressing hunger signals, also has influences on the immune system. Elevated level of leptin contributes to chronic inflammation by up-regulating inflammatory cytokines (like tumor necrosis factor- α , TNF- α ; interleukins, IL-1 β , and IL-6) (43). Elevated levels of pro-inflammatory cytokines could exert a strong effect on protein and energy metabolism by promoting muscle breakdown. Increased catabolism leads to resting energy expenditure culminating to weight loss and reduced lean body mass (44).

Inflammation can also cause a decrease in absorption capacity of the intestine. Elmali *et al.* (2005) (45) reported a restoration of absorption capacity of the intestine upon treatment with anti-inflammatory drugs. Thus, increased body weight of the arthritic rats during the course of treatment with an anti-inflammatory drug (indomethacin) and varied doses of the extract and fractions could be due to the reduction of the inflammatory cytokines and subsequent decrease in protein and muscle breakdown. It could also be due to the restoration of absorption capacity of the intestine.

Edema is one of the fundamental actions of acute inflammation and is an essential parameter to be considered when evaluating compounds with potential anti-inflammatory activity (46, 47). In this study, there was a two-fold increase in paw size in the feet of rats injected with Freund's adjuvant. A significant ($P < 0.05$) reduction in paw size of rats treated with standard drug, ethanol leaf-extract, aqueous and ethyl acetate leaf fractions of *B. coriacea* at varied, was observed.

Previous authors have also reported a significant reduction in paw size of rats on treatment with medicinal plants (48, 49).

Oxidative stress is a situation in the biological science in which there is an imbalance between oxidants and antioxidants in favor of the oxidants, culminating in interference of redox signaling and control and/or molecular injury (50). In normal physiological processes, reactive oxygen species (ROS) are formed and they play crucial functions in cell signaling and tissue homeostasis (51). Nevertheless, their excessive production culminates in severe alterations to cell components and augment various pathogenesis, such as lipids, proteins, and DNA damage (52). Sequel to high level of polyunsaturated fatty acids (PUFAs) in cellular membranes or organelle membrane, they are prone to ROS damage, which is referred to as lipid peroxidation. Thus, lipid peroxidation is a process that involves the removal of electrons from lipids by free radical species such as oxyl radicals, peroxy radicals, and hydroxyl radicals leading subsequently to the production of reactive intermediates that can undergo further reactions. This causes damage to phospholipids and function as cell death signal which induces programmed cell death. Hence, oxidized phospholipid mediates important function in several inflammatory disorders and frequently mediate proinflammatory alteration (53).

There exist three major classes of antioxidant enzymes in all body cells which include the catalases, superoxide dismutases (SOD), and glutathione peroxidases (GPX). These enzymes play vital functions in cells' homeostasis. Their induction is a reflection of specific response to pollutant oxidative stress (54). These enzymic and non-enzymic antioxidants such as glutathione (GSH) have the ability to prevent or retard the oxidation of macromolecules. These antioxidants retard or terminate these chain reactions by mopping up free radicals or via inhibition of other oxidation reactions by being oxidized themselves (55).

Chemical composition analysis of *B. coriacea* has been investigated by previous studies. Phytochemicals present in *B. coriacea* ethanol leaf-extract and fractions are terpenoids, phenols, alkaloids, flavonoids, tannins, saponins and steroids (56). Other authors have also corroborated this finding (57, 58, 59, 60, 61). Phytochemicals can act as antioxidants (e.g, flavonoids, alkaloids, tan-

nins, saponins and terpenoids) antiproliferative and anti-inflammatory compounds for prevention of chronic diseases (62, 63).

The results of our study revealed increased oxidative stress in adjuvant-induced arthritic rats as evidenced by increased lipid peroxidation product-MDA and NO production, and impaired enzymatic and non-enzymatic antioxidant defense system of the body (Tables 3- 8). The raised MDA (a consequence of increased extent of lipid peroxidation) might be due to the increased formation of ROS which tends to increase abundantly during chronic inflammation and could result to damage to tissues. Other authors have also reported increase in MDA in liver and brain of rats with arthritis (64, 65, 66). Elevated level of MDA has been found in the serum, plasma and erythrocytes of RA patients (13, 67). Administration of standard drug and samples reduced levels of MDA as shown in Table 3.

Some functions of NO include immune response, neural communication and blood pressure maintenance (68). In this study, increased NO level portends oxidative stress and this corroborates previous findings as reported by other authors (64, 13, 67). However, Veselinovic *et al.* (69) reported unaltered level of NO in the plasma of RA patients. The increase in the level of NO in the plasma of arthritic rats might be due to the hyperactivity of the NO forming enzyme, nitric oxide synthase (70). The observed reduced SOD activity might be due to its depletion caused by increased oxidative stress that occur during inflammatory process.

This decreased erythrocyte SOD activity is in agreement with other studies as well (13, 71, 72, 73, 74, 75). However, increased (69, 76), or even unaltered SOD activity (77) has also been reported by some groups.

Catalase is an enzyme that catalyses the conversion of hydrogen peroxide into water and oxygen. This protects cells from harmful effects that accumulated hydrogen peroxide could have caused. From our result, catalase activity is lowered in arthritic rats and this might be as a result of catalase being used up by hydrogen peroxide.

Diminished catalase activity in brain and liver of arthritic rats have been reported by previous authors (65, 66). This result is in tandem with other findings using human beings (54, 13, 71, 74). However, some groups have also reported unaltered catalase activity in RA patients (64, 75, 78, 72).

We also observed a significant decrease in GPx activity in the adjuvant-induced arthritic rats (Table 7). This is in line with the findings of other studies using humans (79, 73, 72). However, two studies reported an increase on GPx activity in RA patients (80, 76). However, three study groups did not report any differences between cases and controls in GPx activity in RA patients (75, 78, 74).

We noticed significantly low levels of GSH in arthritic rats as compared to the control rats (healthy rats) (Table 8). GSH is a sulfhydryl molecule that acts as a defense system in the body. It functions as an intracellular reductant in redox reactions taking place in the human body by protecting cellular components from damage caused by ROS. The observed low level of GSH in plasma of arthritic rats has also been reported by previous authors (64, 75, 79, 74, 81, 82, 13). Veselinovic *et al.* (69) reported that GSH level was unchanged in RA patients. This contradicting report by various authors could be due to differences in RA severity and response to treatments by rats/or patients.

ROS are highly reactive chemical species that have the potential to damage lipids, proteins and deoxyribonucleic acid (DNA) in joint tissues. ROS are required in maintaining redox state of cells. Other functions include cell signaling, differentiation, proliferation, growth, apoptosis and phagocytosis. However, if the concentrations of ROS are increased beyond physiological conditions they can damage macromolecules like lipids in the cell membranes, proteins and nucleic acids (83, 81). Oxidative stress results if the concentration of oxidants is higher than antioxidants (84). Under this condition, redox signaling is disrupted leading to macromolecular damage (85, 86, 87). The damaging ROS is annulled by the action of antioxidants. Enzymatic antioxidant response is carried out by SOD, catalase, GPx, glutathione reductase and transferase while non-enzymatic antioxidant includes the action of vitamins (e.g. A, C, and E), β -carotene, some minerals (e.g. copper, zinc, manganese, and selenium), GSH and some phytochemicals (e.g. flavonoids, terpenoids, alkaloids) (85, 64).

A large number of authors reported that ROS are implicated in inflammation and destruction in the joints of arthritic animals and RA patients (64, 88, 13, 85, 89, 90, 91). Oxidative stress is implicated in damaging of joints due to RA (87). There is strong evidence that ROS are highly involved in cartilage degradation in experimental arthritic rats (92).

Oxidative stress can also impair DNA mismatch repair mechanism which may result to an increase in the formation of DNA adducts in the joints thereby aggravating the disease symptoms (93). Phytochemicals serve as sources of natural compound for developing novel drugs. This is because of their antioxidant and anti-inflammatory roles in management of inflammatory diseases such as RA. The presence of antioxidant minerals (e.g., copper, zinc, manganese, and selenium) and vitamins (A, C and E) in *B. coriacea* is well-documented (56, 94). Thus, the anti-RA effect exerted by the samples could be attributed to the action of these antioxidants present in the plant. However, limitation of this study is the small number of rats sampled at each point.

Conclusion

Induction of RA caused oxidative stress evidenced by elevation of MDA and NO and diminished activities of antioxidants: SOD, GPx, Catalase and GSH. Administration of samples to rats led to decrease in oxidative stress and boosting of the antioxidants. This study has demonstrated *in vivo* the therapeutic potential of leaf extract and ethyl acetate fraction of *B. coriacea* at curbing oxidative stress and hence a potential alternative to synthetic drugs in the management of oxidative stress in RA patient.

Ethics approval and consent to participate was adequately sought.

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ANTIOKSIDATIVNI UČINEK ETANOLNEGA IZVLEČKA IN FRAKCIJ LISTOV *Buchholzia coriacea* NA FREUNDOV ADJUVANTNI ARTRITIS PRI ALBINO PODGANAH: PRIMERJALNA ŠTUDIJA

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Izveček: V več študijah je bil nakazan pomen reaktivnih kisikovih zvrsti pri ohranjanju vnetja in poznejšem uničenju sklepov pri pacientih z osteoartritisom. Proučevali smo antioksidativne učinke etanolovega izvlečka ter vodnih in etil acetatnih frakcij listov *Buchholzia coriacea* na kazalce oksidativnega stresa pri samih albin podgan Wistar z revmatoidnim artritisom. 180 podgan smo naključno porazdelili v 12 skupin. V vsaki skupini je bilo 15 podgan. Podgane brez artritisa so bile uvrščene v skupino 1. V skupinah 2 do 12 so bile podgane z revmatoidnim artritisom, povzročnim z intradermalno injekcijo 0,1 ml Freundovega kompletnega adjuvansa v zadnjo levo tace podgan. Skupina 2 (podgane z artritisom) ni bila zdravljena, prejela je fiziološko raztopino, skupina 3 (podgane z artritisom) pa je prejela 5 mg/kg indometacinaTM (standardno zdravilo). Podgane v skupinah 4 do 12 so prejele vzorce adjuvansa v odmerkih 200, 400 in 800 mg/kg telesne mase. Aplikacija Freundovega adjuvansa je povzročila vnetje in oksidativni stres, kar se je kazalo v značilnem ($p < 0.05$) povišanju velikosti tac in kazalcev oksidativnega stresa ter zmanjšanju telesne teže podgan. Podgane z artritisom so bile zdravljene s standardnim zdravilom in vzorci adjuvansa (v različnih odmerkih), kar je vodilo v obrat trenda teh parametrov v odvisnosti od časa in odmerka. Najboljši rezultat, podoben učinku indometacina, je bil pri podganah, ki so prejele 800 mg/kg vodne frakcije adjuvansa. Etanolni izvleček in frakcije listov *Buchholzia coriacea* bi zato lahko bili uporabni pri obvladovanju oksidativnega stresa, ki se zelo pogosto pojavlja pri posameznikih z revmatoidnim artritisom.

Ključne besede: revmatoidni artritis; *Buchholzia coriacea*; prosti radikali; oksidativni stres; reaktivne kisikove zvrsti