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Brenania brieyi Root Bark Extracts Ameliorate Chronic Inflammation-Mediated Oxidative Stress in Rats †

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Abstract: Oxidative stress is implicated in the pathogenesis of many chronic diseases. This study investigated the effect of methanol and chloroform extracts of root bark of *Brenania brieyi* on inflammation-induced oxidative stress in rats. Cotton pellet-induced inflammatory model was used to induce oxidative stress. The rats were treated with varying concentrations of each extract and indomethacin (standard drug) for 7 days. On day 8, their blood samples collected by cardiac puncture was used for the determination of biochemical parameters of oxidative stress such as the malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities along with reduced glutathione (GSH), vitamins C, and E. using standard methods. The bioactive compounds responsible for bioactivity were determined with gas chromatography-mass spectrometry (GC-MS) techniques. Results obtained showed a significant ($p < 0.05$) decreases in malondialdehyde level, an increase in superoxide dismutase and catalase activities, reduced glutathione with a significant increase in vitamin C in groups 6 and 9 was observed in the extracts treated groups compared with the untreated group. A total of sixteen bioactive compounds with known antioxidant and anti-inflammatory activities were identified in the extracts with 9-Ocadecenoic acid being the most abundant. The result of this study suggests that *Brenania brieyi* has antioxidant activities, and hence could be used in the management and treatment of oxidative stress-related diseases.

Keywords: *Brenania brieyi*; oxidative stress; antioxidants activity; anti-inflammatory activity

1. Introduction

Inflammation protects the body from injuries caused by chemicals, mechanical or thermal stimuli, trauma, microbial agents, or autoimmune diseases [1]. In a normal state, inflammation is usually resolved to prevent tissue damage. However, in chronic inflammatory disorders and granuloma condition, there is an excessive inflammatory response characterized by the presence of activated, and dysregulated mediators, and cells associated with the release of reactive oxygen species (ROS,) and reactive nitrogen species (RNS) from activated neutrophils, and macrophages [2,3].

Paradoxically, the balance between the production, and neutralization of ROS, and RNS by antioxidants is very delicate, and if this balance tends to overproduction of ROS or RNS, the cells

start to suffer the consequences of oxidative stress, which is implicated in the pathogenesis of chronic diseases [4]. Management and treatment options for some of these diseases have severe side effects such as gastrointestinal bleeding, ulcer, and opportunistic infections due to immune suppression [2] Recently, there is intensified research in the exploration of natural agents which have high therapeutic value, and biocompatibility [5]. Medicinal plants contain an assortment of compounds with promising biological and pharmacological activities.

Brenania brieyi is used in traditional medicine for the treatment of fever, pain, swelling, and endocrine disorders [6]. Most of the plants used by traditional herbalists in the treatment of ailments are used without scientific investigation. Given all the various trado-medical applications of *B. brieyi*, and the numerous side effects associated with the use of synthetic inflammatory drugs, methodical investigation of the antioxidant potential of *B. brieyi* could help in the search for newer, cheaper, and safer alternative drug for the management, and treatment of several ailments, especially oxidative stress-related diseases. This work was therefore aimed at evaluating the effects of methanol, and chloroform extracts of *B. brieyi* root bark against inflammation-mediated oxidative stress in Wistar rats.

2. Materials and Methods

2.1. Collection and Authentication of Plant Materials

Brenania brieyi root bark used for this research was sourced from Anambra State, Nigeria in 2015. The root bark was identified by Mr. Felix Nwafor, a taxonomist from the Pharmacognosy Department, University of Nigeria Nsukka, Nigeria where a voucher specimen (identification number PCG/UNN/0327) was deposited. Extraction of the shade dried and pulverized sample was done by cold maceration in methanol and chloroform for 48 h with intermittent shaking. This was followed by filtration with cheesecloth and Whatman No. 4 filter paper. The filtrate was separated into two layers with 20% *v/v* distilled water after which a separating funnel was used to separate the two layers. Each layer was dried with a rotary evaporator at 45 °C and stored in a refrigerator.

2.2. Chemicals and Instruments

Chemical used in this research work were of analytical grades. They were sourced from reputable companies: British Drug Houses, England, Sigma-Aldrich Inc., UK, Teco USA, and Evans Pharmaceutical, England.

2.3. Ethics Approval

Ethical approval on the use of laboratory animals was obtained from the committee of the University of Nigeria, Nsukka on the care and use of laboratory animals, in accordance to the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23, revised 1985).

2.4. Induction of Inflammation

Adult male Wistar rats (forty-five) divided into nine groups of five rats each were implanted cotton pellets according to the method of Mosquera et al. [7] except one group which served as a baseline (group 1). Group 1 was not implanted with pellet and given distilled water, group 2 was implanted, and given distilled water (cotton pellet control), group 3 received 10 mg/kg body weight (b. w) of indomethacin (standard control), groups 4–6 were administered 50, 100, and 200 mg/kg b. w of methanol extract respectively while groups 7–9 were also given the same doses of chloroform extract. Treatment lasted for seven days. The animals were anesthetized on the 8 days with chloroform, and blood samples used for the determination of antioxidant parameters were collected through cardiac puncture.

2.5. Biochemical Studies

The following methods were used to investigate biochemical parameters of oxidative stress: Malondialdehyde [8], superoxide dismutase [9], catalase [10], glutathione peroxidase [11], reduced glutathione [12], vitamin E [13], vitamin C [14], while GC-MS analysis was done with GC-MS analyzer (GC-MS-QP 2010 plus Shimadzu, Japan).

2.6. Statistical Analysis

Statistical product and service solutions (SPSS) for windows version 18.0 was used to analyze data obtained. One-way analysis of variance (ANOVA) and *post hoc* multiple comparisons were used to determine differences between the mean values obtained.

3. Results and Discussion

3.1. Effects of the Methanol, and Chloroform Extracts of the Root Bark of B. brieyi on the MDA Level

Oxidative stress plays a very important role in the pathophysiological development of several diseases [15]. Treatment with our extracts and indomethacin reduced the MDA level significantly compared with group 2. Though, all the groups treated with both extracts had lower MDA levels than the indomethacin group, indicating higher inhibition of lipid peroxidation in extracts treated groups (Figure 1). The reduction in MDA level, a known lipid peroxidation index [16], in the extracts treated groups indicates the ability of the extracts to stem down oxidative stress by inhibiting lipid peroxidation in inflammation. This will in turn help in the prevention of health challenges associated with lipid peroxidation. It could be possible that antioxidants nutrients present in the extracts scavenged free radicals thereby preventing initiation of lipid peroxidation or reduced peroxy radicals to hydroperoxide before it can propagate the radical chain.

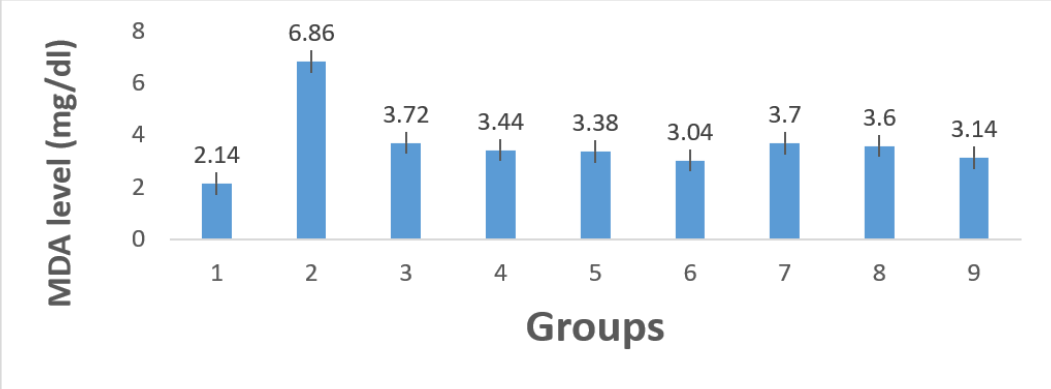

Figure 1. Malondialdehyde level of rats implanted with a cotton pellet.

3.2. Effects of the Methanol and Chloroform Extracts of the Root Bark of B. brieyi on Antioxidant Enzymes Activities

A decrease in antioxidant content may predispose the cells to oxidative stress during several degenerative diseases. Interestingly, the activities of SOD and CAT were significantly ($p < 0.05$) restored in the serum of rats following treatment with all the varying doses of both methanol and chloroform extract of *B. brieyi* when compared with group 2. However, a non-significant ($p > 0.05$) increase in GPx activity was observed in all the extracts treated groups compared with group 2. The effect of the standard drug, indomethacin was comparable with that of the extracts (Table 1). SOD catalyzes the conversion of superoxide ion (O_2^-) radical to O_2 and H_2O_2. H_2O_2 is later acted upon by catalase which transforms it into H_2O [16]. Considering the role antioxidants play in reactive oxygen species deactivation [17], restoration of these antioxidant enzymes unveiled the extract's potential in

mopping up or scavenging free radicals generated under oxidative stress-mediated by cotton pellet implantation. This might be attributed in part to the ability of the extracts to act as a scavenger of free radical products of granular inflammation, performed synergistic roles, or might have played a role in enhancing the synthesis of the antioxidant enzymes which in turn protected the cells from reactive species.

Table 1. Effects of the methanol and chloroform extracts of *B. brieyi* on serum antioxidant enzymes activities of rats implanted with a cotton pellet.

Groups	Treatment	Doses	Superoxide Dismutase (u/L)	Catalase (u/L)	Glutathione Peroxidase (u/L)
1	Normal rats	-	11.46 ± 0.05 ^{c}	3.64 ± 0.16 ^{d}	0.50 ± 0.07 ^{c}
2	Control (N. S)	I mL/kg b. w.	11.34 ± 0.06 ^{a}	1.36 ± 0.15 ^{a}	0.36 ± 0.05 ^{a}
3	Indomethacin	10 mg/kg b. w.	11.40 ± 0.03 ^{bc}	1.80 ± 0.24 ^{ab}	1.80 ± 0.24 ^{ab}
4	Methanol Ext.	50 mg/kg b. w.	11.39 ± 0.04 ^{b}	2.12 ± 0.06 ^{bc}	0.38 ± 0.03 ^{ab}
5		100 mg/kg b. w.	11.42 ± 0.03 ^{bc}	2.18 ± 0.27 ^{bc}	0.40 ± 0.07 ^{ab}
6		200 mg/kg b. w.	11.42 ± 0.02 ^{bc}	3.30 ± 0.06 ^{d}	0.44 ± 0.05 ^{abc}
7	Chloroform Ext.	50 mg/kg b. w.	11.41 ± 0.21 ^{b}	2.16 ± 0.17 ^{bc}	0.36 ± 0.05 ^{a}
8		100 mg/kg b. w.	11.42 ± 0.03 ^{bc}	2.58 ± 0.20 ^{c}	0.38 ± 0.04 ^{ab}
9		200 mg/kg b. w.	11.43 ± 0.02 ^{bc}	2.68 ± 0.30 ^{c}	0.44 ± 0.06 ^{abc}

Values are expressed as mean ± SD (n = 5). Mean values with different letters of the alphabet down the column differed significantly ($p < 0.05$) while mean values with the same letters of the alphabet down the column are not significantly different ($p > 0.05$).

3.3. Effects of the Methanol and Chloroform Extracts of the Root Bark of B. brieyi on Non-Enzymatic Antioxidant Levels of Rats Implanted with a Cotton Pellet

Natural products are key players in maintaining the redox state in the body, cell signaling mediators, and inhibitors of cell proliferation [18]. As is evident in Table 2, treatment with both extracts and indomethacin had significant ($p < 0.05$) restoration in GSH level in groups 3–9 when compared with group 2, with groups 5 and 6 treated with 100 and 200 mg/kg of methanol extract respectively, having restoration in GSH level which is comparable with group 1. In the same vein, groups 6 and 9 had a significant ($p < 0.05$) increase in vitamin C level relative to group 2 while only groups 6 and 8 had higher ($p < 0.05$) vitamin E compared with normal control. The possible reason for the increase in these antioxidants as a result of treatment with the extracts might be that the plant's antioxidants complemented or induced synthesis/or release of antioxidant enzymes which could have work in synergy with the endogenous antioxidants. The increase in reduced glutathione by the extracts could have helped in scavenging radicals since glutathione plays a vital role in protecting the cells against free radical-induced injuries [15]. This further buttresses the antioxidant potential of the plant which could be attributed to bioactive compounds found in the extract as reported by Odo et al. [19]. Thus, the antioxidant property of the extracts might confer an additional therapeutic benefit in averting diseases. Since studies have shown that maintaining redox balance is the basis for preventing metabolic diseases [20].

Table 2. Effects of the methanol and chloroform extracts of root bark of *B. brieyi* on serum non–enzymatic antioxidant concentrations of rats implanted with a cotton pellet.

Groups	Treatment	Doses	Glutathione (mg/dL)	Vitamin C (mg/dL)	Vitamin E (mg/dL)
1	Normal rats	-	2.62 ± 0.08 ^{f}	1.42 ± 0.10 ^{d}	0.62 ± 0.02 ^{cd}
2	Control (N. S)	I mL/kg b. w.	0.68 ± 0.08 ^{a}	1.02 ± 0.04 ^{a}	0.42 ± 0.03 ^{a}
3	Indomethacin	10 mg/kg b. w.	1.14 ± 0.18 ^{b}	1.06 ± 0.05 ^{a}	0.56 ± 0.04 ^{abcd}
4	Methanol Ext.	50 mg/kg b. w.	2.24 ± 0.05 ^{e}	1.08 ± 0.08 ^{ab}	0.46 ± 0.07 ^{ab}
5		100 mg/kg b. w.	2.54 ± 0.13 ^{f}	1.12 ± 0.04 ^{ab}	0.52 ± 0.03 ^{abc}
6		200 mg/kg b. w.	2.58 ± 0.13 ^{f}	1.22 ± 0.08 ^{c}	0.70 ± 0.04 ^{d}
7	Chloroform Ext.	50 mg/kg b. w.	1.58 ± 0.05 ^{c}	1.04 ± 0.05 ^{a}	0.56 ± 0.05 ^{abcd}
8		100 mg/kg b. w.	2.00 ± 0.12 ^{d}	1.04 ± 0.11 ^{a}	0.60 ± 0.05 ^{bcd}
9		200 mg/kg b. w.	2.22 ± 0.16 ^{e}	1.18 ± 0.04 ^{bc}	0.56 ± 0.02 ^{abcd}

Values are expressed as mean ± SD (n = 5). Mean values with different letters of the alphabet down the column are significantly different ($p < 0.05$) while mean values with the same letters of the alphabet down the column are not significantly different ($p > 0.05$).

3.4. Compounds Identified in the Root Bark of B. brieyi with GC-MS

Plants are endowed with numerous compounds with pharmaceutical activities. A total of eight compounds were identified in the methanol extract while ten compounds were detected in chloroform extract. Some of the identified compounds such as pentadecanoic acid, octadecanoic acid, eicosanoic acid, tetradecanoic acid, hexadecenoic acid, 9,12-octadecanoic acid, and squalene have already been reported to have antioxidant/anti-inflammatory activities [19]. Hence, the efficacy of the extracts in ameliorating oxidative stress under inflammatory response could be attributed highly to the presence of his phytonutrients.

Table 3. Compounds identified in the root bark of *B. brieyi* with GC-MS.

S/N	Methanol Extract	Chloroform Extract
1.	Decanoic acid	Undecanoic acid
2.	Pentadecanoic acid	Tetradecanoic acid
3.	9, 12-hexadecadienoic acid	14-methylpenta decenoate
4.	9-ocatadecenoic acid	Hexadecanoic acid
5.	Octadecanoic acid	9,12-Octadecadienoic acid
6.	Eicosanoic acid	9-Octadecenoic acid
7.	6,9-pentadecadien-1-ol	Ocadecanoic acid
8.	4, 8, 12 -tetradecatrienal	Nonadecanoic acid
9.		6,9-Pentadecadien-1-ol
10.		Squalene

4. Conclusions

The findings from this research work unveiled the potency of *Brenania brieyi* in ameliorating inflammation-induced oxidative stress. The extracts inhibited peroxidation of biomembrane, and also restored the endogenous antioxidant status of the inflamed rats. Thus, it could be applied as a therapeutic agent in preventing the onset or management of oxidative stress-related diseases/disorders.

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Conflicts of Interest: There is no conflict of interest to declare.

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