Hydroethanolic Extract of *Buchholzia coriacea* Seeds Alleviates LPS Induced Liver Injury in Rat via Antioxidant and Anti-inflammatory Actions

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**Authors’ contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/JOCAMR/2019/v8i230116

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Complete Peer review History: [http://www.sdiarticle4.com/review-history/50490](http://www.sdiarticle4.com/review-history/50490)

**ABSTRACT**

**Aim:** This study evaluates the hepatoprotective effects of hydroethanolic extract of (defatted) *Buchholzia coriacea* seed (HEBCS) against lipopolysaccharide (LPS) induced inflammatory liver injury in the rat.

**Method:** Thirty male albino rats (120–130 g) were assigned into five groups (n=6/ group). Group I (Control) and II (LPS) received distilled water orally (p.o.) for seven days while animals in groups III, IV and V were administered HEBCS at 125, 250 and 500 mg/kg body weight (b.w.) p.o. daily for 7 days. LPS was injected on the 7th day at a single dose of 4 mg/kg intraperitoneally (i.p.) to the rats in group II, and groups III, IV and V. Six hours after LPS injection, blood was collected to prepare serum, and liver was removed for preparation of homogenate, histopathology and

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immunofluorescence staining. Biomarkers of liver function (ALT, AST and ALP) and oxidative stress (NO, SOD, CAT and MDA) were assessed. Pro-inflammatory cytokine (TNF-α) and inducible protein (C-reactive protein) were also assessed.

**Results:** Results showed significant amelioration of liver function markers and levels of pro-inflammatory proteins by HEBCS. Histopathological studies showed a reduction in inflammatory cells and improvement in liver structure in animals treated with HEBCS.

**Conclusion:** Data from the current study show that HEBCS possesses hepatoprotective effects against LPS induced inflammatory liver injury.

**Keywords:** Lipopolysaccharide; Buchholzia coriacea; liver; antioxidants; inflammation; immunofluorescence; rat.

### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ALD</td>
<td>Acute liver disease;</td>
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<tr>
<td>ALF</td>
<td>Acute liver failure;</td>
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<tr>
<td>ALP</td>
<td>Alkaline phosphatase;</td>
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<tr>
<td>ALT</td>
<td>Alanine aminotransferase;</td>
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<tr>
<td>AST</td>
<td>Aspartate aminotransferase;</td>
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<tr>
<td>CAT</td>
<td>Catalase;</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase 2;</td>
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<tr>
<td>CRP</td>
<td>c-reactive protein;</td>
</tr>
<tr>
<td>DAPI</td>
<td>4′,6-diamidino-2-phenylindole;</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay;</td>
</tr>
<tr>
<td>EIOH</td>
<td>Ethanol;</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione;</td>
</tr>
<tr>
<td>HEBCS</td>
<td>Hydroethanolic extract of Buchholzia coriacea seed;</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneal;</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin 1;</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6;</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase;</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide;</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde;</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease;</td>
</tr>
<tr>
<td>NBF</td>
<td>Neutral buffered formalin;</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide;</td>
</tr>
<tr>
<td>P.o.</td>
<td>Per oral;</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline;</td>
</tr>
<tr>
<td>P-NPP</td>
<td>P-nitrophenylphosphate;</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species;</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species;</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase;</td>
</tr>
<tr>
<td>TLR4</td>
<td>Toll-like receptor-4;</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor alpha;</td>
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### 1. INTRODUCTION

The hepatic disease represents a wide range of conditions that affect the liver. It constitutes a major health challenge throughout the world with a worldwide distribution [1]. Several factors are known to be responsible for liver diseases in the general population, they include viral infection, diet pattern, and exposure to toxins, drugs, and toxicants [2]. Liver disease occurs in various forms of clinical presentations, notable among which is an acute liver failure (ALF) or acute liver disease (ALD).

Acute liver disease (ALD) is a life-threatening condition that is marked by the sudden loss of hepatic function most often in patients without any past medical history of the hepatic disease [3-5]. There are multiple causes of ALD which include hepatitis viruses, drugs, alcohol, and toxins [4,5]. The prevalence of ALD is 8.5% with the highest prevalence (around 12%) found in Europe and the United States [6]. Despite recent advances in intensive care, ALD management remains one of the challenging problems in clinical practice [7,8].

One of the most important causes of ALF are endotoxins such as lipopolysaccharide (LPS) and D-galactosamine (GalN) which constitute major components of the outer membrane of gram-negative bacteria. They have been studied extensively as a major factor contributing to the pathogenesis of gram-negative bacterial infections [9], involving inflammatory response, oxidative stress and severe hepatic injury [10]. The liver plays a significant role in the clearance of LPS from circulation [11], predisposing it to LPS-induced toxicities. Most of the toxicities of LPS, both in the liver and in the systemic circulation, have been related to the induction of inducible enzymes such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and release of acute-phase proteins like C-reactive protein, pro-inflammatory cytokines such as interleukins (IL-1, IL-6), tumour necrosis factor-alpha (TNF-α) and reactive oxygen/nitrogen species (ROS/RNS). The later cause oxidative stress with a resultant increase in the level of lipid peroxidation and elevation of plasma activities of aminotransferases [12]. LPS is known to also play a significant role in other
forms of inflammatory liver diseases. It has been found to accelerate the development of non-alcoholic fatty liver disease (NAFLD) [13].

*Buchholzia coriacea* Engl. (Wonderful Kola) is a forest tree with large, glossy leaves and conspicuous cream-white flowers at the end of the branches [14]. The seed of *B. coriacea* (Fig. 1b) possesses a number of medicinal values. In traditional medicine, it is used for the treatment of fever, trichomoniasis, diabetes mellitus, hypertension, rheumatism, cough, catarrh, and as an anthelmintic agent. It elevates mood, suppresses appetite and hunger, and clean the digestive system. It is also used as a remedy against poison. Indigenous names of *Buchholzia coriacea* in Nigeria are ‘Obi Iyanu’ or ‘Obi ata’ in Yoruba and ‘Okpokolo’ in Igbo.

![Fig. 1. Snapshots of *B. coriacea*: fruit (a) and seed (b)](image)

*Buchholzia coriacea* possess a vast number of medicinal values and wide medicinal applications in traditional medicine [15]. Moreover, it contains minerals and important class of phytochemicals like alkaloids, glycosides, saponin, steroids, tannin, flavonoids, terpenes, reducing sugars and phenols [16,17]. Various solvent extracts of *B. coriacea* have been reported for their antioxidant, anti-inflammatory and immunomodulatory activities [17,18-20].

The pathogenesis of ALD involves a complex mechanism including inflammation and oxidative stress. Hence, therapeutic agents of plant origin have major roles to play due to their multipronged mechanism of action. At present, the treatment or management of liver diseases is generally expensive in terms of hospital cost. Therefore, searching for hepatoprotective substances (with anti-inflammatory and antioxidant actions) from natural sources may offer great potential in the treatment of ALDs. Currently, there is limited information on the potential anti-inflammatory or hepatoprotective potentials of whole extracts/ solvent fractions of *B. coriacea* seed in ALD models. Therefore, an investigation into the anti-inflammatory and hepatoprotective effects of hydroethanolic extract of defatted *B. coriacea* seed is of immense value to the clinical and pharmaceutical community. In this study, we evaluated the hepatoprotective potentials of hydroethanolic extract of defatted *B. coriacea* seeds in a rat model of LPS-induced hepatotoxicity.

2. MATERIALS AND METHODS

2.1 Chemicals

Chemicals used in this research include n-hexane, ethanol, p-nitrophenylphosphate (p-NPP), 4′,6-diamidino-2-phenylindole (DAPI) purchased from Merck®, Darmstadt, Germany. Lipopolysaccharide (from Escherichia coli serotype 055:B5) was purchased from AK Scientific®, Union City, CA. All other reagents used were of analytical grade.

2.2 Assay kits

ELISA Kit for Rat Tumor Necrosis Factor Alpha (TNF-α) was procured from Elabscience® Biotechnology Co. Limited, Houston, Texas, USA, while assay kits for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were products of Fortress® Diagnostics Limited, Antrim, UK.

2.3 Antibody

C-reactive protein (CRP) used for immunofluorescence staining was procured from Elabscience® Biotechnology Co. Limited, Houston, Texas, USA.

2.4 Preparation of the Plant Extract

*B. coriacea* seeds were collected from a farm site in Osi Town in Kwara State, Nigeria. The samples (fresh leaves and fruits) were immediately authenticated at the Department of Biology, the University of Ilorin in Kwara State, Nigeria. A voucher specimen (UIH 1067) has been deposited in the Herbarium of the Department. The name of the plant has also been checked with the plant list database (http://www.theplantlist.org).

Seeds were washed in clean water, cut into thin slices with a knife and air-dried. The seeds were ground to powder and defatted by extracting exhaustively with n-hexane. The residue was air-dried and exactly 500 g of the residue was re-extracted with 80% ethanol. The resulting 80%
ethanol extract was filtered and concentrated under reduced pressure. The concentrated extract was lyophilized and kept refrigerated until used. The solid form obtained (30.1% yield) was termed hydroethanolic extract of (defatted) B. coriacea seeds (HEBCS).

2.5 Experimental Animals and Handling

Thirty male albino rats (120–130 g) were obtained from the experimental animal breeding house, College of Basic Medical Sciences, University of Ibadan, Nigeria. Animals were acclimatized to laboratory conditions, one week prior to the commencement of study. They were contained in wire-meshed cages and supplied with pelleted feed and water ad libitum. The study was approved by the Faculty of Natural Sciences Ethical Review Committee, Ajayi Crowther University (FNS/ERC/201700016) on 10 July 2017. Handling of the experimental animals conforms to international guidelines on the care and use of laboratory animals in research [21].

2.6 Treatments and Experimental Protocol

Rats were randomly assigned into five groups (n=6/group). LPS was suspended in physiological saline (0.9% w/v saline) and was injected on 7th day at a single dose of 4 mg/kg intraperitoneally (i.p.) to the rats, which were being administered HEBCS daily for 7 days. The dose of LPS used (4 mg/kg) has been shown previously to induce hepatic damage and oxidative stress [22,23]. Treatments were administered as presented in Table 1. The doses of HEBCS used in this study were selected on the basis of previous acute toxicity studies done in our laboratory.

Six hours after LPS injection, rats were weighed and blood sample was collected via the retro-orbital vein in plain sample tubes for preparation of serum. They were thereafter euthanized and liver was excised, washed in ice-cold PBS (pH 7.4) to remove residual blood. The liver was blotted to dry and weighed. Section from the liver was fixed in 10% neutral buffered formalin (NBF) for histopathology and the remainder used for the preparation of liver homogenate.

2.7 Preparation of Serum and Liver Homogenate

The blood sample was subjected to centrifugation at 4000 rpm. for 5 min to obtain serum. Approximately 1 g of the liver was minced and homogenized in PBS (10% w/v). The homogenate was centrifuged at 10 000 ×g for 10 minutes at 4°C. The resulting supernatant was collected and stored frozen until used for biochemical analyses. Protein contents of samples (serum and liver homogenate) were determined using the biuret method [24].

2.8 Determination of Tumor Necrosis Factor Alpha (TNF-α) by Sandwich ELISA

Serum level of TNF-α was determined using ELISA kit following the manufacturer’s procedure. Briefly, the ELISA is based on Sandwich-ELISA method where the micro ELISA plate has been pre-coated with an antibody specific to rat TNF-α. Standards or samples

<table>
<thead>
<tr>
<th>Experimental Groups (n=6/group)</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Animals received equivalent volume of distilled water (DW, vehicle for HEBCS) orally (p.o.) for 7 days and were administered saline (vehicle for LPS; i.p.) on 7th day.</td>
</tr>
<tr>
<td>LPS</td>
<td>Animals were administered DW for 7 days and were challenged with LPS (4 mg/kg body weight, b.w.) i.p. on the 7th day.</td>
</tr>
<tr>
<td>LPS + HEBCS 125</td>
<td>Animals received 125 mg/kg b.w. HEBCS p.o. for 7 days and received 4 mg/kg LPS i.p. on the 7th day.</td>
</tr>
<tr>
<td>LPS + HEBCS 250</td>
<td>Animals received 250 mg/kg b.w. HEBCS p.o. for 7 days and received 4 mg/kg LPS i.p. on the 7th day.</td>
</tr>
<tr>
<td>LPS + HEBCS 500</td>
<td>Animals received 500 mg/kg b.w. HEBCS p.o. for 7 days and received 4 mg/kg LPS i.p. on the 7th day.</td>
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</tbody>
</table>
were added to appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibodies specific for rat TNF-α and Avidin-horseradish peroxidase (HRP) conjugate were added to each microplate well successively and incubated. After incubation, free components were washed away. Then the substrate reagent was added to each well, which produced blue colour in wells that contain rat TNF-α, biotinylated detection antibody, and Avidin-HRP conjugate. The enzyme-substrate reaction was terminated by adding stop solution to obtain a yellow colour. The absorbance of the yellow colour developed was measured by spectrophotometry at a wavelength of 450 nm. The absorbance value is proportional to the concentration of rat TNF-α. The concentration of TNF-α in samples was extrapolated from the standard curve.

2.9 Immunofluorescence Assay for CReactive Protein

The method described by Long and Buggs [25] was used for immunofluorescent staining of paraffin-embedded liver sections. Liver section was fixed in 10% neutral buffered formalin (NBF) prior to paraffin embedding. Five micron-sized sections were prepared and mounted on slides. Sections were placed at 60°C for 15 min, incubated in xylene at room temperature (RT) for 15 min, and then transferred sequentially into 100% ethanol (EtOH), 95% EtOH, 70% EtOH, and 50% EtOH for 4 min at RT. Sections were rinsed in deionized water and stored in PBS. Slides were then immersed in antigen retrieval buffer (10 mM citrate, pH 6.2, containing 2 mM EDTA and 0.05% Tween 20) using an uncovered 470 mL tray and heated in a microwave oven for three sequential 5-minute cycles at power levels 4, 5, and then 4. The tray was removed from the microwave oven and allowed to cool to RT. Slides were then rinsed in PBS. Liver sections were outlined with a liquid Blocker Pen to minimize the volume of antibody solution required for staining. CRP antibody was used at the specified dilution ratio. The antibody was diluted in PBS containing 1% BSA and 0.01% Triton X-100 and added to each tissue section. Slides were placed in a tray and water was added to a level within 0.5 inch of the bottom of the slides. The tray was covered and placed in the microwave oven for a three-minute heating cycle at power level 4. Two minutes after the heating cycle was completed, slides were rinsed in PBS and then 1:100 dilution of a secondary antibody was applied (Santa Cruz Biotechnology). Slides were placed back into the tray, containing fresh deionized water, and incubated in the microwave oven for a three-minute heating cycle at power level 4. After incubation, the slides were rinsed in PBS, stained with DAPI and examined using fluorescence microscopy. Image processing and fluorescence intensity measurement were carried out with ImageJ software [26] and relative fluorescence intensity (CRP/DAPI) was calculated from six random field measurements.

2.10 Analysis of Biomarkers of Oxidative Stress

Reduced glutathione (GSH) level was determined according to Jollow et al. [27]. The method described by Hadwan and Abed [28] was used to determine the activity of catalase (CAT) in the serum and liver. Superoxide dismutase (SOD) activity was determined in the liver based on the procedure of Sun and Zigman [29]. Level of lipid peroxidation (LPO) was evaluated by measuring the concentration of malondialdehyde (MDA) in the serum and liver following the method of Varshney and Kale [30]. Nitric oxide (NO) level was determined by the procedure of Green et al. [31].

2.11 Assessment of Liver Function

Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using assay kits (Fortress®, Antrim, UK), following the manufacturer’s protocol. Alkaline phosphatase (ALP) activity was determined according to the method described by Wright et al. [32] based on the conversion of p-nitrophenyl phosphate (p-NPP) to p-nitrophenol.

2.12 Evaluation of Liver Sections by Hematoxylin and Eosin Staining

Liver sections were fixed in 10% NBF prior to paraffin embedding. Paraffin-embedded tissues were sectioned and stained with haematoxylin and eosin (H&E) according to the method described by Fisher et al. [33]. The extent of LPS-induced liver injury was evaluated through the morphological changes.

2.13 Statistical Analysis

Data are expressed as the mean ± standard deviation (SD) for 6 rats. Statistical analysis and graphical constructions were performed using Graphpad® Prism 6.0.1 (Graphpad Software, La Jolla, CA). The statistical significance of
differences between experimental groups was determined by one-way analysis of variance (ANOVA) and complemented with Tukey's test. *P* values less than 0.05 were considered to be statistically significant.

3. RESULTS

3.1 HEBCS Alleviate the LPS-induced Increase in Serum TNF-α Level

The serum and hepatic levels of the pro-inflammatory cytokine, TNF-α was determined after LPS administration in rats by sandwich ELISA. The level of TNF-α was increased significantly in the serum, six hours after LPS treatment (Fig. 2). However, HEBCS at doses of 250 and 500 mg/kg b.w. significantly (*P* < 0.05) alleviated the observed increase in serum TNF-α.

3.2 HEBCS Alleviate the LPS-induced Increase in Hepatic Expression of C-reactive protein

Hepatic expression of the inducible protein, C-reactive protein was determined by immunofluorescence staining following LPS treatment in rats. The expression of C-reactive protein was significantly increased six hours after LPS treatment (Fig. 3). However, pre-treatment with HEBCS at 125, 250 and 500 mg/kg b.w. caused a significant decrease in the expression of this protein in the liver of rats when compared with LPS group.

3.3 HEBCS Alleviates the LPS-induced Oxidative Stress in the Serum and Liver of Rats

The activity of CAT and the levels of GSH, MDA and NO were assessed in the serum and liver of the rats (Figs. 4 and 5). The activity of CAT and GSH level was reduced significantly in the serum of rats six hours after LPS treatment. These were accompanied by a significant increase in serum MDA and NO levels. Pre-treatment with HEBCS caused an increase in GSH level and CAT activity, with a significant increase in the group pre-treated with 250 mg/kg b.w. compared with LPS group. The elevation in serum MDA and NO levels was reduced significantly (*P* < 0.05) by 250 mg/kg b.w. HEBCS.

In the liver, LPS caused a significant reduction in the level of GSH. Contrary to the observation in the serum, hepatic CAT activity increased significantly in response to LPS. SOD activity was decreased by LPS administration followed by elevation in hepatic MDA and NO levels in rats. Pre-treatment with the three doses of HEBCS ameliorated the alterations in oxidative stress biomarkers caused by LPS administration in rat. In all the oxidative stress markers determined, 250 mg/kg b.w. was the most effective dose of HEBCS applied. Except in cases where 250 and 500 mg/kg b.w. significantly ameliorated the LPS induced decrease and increase in SOD activity and MDA level respectively (Fig. 5c and 5d).
Fig. 3. Inhibitory effects of three doses of HEBCS on the LPS-induced expression of CRP in the liver of rat determined by immunofluorescence labeling. Plates in (a) show the immunofluorescence images for expression of CRP in formalin-fixed paraffin-embedded liver sections, and the bar chart, (b) shows the corresponding relative fluorescence intensity (CRP/DAPI).

The values shown are mean ± SD of six measurements. * Significant compared with control alone, P < 0.05. # Significant compared with LPS alone, P < 0.05.

Fig. 4. HEBCS alleviate LPS-induced depletion of serum GSH level (a), reduction in serum CAT activity (b), elevation of serum MDA level (c) and nitric oxide level (d) in rats.

Each bar represents the mean ± S.D. (n = 6). * Significant compared with control alone, P < 0.05. # Significant compared with LPS alone, P < 0.05.
3.4 HEBCS Attenuates LPS-induced Liver Injury

The liver-body weight ratio (LBWR) was calculated and activities of ALP, AST and ALT were determined in the serum of rats following LPS intoxication for six hours. From data obtained from this study (Fig. 6), the LBWR and activities of these enzymes were significantly increased as a result of LPS administration. However, pre-treatment with the three graded doses of HEBCS prevented the observed increase in LBWR and activities of ALP, AST and ALT in the serum of rats. In most cases, 250 mg/kg b.w. HEBCS showed significant (P < 0.05) effects on LBWR and activities of ALP, AST and ALT. An exception was observed in the group pre-treated with 500 mg/kg b.w. (Fig. 6d), where ALT activity was also significantly (P < 0.05) reduced in the serum.

3.5 HEBCS alleviate the LPS-induced Histopathological Alterations in the Liver of Rats

Histopathological evaluation was done by haematoxylin and eosin staining of liver sections after LPS induction. Six hours after LPS treatment, hepatocellular vacuolar degeneration and necrosis were observed, with a moderate portal and central venous congestion, and with severe periportal infiltration by inflammatory cells. (Fig. 7b). In the group pre-treated with 125 mg/kg b.w. HEBCS (Fig. 7c), a moderate portal and central venous congestion, accompanied by severe periportal infiltration by inflammatory cells was observed. However, in the group given 250 mg/kg b.w. HEBCS, (Fig. 7d), a very mild periportal cellular infiltration was observed in liver sections. Also, in the group pre-treated with 500 mg/kg b.w.
HEBCS (Fig. 7e), there is a mild periportal cellular infiltration.

4. DISCUSSION

Liver diseases represent one of the major world public health concern and the state of liver health of an individual is the reflection of the person’s overall health status. Liver diseases are extremely expensive in terms of treatments and management. One option is liver transplant which is unaffordable to many liver patients. It is believed that herbal medicine has a major role to play in the treatment and management of liver diseases, due to their multi-mechanism of action. In this study, we investigate the hepatoprotective potential of orally administered hydroethanolic extract of B. coriacea seeds (HEBCS) in a rat model of acute liver disease (ALD).

LPS-induced acute liver injury is a well-established model to investigate the liver function and the hepatoprotective role of potential drug candidates. LPS induced hepatotoxicity is known to be associated with severe inflammatory responses and hepatic oxidative stress [34]. These are due to the involvement of the liver in the regulation of entry, metabolism, and clearance of LPS during exposure [35]. It has been reported that the hepatic and systemic toxicities exhibited by LPS have been due mainly to the release of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6. These cytokines are produced following the binding of LPS to the CD14/LPS-binding protein and Toll-like receptor-4 (TLR4) on the surface of Kupffer cells [36,37]. Subsequent interactions cause an increase in the expression of inducible enzymes/proteins including COX-2, iNOS, and CRP and production of chemical oxidants such as superoxide radical anion (O2⁻) and NO [34,38,39]. All these contribute to the hepatic injury in LPS - induced toxicity characterized by elevation of serum activities of ALP, AST, and ALT [40].

Previous studies have reported that cytokines like TNF-α, IL-1, and IL-6 are secreted from activated Kupffer cells during LPS endotoxicity [36]. Evidence has also suggested that up-regulation of pro-inflammatory cytokines from neutrophils in the liver were associated with hepatic damage [41]. In the present study, an elevated level of TNF-α was observed in rats following LPS administration. However, pre-treatment with three doses of HEBCS significantly reversed the observed increase in serum TNF-α to a level comparable to those of control animals. These observations are similar to those reported previously by Ajuwon et al. [42] and Zhao et al. [43].

![Fig. 6. HEBCS alleviate LPS-induced elevation of liver-body weight ratio (a), serum activities of ALP, AST and ALT (b-d) in rats](image_url)

Each bar represents the mean ± S.D. (n = 6). * - Significant compared with control alone, P < 0.05. #-Significant compared with LPS alone, P < 0.05
We also observed a significant increase in the expression of CRP in the liver of rats, six hours after LPS administration. LPS signalling has been linked to an elevation of acute-phase proteins, most especially the CRP [34,44]. CRP is an acute-phase protein produced by hepatocytes and it plays a vital role in liver innate immunity [44]. Findings from this study show that the three doses of HEBCS applied significantly alleviate LPS-induced inflammatory response by reducing the expression of these proteins in the liver of rats.

LPS-induced acute liver injury is frequently accompanied by a high level of oxidative stress due to the production of ROS and RNS such as: $\Delta\Delta^2$, $H_2O_2$, NO and resulting in depletion of hepatic redox regulatory molecules most importantly, the GSH [43,45,46]. The NADPH – Oxidases (NOXs) have been identified as vital components involved in the initiation of the immune response to bacterial LPS [47]. As a kill mechanism, NOX rapidly generate high level of superoxide radical when phagocytes are exposed to bacteria [48]. NOX can also be stimulated by the presence of substances such as IFN-γ, TNF-α or IL-1β resulting in a high level of $\Delta\Delta^2$ generation [47,49]. $\Delta\Delta^2$ is converted to $H_2O_2$ through the action of SOD, while the $H_2O_2$ produced is transformed to $H_2O$ and $O_2$ by the action of CAT. In this study, we observed a significant increase in the serum and hepatic NO level in LPS –treated animals when compared with control. The increase observed may be related to the elevated expression of iNOS. However, HEBCS at a dose of 250 mg/kg b.w. significantly reduced the level of NO in the serum and liver of rats.

Data from this study also revealed a significant decrease in the serum and hepatic reduced glutathione (GSH) level in LPS treated rats. GSH is the major non-enzymic antioxidant in tissues and circulation where it plays an essential function in redox regulation. Because of its sulphydryl (–SH) group, it serves as a reductant
in the metabolism of hydrogen peroxide and other organic peroxides and as a free radical scavenger. Depletion of GSH level is usually associated with an excessive free radical generation and oxidative stress [50]. In this study, administration of HEBCS at 250 mg/kg b.w. to LPS-treated rats was able to restore the level of GSH in the serum and liver of rats. This indicates that HEBCS is able to protect against LPS-induced depletion of GSH. The protection displayed by HEBCS in this study is in agreement with previous studies where fruit and seed extracts of B. coriacea were reported to improve the level of GSH in rats [51,52]. LPS treatment also caused a significant decrease in hepatic SOD activity and serum CAT activity six hours after LPS challenge. In the liver, however, LPS treatment caused a significant increase in the activity of CAT. The later observation is in agreement with Ajuwon et al. [42] in LPS induced liver injury model. Increase in CAT activity may be an adaptive response to a high level of \( \text{H}_2\text{O}_2 \) in the liver. Treatment with HEBCS was able to ameliorate the alteration in the activities of SOD and CAT most effectively at 250 mg/kg b.w.

LPS treatment also caused a significant increase in the level of LPO as indicated by the serum and liver MDA levels. Increase in MDA level has been reported to be associated with LPS toxicity [53]. The increase may be due to the decrease in the level of serum and hepatic antioxidant defences. The anti-lipid peroxidation activity exhibited by HEBCS in this study supports the recent report by Salami et al. [54] on reduction in MDA levels after ingestion of diets supplemented with Seeds of Buchholzia coriacea.

In this study, we also observed a significant increase in liver-body weight ratio and activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum of rats following LPS challenge. High serum activities of ALP, AST and ALT has been associated with liver injury. These hepatic function biomarkers are cytoplasmic enzymes which are usually leaked into the blood due to damage to hepatocytes. This observation agrees with the previous report by Depboylu et al. [55], where LPS caused a significant increase in activities of serum aminotransferases. Data from this study show that pre-treatment with HEBCS significantly alleviate the hepatic damage induced by LPS. Previous studies have demonstrated the hepatoprotective potentials of some seed-extracts such as Lepidium sativum [56] and Nigella sativa [57] in LPS-induced hepatic injury. The hepatoprotective effect displayed by HEBCS in this study may be due to its ability to stabilize membrane integrity and prevent attack by reactive species on the hepatocytes.

Histopathological evaluation of hepatic tissues revealed severe hepatocellular degeneration with a high level of inflammatory cells following LPS treatment. This further support the observed increase in the expression of pro-inflammatory proteins and high activities of hepatic function biomarkers in the serum of LPS treated animals. Improvement in liver appearance and decrease in cellular infiltration were observed in the groups that were pretreated with various doses of HEBCS. These further explain the extent of protection by HEBCS on the liver after LPS induced hepatocellular injury.

5. CONCLUSION

In this study, we provide the first \textit{in vivo} evidence of anti-inflammatory activity of hydroethanolic extract of defatted \textit{B. coriacea} seeds in an acute inflammatory liver disease model. The hepatoprotective effect exhibited by this extract was achieved through inhibition of pro-inflammatory cytokines and inducible enzymes as well as through antioxidant actions. In this model, the best hepatoprotective activity was obtained at 250 mg/kg b.w. These suggest that HEBCS may be of immense benefit in the prophylactic management of immune mediated-liver diseases. The extract will also find applications in the treatment of other forms of liver diseases with inflammatory and oxidative stress mechanism, most especially NAFLD. However, future studies are required to further elucidate detail mechanisms underlying the anti-inflammatory and hepatoprotective effects displayed by this extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee."
COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

   DOI: 10.7326/0003-4819-134-3-20010206 0-00013
   DOI: 10.1038/cdddis.2013.499
   DOI: 10.1016/j.cld.2006.05.010
   DOI: 10.1002/hep.22177
   DOI: 10.1056/NEJMra1208937
   DOI: 10.1016/j.jhep.2012.04.004
   DOI: 10.1016/j.jceh.2014.11.005
   DOI: 10.1016/j.ejphar.2015.12.006
    DOI: 10.1152/ajpgi.00550.2001
    DOI: 10.1371/journal.pone.0155030
   DOI: 10.3164/jcbn.13-49
   DOI: 10.1515/jcim-2016-0159.
   DOI: 10.1515/jcim-2014-0046
   DOI: 10.11648/j.ajls.20160405.12


38. Qin L, Li G, Qian X, Liu Y, Wu X, Liu B, Hong JS, Block ML. Interactive role of the

DOI: 10.1002/glia.20225


DOI: 10.1002/glia.20545


DOI: 10.1016/j.tox.2006.06.018


DOI: 10.1152/ajpregu.2000.278.5.R1196


DOI: 10.1186/1472-6882-14-392


DOI: 10.1111/asj.12717


DOI: 10.1111/j.1365-2249.2006.03224.x


DOI: 10.1007/s11010-005-6936-x


DOI: 10.1097/SHK.0b013e318257ed62


DOI: 10.1111/j.1742-4658.2008.06488.x


DOI: 10.1189/jlb.0603252


DOI: 10.1097/WOX.0b013e3182439613


DOI: 10.3923/jpt.2012.181.191


DOI: 10.4103/2221-1691.227998


DOI: 10.3892/mmr.2017.7414


DOI: 10.1080/19390211.2017.1404544

55. Depboylu B, Giriş M, Olgac V, Doğru-Abbasoğlu S, Uysal M. Response of liver to lipopolysaccharide treatment in male


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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/50490