International Journal of Biochemistry Research & Review
3(3): 190-199, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Anti – Atherogenic Activity of Ethanol Extract of *Buccholzia coriacea* Seeds on Hypercholesterolemic Rats

T. O. Omolekan[1*] and C. O. O. Olaiya[1]

[1]Laboratories of Nutritional and Industrial Biochemistry Research, Department of Biochemistry, University of Ibadan, Oyo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author *TOO designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author COOO managed the analyses of the study. These two authors read and approved the final manuscript.*

Research Article

Received 21st December 2012
Accepted 1st March 2013
Published 10th May 2013

ABSTRACT

Aims: To scientific determine the phytochemical constituents of ethanol extract of *B. coriacea* (EEBC) powdered seeds and its effects on the serum lipid profile, serum atherogenic index, and lipid peroxidation in the serum of hypercholesterolemic rats.
Study Design: Twenty male albino rats were used for the experiment. They were divided into four groups, 5 rats in each. The administration is as follows: **GROUP 1 (Normal control):** 0.3ml of corn oil only, **GROUP 2 (experimental control):** 40 mg/kg body weight of cholesterol only, **GROUP 3 (standard drug treatment):** 40 mg/kg body weight of cholesterol and 260mg/kg body weight of cholestyramine, GROUP 4 (extract treatment): 40 mg/kg body weight of cholesterol and 250mg/kg body weight of EEBC.
Place and Duration of Study: Department of Biochemistry, University of Ibadan, Nigeria for eight weeks.
Methodology: At the end the 6 weeks of administration, the animals were kept fasting overnight. They were then made unconscious by cervical dislocation and dissected. The blood was then collected into clean vials. The Lipid profile, malondialdehdye levels were measured in the serum of the rats in the four groups while the atherogenic index was calculated. The qualitative phytochemical screening of EEBC seeds powder was

**Corresponding author: Email: toluomon@yahoo.com;*

performed. Data were statistically analyzed by one way ANOVA followed by post hoc Duncan multiple range comparison tests.
Results: EEBC powdered seeds contains tannins, carbohydrates, saponins, flavonoids, saponins, terpenoids and alkaloids. The level of serum total cholesterol, triglyceride, LDL – cholesterol, atherogenic index significantly decreased (*P=.05*) by 29.5%, 41.5%, 39.2%, 77.4% respectively in the extract – treated group compared with experimental group. Whereas the experimental group showed a significant (*P=.05*) increase compared with normal control. There was a significant (*P=.05*) increase of 67.9% in the serum HDL - cholesterol of the extract – treated group compared with the experimental group. Whereas the experimental group showed a significant (*P=.05*) decrease in comparison with the normal control. A significant (*P=.05*) decrease of 36% in the amount of serum malondialdehyde was produced in the extract – treated groups compared with the experimental control which showed a significant (*P=.05*) increase compared with the normal control.
Conclusion: EEBC seed powder has potent anti- atherogenic properties.

Keywords: Ethanol extract of Buccholzia coriacea (EEBC); hypercholesterolemia; cholestyramine; atherogenic index; lipid profile; lipid peroxidation.

1. INTRODUCTION

Atherosclerosis as a leading cause of mortality worldwide and a cause of chronic non – infectious diseases like stroke and cardiovascular diseases is a pathological disease of the arteries characterized by a gradual accumulation of fatty streaks containing foam cells in the arterial wall. The main cause of atherosclerosis is hypercholesterolemia. Epidemiologic studies have demonstrated a positive significant relationship between plasma cholesterol concentrations and coronary artery disease [1].

Synthetic anti - hyperlipidemic drugs like statins and bile acid sequestrant have been successfully used in the amelioration of hypercholesterolemia associated with this disease. But, statins and bile acid sequestrant have various side effects [2] that could lead to the malfunctioning of the body function e.g. formation of gall stones, gall bladder diseases, itching and diarrhea. Therefore, much attention has been shifted to the use of natural products from plants with very few side effects in the treatment of atherosclerosis [3]. One of such plants is *Buccholzia coriacea* (Family: Capparaceae, common name: magic/wonderful cola, English name: Musk tree). Different parts of the plant are traditionally used for myriads of therapeutic purposes. The sap exudes with a violently spicy pungent smell that causes sneezing [4] and can be turned into a pulp for inhalation or snuff to relief head ache, sinusitis, bronchitis, otitis, nasal congestion in Ivory Coast. It is used in Garbon for treatment of small pox and skin itch and in Ghana for ear ache [5]. The seed decoction is usually made in lime or local gin by traditional healers in Edo (Southern Nigeria) for the treatment of diabetes mellitus, hypertension and cold [6]. Its hypoglycemic activity [6], phytochemical analysis, proximate composition and antimicrobial studies [7], anthelmintic properties [8], cytotoxicity evaluation [9], antiplasmodial activity [10], antibacterial activity [11] of various parts of the plant have been reported. The phytochemical screening of the stem barks reveals the presence lupeol, flavonoid, terpenes and steroids [7].

There has been a dearth of scientific evidences of its anti – atherogenic activities. Therefore, this study has been undertaken to evaluate the anti – atherogenic activities of ethanol extract

of *B. coriacea* seeds powder in the serum of cholesterol – induced hypercholesterolemic rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Questran (a statin drug that contains the active ingredient - cholestyramine) was obtained from Bristol – Meyers Squibb Pharmaceutical Company Ltd, New York, NY10154. HDL – Cholesterol, Total Cholesterol and Triglyceride Kits were obtained from RANDOX Laboratories Ltd., 55 Diamond Road, Crumlin, Co., Antrim, United Kingdom, BT29 4QY. Potassium Phosphate buffer, Cholesterol, glacial acetic acid, lead acetate, chloroform, $FeCl_3$ solution, H_2SO_4 solution, Thiobarbituric acid, Tris – KCl buffer and Tricarboxylic acid were obtained from Sigma Chemical Co., Saint Louis, 62 MO, USA or BDH Chemical Ltd, Poole, England.

2.2 Plant Material

Fresh seeds of *B. coriacea* were obtained from a local market in Ibadan, Oyo State, Nigeria. The plant seeds were authenticated at the herbarium of Botany and Microbiology Department, University of Ibadan. A voucher specimen was already available.

2.3 Preparation of Plant Extract

The seeds were immediately rinsed of debris, peeled, chopped and shade – dried for 1 week in laboratory trays. The dried seeds were powdered and weighed. 4 kg of the powdered seeds were macerated in ethanol (80% v/v) for 24 hours with intermittent shaking. The extract obtained from percolation was collected in a flask and then concentrated to a dark brown residue (EEBC) using rotary evaporator at 66^{o}C until the solvent part was evaporated. The dark brown residue was dissolved in small amount of dimethyl sulfoxide (DMSO)

2.4 Phytochemical Screening

EEBC seeds powder was screened qualitatively for the presence of bioactive phytochemicals such as cardiac glycosides, saponins, alkaloids, anthraquinones, flavones glucosides and tannins using standard procedures outline by Evans and Trease [12].

2.4.1 Test for saponins

5 ml of ethanol extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins.

2.4.2 Test for terpenoids

2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicates the presence of terpenoids.

2.4.3 Tests for steroids

I. A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid added indicates the presence of steroids.
II. The development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acids indicates the presence of steroids.

2.4.4 Test for tannins

About 2 ml of the ethanol extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$ solution were added. The formation of a green precipitate was an indication for the presence of tannins.

2.4.5 Tests for glycosides

2 ml of each extract was dissolved in 2 ml of glacial acetic acid containing one drop of $FeCl_3$ solution. The mixture was then poured into a test tube containing 1 ml of concentrated H_2SO_4. A brown ring at the interphase indicates the presence of a deoxy-sugar, characteristic of cardenolides.

2.4.6 Test for alkaloids

3 ml of ethanol extract was stirred with 3 ml of 1% HCl on a steam bath. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.4.7 Test for flavonoids

To 1 ml of ethanol extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

2.5 Animals

Twenty male albino rats weighing between 75 -175 g were used for the experiment. They were obtained from the Central Animal House of Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan, Nigeria. The animals were housed in standard cages and maintained under normal room temperature at the animal house, Department of Biochemistry, University of Ibadan, Nigeria where the whole experiment was carried out. The rats were acclimatized for 2 weeks and were fed on normal diet of pelletized mouse chow, with water given *ad libitum* at room temperature with 12 – h light and dark cycle before the experiment commenced.

2.6 Experimental Design

The twenty male albino rats were divided into four groups, 5 rats in each. They were then administered with different doses of the tested compounds as follows:

a. **GROUP 1 (Normal control)**: received only 0.3ml of corn oil.

b. **GROUP 2 (experimental control)**: received 40 mg/kg body weight of cholesterol only.
c. **GROUP 3 (standard drug treatment)**: received 40 mg/kg body weight of cholesterol and 260mg/kg body weight of cholestyramine (questran).
d. **GROUP 4 (extract treatment)**: received 40 mg/kg body weight of cholesterol and 250mg/kg body weight of EEBC.

All the animals used for the experiment were kept under daily cage - side observation for daily food intake, general health and behavior. The administration of cholestyramine, EEBC and cholesterol was done orally five times in a week for 6 weeks by means of intra – gastric feeding tube in the volume of 5ml/kg body weight of corn oil. Corn oil served as the vehicle for EEBC, cholestyramine and cholesterol. The dosage of administration of EEBC was obtained from the LD_{50} study and acute toxicity test as reported by Adisa, Choudary and Olorunsogo [6]. The dosage for cholesterol, cholestyramine and the period of treatment were selected on the basis of previous studies by Erukainure, Abovwe, Adefegha, Egwuche and Fafunso [13].

At the end of the 6 weeks, all the animals were kept fasting overnight. The animals were made unconscious by cervical dislocation. Then, animals were dissected and the blood was collected from inferior vena cava into different vials for biochemical analysis. The animals were then buried.

2.7 Serum Preparation

The blood was centrifuged at 3000 rpm for 10min and the serum (supernatant) was analyzed to evaluate some biochemical parameters.

2.8 Lipid Profile Estimation and Lipid Peroxidation Assessment

Total cholesterol was measured by CHOP/PAP method [14], Triglyceride was measured by GPO/PAP method [15], HDL – Cholesterol was measured by PEG precipitation method [16] and colorimetric method and LDL –Cholesterol was calculated by using Freidewald's formula [17]. Malondialdehyde (MDA) level was measured by colorimeter using thiobarbituric acid reactive substance (TBARS) method described by Varshney and Kale [18].

2.9 Atherogenic Index Calculation

Atherogenic Index was calculated by using the following formula [19],

$$AI = \frac{total\ serum\ cholesterol}{total\ HDL{-}cholesterol}$$

2.10 Statistical Analysis

The statistical significance between groups was analyzed using one – way Analysis of Variance (ANOVA), followed by post hoc Duncan multiple range comparison tests using SPSS version 17.0. The level of significance was expressed by '*P'* values as mentioned in the tables. '*P'* values of =.05 were considered as significant

3. RESULTS AND DISCUSSION

The phytochemical screening of EEBC seeds revealed the presence of tannins, carbohydrates, saponins, flavonoids, saponins, terpenoids and alkaloids.

There was a significant (*P=.05*) decrease in the level of serum total cholesterol, triglyceride, low density lipoprotein (LDL) cholesterol and atherogenic index Table 1 of the extract and standard drug treated groups when compared with those in the experimental group. Whereas the level of serum total cholesterol, triglyceride, low density lipoprotein (LDL) cholesterol and atherogenic index in the experimental group showed a significant (*P=.05*) increase as compared with those in the normal control. Also, there was a significant (*P=.05*) increase in the serum high density lipoprotein (HDL) cholesterol in extract and standard drug treated groups in comparison with the experimental group. Whereas the level of serum HDL in the experimental group showed a significant (*P=.05*) decrease in comparison with the normal control.

There was a significant (*P=.05*) decrease in the amount of serum malondialdehyde produced in the extract and standard drug treated groups in comparison with the experimental control. Whereas there was a significant (*P=.05*) increase in the amount of serum malondialdehyde produced in the experimental control in comparison with the normal control as shown in Fig. 1.

Atherosclerosis is a pathological disease of the arteries characterized with gradual accumulation of fatty streaks – containing foam cells in the arterial wall [20]. A primary cause of atherosclerosis is the presence of high level of oxidized LDL – cholesterol in the blood as well as a deficiency of LDL membrane receptor on the surface of the adiposites [20]. When LDL – cholesterol particles are not efficiently removed from the blood, there is an increased chance of the formation of oxidized LDL – cholesterol which will invade the lining of the arteries and participate in plaque formation.

Therefore, the reduction of LDL – cholesterol in the EEBC treated hypercholesterolemic rats shows that the extract is potent in reducing the formation of plaques in the coronary arteries and also the generation of atherosclerosis. This study also supports other clinical studies that have shown that decreasing the amount of plasma LDL – cholesterol significantly reduces the risk of coronary heart disease morbidity and mortality and also decreases the progression of atherosclerosis lesions [21].

Table 1. Effect of ethanol extract of *B. coriacea* and cholestyramine on serum lipid profile and atherogenic index

GROUPS	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Atherosclerosis Index
Normal control	96.2 ± 2.4	285.6 ± 6.7	48.9 ± 0.8	48.8 ± 3.2	1.97
Experimental control	170.2 ± 7.2*	525.6 ± 5.2*	25.8 ± 1.6*	78.6 ± 0.4*	6 .60*
Standard drug treatment	152.2 ± 9.0**	358.2 ± 2.4**	72.8 ± 1.6**	54.2 ± 1.3 **	2.09**
Extract treatment	120.0 ± 8.8**	307.4 ± 9.3**	80.4 ± 0.5**	47.8 ± 2.2 **	1.49**

*Values are expressed as MEAN ± SEM (n=5), ANOVA followed by post hoc Duncan multiple range comparison test. *P =.05, when compared to the Normal control. **P =.05, when compared to the Experimental Control.*

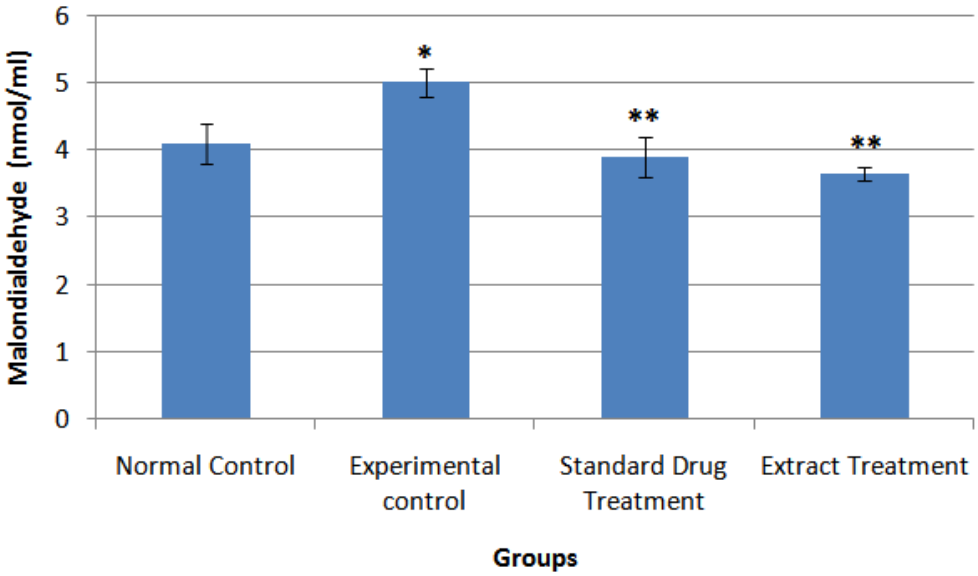

Fig. 1. Effect of ethanol extract of *B. coriacea* and cholestyramine on serum lipid peroxidation

*Values are expressed as MEAN ± SEM (n=5), ANOVA followed by post hoc Duncan multiple range comparison test. *P =.05, when compared to the Normal control. **P =.05, when compared to the Experimental Control.*

The presence of saponins in the extract also suggests that the reduction in the serum total cholesterol in the extract might be due to competitive inhibition of the adsorption of dietary cholesterol by saponins in the intestine of the animal or through the stimulation of biliary excretion of bile acid or cholesterol in feaces by saponins [22]. This is due to the fact that saponins are glucosides of steroids and steroid alkaloid which bear chemical similarity with cholesterol and may either block the absorption of cholesterol from by the intestinal lumen or enhance its excretion.

The reduction noticed in the amount of malondialdehyde in the serum of the extract treated group as compared with the experimental control might be due to the presence of non – enzymatic antioxidants like flavonoids in the extract. The protective role of flavonoid has been attributed to many mechanisms i.e, antioxidant properties and also anti – inflammatory activity [23]. Epidemiological studies have reported a reduced risk of coronary heart disease in subjects with high flavonoid intake [24]. It is also possible that the extract works synergistically in inducing the expression on the host enzymatic anti – oxidants like catalase, superoxide dismutase.

4. CONCLUSION

This present study suggests that the ethanol extract of *B. coriacea* seeds powder has significant anti – atherogenic activity when administered to hypercholesterolemic rats. The anti – atherogenic effects of ethanol extract of *B. coriacea* seeds may be due to the presence of flavonoids, saponins and plant sterols. To determine the precise mechanism of action of some specific biological moiety, further research work is required.

ETHICAL APPROVAL

The principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the ethical committee of animal care and human subjects, University of Ibadan, Nigeria.

COMPETING INTERESTS

No competing interest was declared by the authors.

REFERENCES

1. Moarreaf AR. Risk factor modification of coronary artery disease. SEM J. 2004;5:1–7.
2. Lankin VZ, Tikhaze A, Kukharchuk V, Belenkov Y. Antioxidant decreases the intensification of LDL *in vivo* peroxidation during therapy with statins. Mol Cell Biochem. 2003;249:126–40.
3. Cooke JP. Nutriceuticals for cardiovascular health. Am. J. Cardiol. 1998;82:433–65.
4. Kerharo J, Bouquet A. Plantes Medicinales et toxiques, de la Cote d'Ivoire et Haute – Volta, Orstom edit. Paris. Vigot. 1950;295. French
5. Bouquet A, Debray M. Plantes Medicinales de Cote d'Ivoire. Doc Otrstom. Bufkill, H.M. 1985;32:63. French
6. Adisa RA, Choudary MI, Olorunsogo OO. Hypoglycemic activity of *Buchholzia coriacea* (Capparaceae) seeds in streptozotocin – induced diabetic rats and mice. Exp. Toxicol. Pathol. 2011;63(7-8):619-625.
7. Ajaiyeoba EO. Phytochemical and antimicrobial studies of *Gynandropsis gynandra* and *Buccholzia coriacea*. Afri. J. Biomed. Res. 2000;3:161–165.
8. Ajaiyeoba EO, Onocha PA, Olanrewaju O. *In vitro* anthelmintic properties of *Buccholzia coriacea* and *Gynandropsis gynandra* extracts. Pharmaceutic. Biol. 2001;39(1):28-31.
9. Ajaiyeoba EO, Onocha PA, Nwozo SO, Sama W. Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. Fitoterapia. 2003;(74):706-709.
10. Okoli BJ, Okere OS, Adeyemo SO. The antiplasmodial activity of *Buccholzia coriacea*. Jour of Medical and Applied Biosciences. 2010;2:21–29.
11. Mbata TI, Duru CM, Onwumelu HA. Antibacterial activity of crude seed extracts of *Buchholzia coriacea* E. on some pathogenic bacteria. *Journal of Developmental* Biology and Tissue Engineering. 2009;1(1):001-005
12. Evans WC, Trease GE. Test book of pharmacognosy. 11th ed.: Balliere Tindall and Macmillan Publishers, London. 1989;pp.176–180
13. Erukainure OL, Abovwe JA, Adefegha AS, Egwuche RU, Fafunso MA. Antilipemic and hypocholesteremic activities of *Globimetula braunii* in rats. Exp. Toxicol. Pathol. 2011;63(7/8):657-661.
14. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic Determination of Total Serum Cholesterol. Clin. Chem. 1974;20(4):470-5.
15. Fossati P, Prencipe L. Serum Triglycerides Determined Colorimetrically with an Enzyme That Produces Hydrogen Peroxide. Clin. Chem. Oct 1982;28(10):2077-80.
16. Grillo F, Izzo C, Mazzotti G, Murador E. Improved Method for Determination of High - Density-Lipoprotein Cholesterol II. Enzymatic Determination of Cholesterol in High - Density Lipoprotein Fractions with a Sensitive Reagent. Clin. Chem. 1982;28(10):2077-80

17. Friedewald CoT, Levy RT, Friedrukson OS. Estimation of the concentration of LDL in plasma without the use of preparative concentrating Clin. Chem. 1972(18):499-502.
18. Varshney R, Kale RK. Effect of calmodulin antagonist on radiation 306 induced – lipid peroxidation in microsomes. Int. J. Rad. Biol. 1990;58(5):733-43.
19. Mazumdar PM, Farswan M, Parcha V. Effect of an isolated active compound (Cg- 1) of *Cassia glauca* on blood glucose, lipid profile and atherogenic index in diabetic rats. Indian J. Pharmacol. 2009;41(4):182-186.
20. Ross R. Atherosclerosis – an inflammatory disease. N. Engl. J. Med. 1999;340:115-126.
21. Moghadasian MH, Mancini GB, Frohlich JJ. Pharmacotherapy of hypercholesterolaemia: Statins in clinical practice. Expert Opin. Pharmacother. May 2000;1(4):683-95.
22. Durrington P. Dyslipidaemia. The Lancet August. 2003;362(9385):717-731.
23. Ishikawa Y, Sugiyama H, Stylianou E, Kitamura M. Bioflavonoid quercetin inhibits interleukin-1-induced transcriptional expression of monocyte chemoattractant protein-1 in glomerular cells via suppression of nuclear factor-B. J Am Soc Nephrol. 1999;10:2290–6.
24. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet. 1993;342:1007-11.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=231&id=3&aid=1366