

Anti – atherogenic Activity of Ethanolic Extract of *Bucchozia coriacea* Seeds on Hypercholesterolemic Rats

Omolekan, T.O.^{1*}, Olaiya C.O.O.²

^{1,2} Laboratories of Nutritional and Industrial Biochemistry Research, Department of Biochemistry, University of Ibadan, Oyo State, Nigeria

ABSTRACT

Aims: To scientific determine the phytochemical constituents of EEBC powdered seeds and the effect of ethanolic extract of *B. coriacea* (EEBC) seeds powder on the serum lipid profile, serum atherogenic index, and the extent of 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, dissected and the blood was collected. Lipid profile and malondialdehyde levels were measured. The anti – atherogenic effect was assessed by the atherogenic index. The qualitative phytochemical screening of EEBC seeds powder was performed. Data were statistically analyzed by one way ANOVA followed by post hoc Duncan multiple range comparison tests.

RESULTS: The qualitative phytochemical screening of EEBC seeds revealed the presence of 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 which showed a significant ($P=.05$) increase compared with normal control. There was a significant ($P=.05$) increase in the serum HDL - cholesterol by 67.9% in extract treated groups compared with the experimental group which showed a significant ($P=.05$) decrease in comparison with the normal control. A significant ($P=.05$) decrease from 36% in the amount of serum malondialdehyde produced in the extract treated groups compared with the experimental control which showed a

* Tel.: +2348058745232.

E-mail address: toluomon@yahoo.com.

significant ($P=.05$) increase compared with the normal control.

Conclusion: EEBC seed powder has potent anti- atherogenic properties.

Keywords: Ethanolic extract of *Bucchozia 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 system 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 *Bucchozia 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 has been reported. The phytochemical screening of the stem barks reveals the presence lupeol, flavonoid, terpenes and steroids (8).

There has been a dearth of scientific evidences of its anti – hypercholesterolemic and anti – atherogenic activities. Therefore, this study has been undertaken to evaluate the anti – hypercholesterolemic and anti – atherogenic activities of ethanolic extract of *B. coriacea* seeds powder in cholesterol – induced hypercholesterolemic rats.

2. MATERIAL 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, Thiobarbituric acid, Tris – KCl buffer and

Tricarboxylic acid were obtained from Sigma Chemical Co., Saint Louis, 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, University of Ibadan. A voucher specimen was already there.

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 powered and weighed. 4 kg of the powered 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°C until the solvent part was evaporated. The dark brown residue was dissolved in small amount of dimethyl sulphuroxide (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, 1989 (12). Frothing test for saponins, salkowskistest for terpenoids, Liebermann – Burchard tests for steroids, ferric chloride test for tannins, Keller – Killiani test for cardiac glycosides, Dragendorff's and Mayer's test for alkaloids, Fehling's test for reducing sugars. Ammonia test for the detection of flavonoids were also performed.

2.5 Animals

Healthy male albino rats weighing between 75 -175kg 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

Twenty male albino rats were used and all fed with normal diet. They 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 for EEBC was obtained from its LD₅₀ study and acute toxicity test as reported by Adisa *et. al.*, 2010 (6). The dosage for cholesterol, cholestyramine and the period of treatment were selected on the basis of previous studies by Erukainure *et. al.*, 2011 (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, 1990 (18).

2.9 Atherogenic index calculation

Atherogenic Index was calculated by using the following formular (19),

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

2.10 Statistical analysis

The statistical significance between groups was analysed 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 serum total cholesterol, triglyceride, low density lipoprotein (LDL) cholesterol, atherogenic index "Table 1" in the extract and standard drug treated groups compared to experimental group which showed a significant ($P=.05$) increase as compared to normal control. 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 which showed a significant ($P=.05$) decrease in comparison with the normal control.

Table 1: Effect of ethanolic 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.

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 which showed a significant ($P=.05$) increase in comparison with the normal control as shown in "Figure 1".

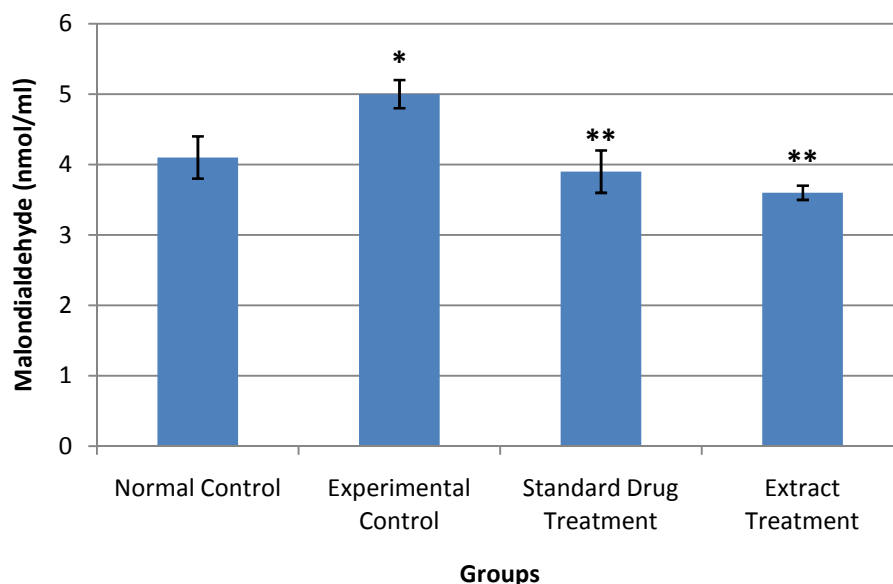


Figure 1: Effect of ethanolic extract of *B. coriacea* and cholestyramine on serum lipid peroxidation

Values are expressed as $MEAN \pm SEM$ ($n=5$), 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.

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 adiposities (20). Therefore the LDL – cholesterol particles are not efficiently removed from the blood thereby leading to an increased chance of the oxidized LDL – cholesterol invading the lining of the arteries and participating in plaque formation.

Insult of the endothelium of the artery through mechanical trauma and oxidative stress facilitates the deposition of immune complexes that will further lead to oxidation of polysaturated fatty acids in the LDL – cholesterol (20) and subsequently lipid peroxidation in the artery. The product of lipid peroxidation react with the lysine residues on the apolipoprotein – 100 of LDL – cholesterol thereby modifying its structure and altering the charge on the molecule making it no recognizable by normal receptors. It is instead recognized by “scavenger” receptors on macrophages and smooth muscles and these are capable of releasing its stock of cholesterol and cholesterol ester.

Clinical studies 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). The reduction in the serum total cholesterol in the extract and standard drug treatment groups may be due to the competitive inhibition of the adsorption of dietary cholesterol in the intestine of the animal or through the stimulation of biliary excretion of bile acid or cholesterol in feces (22). The phytochemical

screening of ethanolic extract of *B. coriacea* seeds shows the presence of saponins which are glucosides of steroids and steroid alkaloid. They 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 ethanolic extract of *B. coriacea* seeds powder has significant anti – atherogenic activity when administered to hypercholesterolemic rats. The anti – atherogenic effects of ethanolic 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 processing of the ethanolic extract of *B. coriacea* seeds is required.

COMPETING INTERESTS

No competing interest was declared by the authors.

AUTHORS' CONTRIBUTIONS

Omolekan T.O. designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Dr. Olaiya C.O.O. managed the analyses of the study. These two authors read and approved the final manuscript.

REFERENCES

- 1) Moarreaf A.R. Risk factor modification of coronary artery disease. SEM J 2004;5:1–7.
- 2) Lankin V.Z., Tikhaze A., Kukharchuk V., et. al. Antioxidant decreases the intensification of LDL in vivo peroxidation during therapy with statins. Mol Cell Biochem 2003; 249:126–40.
- 3) Cooke, J.P. Nutraceuticals for cardiovascular health. Am J Cardiol 1998; 82:433–65.
- 4) Kerharo J. and Bouquet A. (1950). Planter Medicinales et toxiquet, de la Cote d'Ivoire et Heute – volti, Ministere de la France d'iytremerum. Paris: Office de la Recherche Scientifique Outramer. p. 289. French
- 5) Bouquet A. and Debray M. (1974). Plantes Madiinalia de Cote d'Ivoire. Doc Orstrom; 32: 63. French
- 6) Adisa R.A., Choudary M.I. and Olorunsogo O.O. hypoglycemic activity of *Buchholzia coriacea* (Capparaceae) seeds in streptozotocin – induced diabetic rats and mice. Exp. Toxicol. Pathol (2010), doi:10.1016j.erp.2010.05.002

- 259 7) Ajaiyeoba E.O. Phytochemical and antimicrobial studies of *Gynandropsis gynandra*
260 and *Bucchozia coriacea*. Afri. J. Biomed. Res. 2000: 5, 125 – 128.
261
262
- 263 8) Ajaiyeoba E.O., Onocha P.A. and Olanrewaju O. *In vitro* anthelmintic properties of
264 *Bucchozia coriacea* and *Gynandropsis gynandra* extracts. Pharmaceutic Biol. 2001:
265 39
266
- 267 9) Ajaiyeoba E.O., Onocha P.A., Nwozo S.O. and Sama W. Antimicrobial and
268 cytotoxicity evaluation of *Bucchozia coriacea* stem bark. Filoterapia (2003). 74, 706
269 – 709.
270
271
- 272 10) Okoli B.J., Okere O.S. and Adeyemo S.O. The antiplasmodial activity of *Bucchozia*
273 *coriacea*. Jour of Medical and Applied Biosciences 2010. Vol.2
274
- 275 11) Mbata T.I., Duru C. M. and Onwumelu H. A. Antibacterial activity of crude seed
276 extracts of *Bucchozia coriacea* E. on some pathogenic bacteria. *Journal of*
277 *Developmental Biology and Tissue Engineering* 2009: 1 (1): 001–005,
278 www.academicjournals.org/ajdbte
279
- 280 12) Evans W.C. and Trease G. E. Test book of pharmacognosy. 11th ed.: Balliere
281 Tindall and Macmillan Publishers, London, 1989; pp. 176 - 180
282
283
- 284 13) Erukainure O.L., Abovwe J.A., Adefegha A.S., *et. al.* Antilipemic and
285 hypocholesteremic activities of *Globimetula braunii* in rats. Experimental and
286 Toxicologic Pathology 2011, 63 (7 – 8): 657 – 661.
287
- 288 14) Allain C.C., Poon L.S., Chan C.S.G., *et. al.* Enzymatic Determination of Total Serum
289 Cholesterol. Clin. Chem. 1974; 20(4): 470-5.
290
- 291 15) Fossati P. and Prencipe L. Serum Triglycerides Determined Colorimetrically with an
292 Enzyme That Produces Hydrogen Peroxide. 1982. Clin. Chem. 1982; 28(10): 2077-
293 80.
294
295
- 296 16) Grillo F., Izzo C., Mazzotti G., *et. al.* Improved Method for Determination of High-
297 Density-Lipoprotein Cholesterol II. Enzymatic Determination of Cholesterol in High-
298 Density Lipoprotein Fractions with a Sensitive Reagent. 1981. Clin. Chem. 27(3).
299 375-9.
300
301
- 302 17) Friedewald, Co. T, R.T. Levy and O.S. Friedrukson. Estimation of the concentration
303 of LDL in plasma without the use of preparative concentrating Clin. Chem., 1972: 18:
304 499-502.

305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332

- 18) Varshney R. and Kale R. K. Effect of calmodulin antagonist on radiation induced – lipid peroxidation in microsomes. Int. J. Rad. Biol. 1990; 58(5): 733 – 43.
- 19) Mazumdar P.M., 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 M.H., Mancini G.B. and Frohlich J.J. Pharmacotherapy of hypercholesterolaemia: Statins in clinical practice. Expert Opin. Pharmacother., 2000: 1. Pg 77 – 80.
- 22) Durrington P., 2003. Dyslipidaemia. The Lancet 362 (9385): 717 – 731.
- 23) Ishikawa Y., Sugiyama H., Stylianou E., et. al. 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 M.G., Feskens E.J., Hollman P.C., et. al. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993; 342:1007–11.

333
334