Antihypercholesterolemic activity of ethanolic extract of *Buchholzia coriacea* in rats

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Abstract

Background: Hypercholesterolemia is a condition characterised with high level of cholesterol in the blood.

Objectives: The effect of ethanolic extract of *Buchholzia coriacea* (EEBC) on the lipid profile levels and extent of lipid peroxidation in hypercholesterolemic albino rats was investigated in this study.

Methods: Thirty albino rats were divided into six different groups which consist of group 1 (control), group 2 (hypercholesterolemic rats), group 3 (hypercholesterolemic rats treated with ethanolic extract of EEBC), group 4 (hypercholesterolemic rats treated with questran), group 5 (normal rats treated with EEBC) and group 6 (normal rats treated with questran). The rats were sacrificed at the end of the sixth week and assay conducted for Aspartate Transaminase (AST), Alanine Transaminase (ALT), lipid profile and biomarker of oxidative stress.

Results: The serum and liver total cholesterol and LDL – cholesterol levels as well as lipid peroxidation in the EEBC–treated hypercholesterolemic rats were significantly reduced ($p < 0.05$) when compared with the untreated hypercholesterolemic rats. The activities of AST and ALT in EEBC – treated hypercholesterolemic rats were not significantly different ($p > 0.05$) from the control.

Conclusions: The results suggest that *Buchholzia coriacea* seeds contain potent antihypercholesterolemic agent which may find clinical application in ameliorating hypercholesterolemia and its attendant complications.

Keywords: *Buchholzia coriacea*, hypercholesterolemia, oxidative stress

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Introduction

Hypercholesterolemia is a condition characterized with high level of cholesterol in the blood of animals. It is caused by a variety of factors which can either be environmental or genetic.[1] It is a major risk factor in disease conditions like atherosclerosis, hypertension and other cardiovascular diseases.[2] Epidemiological and metabolic studies have shown that serum cholesterol levels are strongly influenced by the amount and type of dietary fatty acids as well as the daily intake of cholesterol both of which are dietary factors.[3] Herbal medicine, which is the use of plants for therapeutic or medicinal purpose has been practiced by all cultures throughout history.[4] This practice is becoming common in Africa because it is relatively cheap, affordable and claimed to be effective. One of such herbal (plants) remedies is *Buchholzia coriacea* (Capparaceae, common name – magic/wonderful kola, English name – musk tree).

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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.[5] It is therefore turned into a pulp for inhalation or into snuff to relieve head ache, bronchitis, sinusitis, otitis and nasal congestion in Ivory Coast. It is used in the treatment of small pox and skin itch in Gabon and ear-ache in Ghana.[6] Topical application of the leaves and fruits on the body is used to relieve fever in Sierra Leone while the seeds are applied over the stomach to manage difficulty in child birth.[7] In Nigeria, the Edos boil and eat the fruit after storage for a few days.[8] The seed decoction is usually made in lime or local gin for the treatment of diabetes mellitus, hypertension, cold by traditional healers. Its hypoglycemic activity,[8] phytochemical analysis, proximate composition and antimicrobial studies[9, 10] anthelmintic properties,[11] cytotoxicity evaluation,[12] antiplasmodial activity,[10] antibacterial activity[13] of various parts of the plants have been reported. Its phytochemical screening revealed that the seeds contain alkaloids, stem barks contain lupeol, flavonoid, terpenes and steroids.[11] This study was designed to investigate the effects of the ethanolic extract of *Buchholzia coriacea* (EEBC) on the lipid profile, liver activity and the degree of lipid

peroxidation in the serum and tissues of normal and hypercholesterolemic wistar albino rats.

Methods

Plant Material

Fresh seeds of *Buchholzia coriacea* were purchased at Bodija market in Ibadan, Oyo State, Nigeria. Confirmatory identification of the plant seeds was done at the herbarium of Botany Department, University of Ibadan where voucher specimen was deposited. 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. Four kilograms of the powdered seeds were macerated in ethanol (80% v/v) for 48 hours with intermittent shaking. The extracts were filtered and concentrated using a rotary evaporator and then stored in a refrigerator for subsequent use.

Experimental design

Thirty male albino rats weighing between 75 – 175 g were used for this investigation. They were obtained from the Central Animal House of the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan, Nigeria. They were acclimatized for 2 weeks on normal diet of pellitized mouse chow, with water given *ad libitum* at room temperature with a 12-h light and dark cycle before the commencement of the experiment. They were divided into six groups, each consisting of five animals.

Group 1 = corn oil only 0.3 ml (control)
Group 2 = questran (cholestyramine) (260 mg/kg body weight)
Group 3 = cholesterol only (40 mg/kg body weight)
Group 4 = cholesterol + questran (cholestyramine) (260 mg/kg body weight)
Group 5 = cholesterol + ethanolic extract of *Buchholzia coriacea* (EEBC) (250 mg/kg body weight)
Group 6 = ethanolic extract of *Buchholzia coriacea* (EEBC) (250 mg/kg body weight)

Corn oil served as the vehicle for EEBC, questran (cholestyramine) and cholesterol. EEBC was dissolved in 1 ml of dimethyl sulphoxide (DMSO). Cholestyramine, EEBC and cholesterol were administered five times a week for six consecutive weeks. The dose of cholestyramine, cholesterol and the period of treatment were selected on the basis of previous studies by Okoro,[14] while that of EEBC was from Adisa et al. [8] At the end of the sixth week, the rats were fasted overnight and sacrificed.

Biochemical assays

The serum levels of ALT, AST were assayed by the method of Reitman and Frankel.[15] Triglyceride was measured by colorimetric method as described by Fossati and Prencipe,[16] total cholesterol levels were measured by a method of enzyme hydrolysis as described by Richmond,[17] and Roschlau *et. al.*[18] HDL – cholesterol was determined in both the serum and post mitochondrial fraction (supernatant) by the method of Jacobs *et. al,*[19] and LDL – cholesterol level was determined by the method of Friedewald *et. al.*[20]

Protein estimation

Serum total protein was estimated spectrophotometrically according to the method described by Gornal et al,[21] with slight modifications, using bovine serum albumin as standard.

Assay for biomarker of oxidative stress

Serum lipid peroxidation was determined spectrophotometrically by the thiobabituric acid reactive substances (TBARS) method as described by Varshney and Kale,[22] and was expressed in terms of TBARS formed per mg protein.

Stastistical analysis

The results are presented as mean ± SD of five animals per group and statistical levels of significance were determined using the one – way ANOVA followed by the post hoc Duncan multiple range tests for analysis using Statistica package, version 7.1, Statsoft Inc. ($p < 0.05$ was considered significant).

Results

Table 1 shows the effects of EEBC, questran, on body weights and the relative weight of visceral organs in normal and hypercholesterolemic rats.

Table 1: Effects of EEBC, questran, on body weights and the relative weight of visceral organs of the normal and hypercholesterolemic rats

Treatment groups	Initial weight (g)	Final weight (g)	Weight gain	Kidney weight (g)	Liver weight (g)	Heart weight (g)	Relative organ weight = organ weght/ body weight (%)		
							Kidney	Liver	Heart
Control	75±0.0	175±10.31	100±18.76[a]	0.59±0.39	5.53±0.08	0.60±0.80[a]	0.34±0.14	3.16±0.08	0.34±0.01
Qu*	150±0.0	195±7.56	45±10.81	0.74±0.18	6.50±1.13	0.62±0.14	0.38±0.04	3.33±0.20	0.32±0.04
Ch*	125±0.0	348±18.75	223±7.81[ab]	0.82±0.07[b]	6.72±0.08	0.96±0.06[ab]	0.33±0.02	2.71±0.19	0.39±0.39
Ch + Qu*	175±0.0	215±9.81	40±3.41[ab]	0.06±0.08	5.22±0.58	0.65±0.09	0.28±0.02	2.43±0.19	0.30±0.02
Ch+ EEBC*	150±0.0	185±1.52	35±17.01[b]	0.59±0.12[b]	5.85±0.96	0.58±0.10[b]	0.32±0.03	3.16±0.20	0.31±0.05
EEBC*	100±0.0	120±0.93	20±6.02	0.50±0.07	3.89±0.67	0.48±0.20	0.42±0.05	3.24±0.22	0.40±0.1

Values are mean ± SD of five rats in each group; means with the same letters are significantly different ($p < 0.05$) from the other * Qu = questran; Ch = cholesterol; EEBC = ethanolic extract of *Buchholzia coriacea* (EEBC)

Although cholesterol caused a significant increase in the body weight gain of hypercholesterolemic rats compared with the control, administration of EEBC to hypercholesterolemic rats caused a 91.0% significant reduction ($p < 0.05$) in their body weight gain when compared with the untreated hypercholesterolemic rats.

There was no significant difference ($p > 0.05$) in the relative organ weight of the organs of all groups except for a significant reduction ($p < 0.05$) in the relative liver weight of hypercholesterolemic rats compared with the control.

Biochemical parameters

Figures 1, 2, 3 and 4 show the effect of EEBC, Questran in the serum, liver and kidney of the normal and hypercholesterolemic rats.

The concentration of total cholesterol, triglyceride, HDL – cholesterol and LDL – cholesterol in the serum and liver of the hypercholesterolemic rats which were significantly increased ($p < 0.05$) when compared with the control. The serum and liver total cholesterol concentrations of the EEBC treated – hypercholesterolemic rats were significantly reduced ($p < 0.05$) by 45.9% and 44.2% respectively, when compared with the untreated hypercholesterolemic rats. The liver and serum LDL – cholesterol concentration of EEBC treated – hypercholesterolemic rats were significantly reduced ($p < 0.05$) by 14.8% and 73.5% respectively, in comparison with the untreated hypercholesterolemic rats. The triglyceride concentration in the liver and serum of hypercholesterolemic rats treated with EEBC were significantly reduced by 74.6% and 48.4% respectively when compared with the untreated hypercholesterolemic rats but there was a significant increase ($p < 0.05$) in kidney triglyceride concentration of the hypercholesterolemic rats treated with EEBC when compared with the hypercholesterolemic rats. Significant increases ($p < 0.05$) were noticed in the liver and kidney HDL – cholesterol of hypercholesterolemic rats treated with EEBC when compared with the untreated hypercholesterolemic rats but the serum HDL – cholesterol in the hypercholesterolemic rats treated with EEBC was significantly reduced when compared with untreated hypercholesterolemic rats. Similar results were seen in the lipid profile levels of hypercholesterolemic rats treated with questran when compared with untreated hypercholesterolemic rats as that of hypercholesterolemic treated with EEBC except that significant decrease in the serum, liver and kidney HDL – cholesterol were seen in the hypercholesterolemic rats treated with questran.

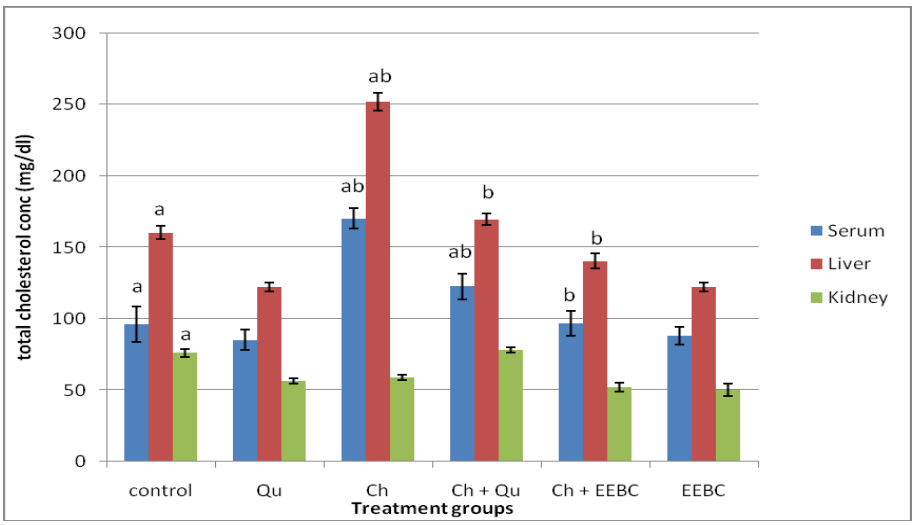

Figure 1: Effects of EEBC, questran, on the serum, liver and kidney total cholesterol levels of the normal and hypercholesterolemic rats

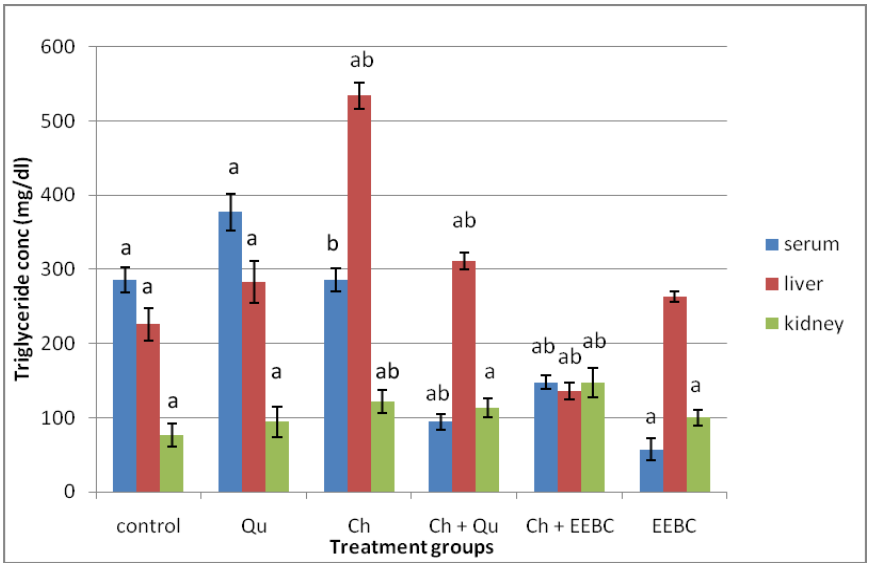

Figure 2: Effects of EEBC, questran, on the serum, liver and kidney triglyceride levels of the normal and hypercholesterolemic rats

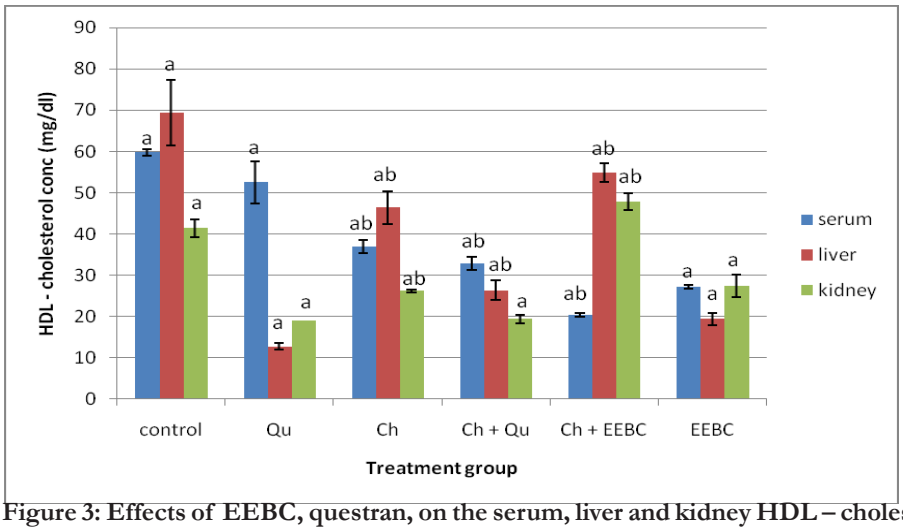

Figure 3: Effects of EEBC, questran, on the serum, liver and kidney HDL – cholesterol levels of the normal and hypercholesterolemic rats

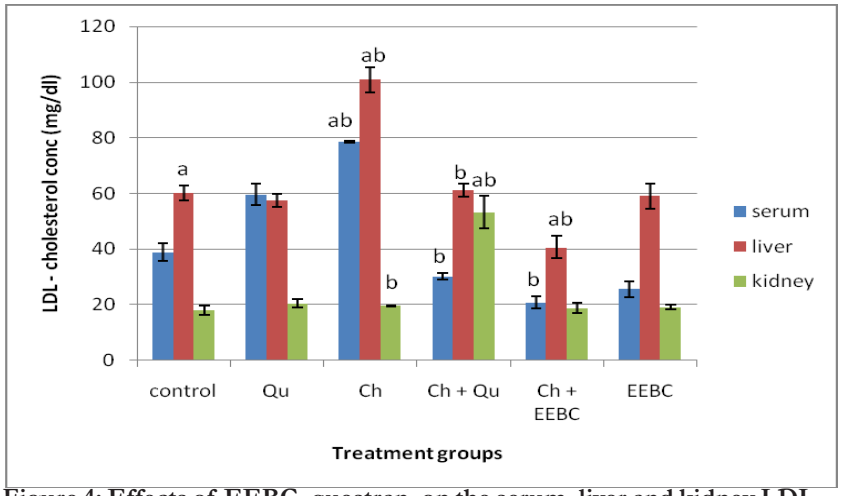

Figure 4: Effects of EEBC, questran, on the serum, liver and kidney LDL – cholesterol levels of the normal and hypercholesterolemic rats

Biomarker of oxidative stress and liver damage

Table 2 shows the effects of EEBC, questran, on degree of lipid peroxidation, AST and ALT levels in normal and hypercholesterolemic rats

The extent of lipid peroxidation in the serum and liver of hypercholesterolemic rats when compared with control was significantly increased ($p < 0.05$) by 18.0% and 50.9% respectively. This was significantly reduced ($p < 0.05$) by 28.2% and 30.2% in hypercholesterolemic rats treated with EEBC respectively. There was a similar reduction in hypercholesterolemic rats treated with questran. It was observed that lipid peroxidation in the hypercholesterlemic rats treated with EEBC and those treated with questran were not significantly different ($p > 0.05$) from the control.

The activities of AST and ALT in the serum of hypercholesterolemic rats were not significantly different ($p > 0.05$) when compared with that of normal rats. Also the activities of AST and ALT in the serum of EEBC – treated hypercholesterolemic rats were not significantly different ($p > 0.05$) from the normal and hypercholesterolemic rats.

Table 2: Effects of EEBC on biomarker of oxidative stress and liver damage*

		Control	Questran	Cholesterol	Cholesterol + Questran	Cholesterol + EEBC	EEBC
Lipid peroxidation	Serum	4.1±0.3^{a}	3.5±2.0^{a}	5.0±0.2^{ab}	3.9±0.3^{b}	3.9±0.1^{b}	4.1±0.2
TBARS(nmol/ mg protein)	Liver	2.7±0.7^{a}	2.1±0.	5.6±0.4^{ab}	3.8±0.2^{b}	4.3±0.4^{b}	3.3±0.5
Aspartate Transaminase (U/l)	Serum	54.2±5.3	49.4±1.5	35.2±5.0	26.2±2.7	39.4±2.4	31.2±2.6
Alanine Transaminase (U/l)	Serum	12.4±3.6^{a}	10.2±2.9	9.0±3.6	5.0±1.6	5.6±1.8^{a}	11.2±2.8

*Values are mean±SD of five rats in each group; mean values with superscripts a and b are significantly different ($p < 0.05$) from the control and hypercholesterolemia (cholesterol-fed rats) respectively per parameter.

Discussion

The process of atherogenesis has been considered by many to be as a result of the acccumulation of lipids within the artery wall. High plasma concentrations of cholesterol (LDL – cholesterol in particular) are one of the risk factors for atherosclerosis and cardiovascular diseases.[23] Medicinal plants are gradually gaining wide acceptability worldwide because they are potential sources of bioactive agents used as pharmaceuticals.[24] The ethanolic extract of *Buchholzia coriacea* has been shown to reduce fasting blood sugar (FBS) in streptozotocin – induced diabetic rats and mice.[8] The present study investigated the antihypercholesterolemic effects of ethanolic extract of *Buchholzia coriacea* in cholesterol – induced hypercholesterolemic rats.

The weight gained observed in the hypercholesterolemic rats might be as a result of the high rate of lipid deposit on the adipose tissue of the rats. This might further be confirmed by the insignificant changes observed in the relative organ weight of the hypercholesterolemic rats when compared with the control. The increase in the total – cholesterol, triglycerides and LDL – cholesterol concentration in the serum of hypercholesterolemic rats is in agreement with a previous report, [25] confirming that serum cholesterol levels are strongly influenced by the amount and type of dietary fatty acid as well as the daily cholesterol intake.[3] The significant decrease observed in the serum and liver total cholesterol in the EEBC – treated and questran – treated hypercholesterolemic rats may be due to the competitive inhibition of the absorption of dietary cholesterol in the intestine of the animal or through the stimulation of biliary excretion of bile acid or cholesterol in faeces.[26] LDL – cholesterol which has been reported to be the major transporter of cholesterol and also encourage the deposit of cholesterol in the arteries[27] was significantly lowered in the hypercholesterolemic rats treated with EEBC and questran. Clinical studies have shown that decreasing plasma LDL – cholesterol significantly reduces coronary heart disease morbidity and mortality, and also decreases the progression of atherosclerosic lesions.[28] In earlier studies, EEBC was able to reduce the total – cholesterol and triglycerides in streptozotocin – induced diabetic rats.[8] The plant extract has alkaloids, saponins, cardiac glycosides and flavone glycosides.[10,11] Saponins are glycosides of steroids and are also triterpenes found in plants. They bear a chemical similarity with cholesterol and may either block the absorption of cholesterol or enhance its excretion. Lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, reduced erythrocyte survival and perturbation in lipid fluidity.[29] The significant reduction in lipid peroxidation ($p<0.05$) by 28.2% and 30.2% in hypercholesterolemic rats treated with EEBC in this study shows that EEBC is rich in antioxidant compounds.

Conclusion

This study therefore suggests that *B. coriacea* seeds contain potent antihypercholesterolemic agent which either act synergestically or individually in ameliorating hypercholesterolemia and its secondary complications.

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