Experimental and Toxicologic Pathology 63 (2011) 657–661

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Antilipemic and hypocholesteremic activities of *Globimetula braunii* in rats

Ochuko L. Erukainure [a,*], Joseph A. Abovwe [b], Adeniyi S. Adefegha [c], Rabiat U. Egwuche [d], Michael A. Fafunso [b]

[a] Food Technology Division, Federal Institute of Industrial Research, P.M.B. 21023, Ikeja, Lagos, Nigeria
[b] Department of Biochemistry, University of Ibadan, Ibadan, Nigeria
[c] Department of Biochemistry, Federal University of Technology, Akure, Nigeria
[d] Department of Chemistry/Biochemistry, IBB University, Lapai, Nigeria

ARTICLE INFO

Article history:
Received 16 February 2010
Accepted 16 May 2010

Keywords:
Cholesterol
Hypercholesteremia
Lipid peroxidation
Cholestyramine
CVD
LDL

ABSTRACT

Antilipemic/hypocholestermic activities of the methanolic extracts of *Globimetula braunii* were studied in the tissues of normo and hypercholesteremic rats. Hypercholesteremia was induced in the rats by feeding with dietary cholesterol at a dose of 40 mg/kg body weight. A significant increase ($p < 0.05$) was observed in the body and the visceral organs relative weights in all the groups. There was a significant decrease in HDL-cholesterol and a significant increase in the levels of LDL, total cholesterol and triglycerides in hypercholesteremic rats when compared to normo rats. Administration of the methanolic extracts restored the elevated levels of serum lipids to normal. The methanolic extracts of *G. braunii* produced a significant ($p < 0.05$) decrease in the levels of total cholesterol, triglyceride, LDL-cholesterol and lipid peroxidation of the extract treated group compared to hypercholesteremic control. There was a significant ($p < 0.05$) increase in the levels of HDL-cholesterol as compared to hypercholesteremic control. The results obtained from this study are indications of the antilipemic and hypocholestermic activities of *G. braunii* as well as its cardio-protective potential in normo and hypercholesteremia conditions.

1. Introduction

From the beginning of the last century, evidences on the cholesterol-lowering properties of medicinal plants have been accumulating. The importance of such investigations, are confirmed in the treatment of obesity, diabetes mellitus, heart failure, and atherosclerosis (Ross, 1986). Scientists have reported the role of medicinal plants in the control of elevated serum cholesterol, and the reduction of morbidity and mortality due to vascular diseases associated with it.

The elevation of serum total cholesterol and low-density lipoprotein (LDL) cholesterol as well as alteration of other lipid parameters has been implicated as a primary risk factor for cardiovascular disease (Jensen et al., 1988; Motta et al., 2001; Maghrani et al., 2004; Edijala et al., 2005; Jaouad et al., 2006). Varieties of plants have been used traditionally in the treatment of various cardiovascular diseases.

The *Globimetula braunii* is a parasitic plant, commonly known as mistletoe. It grows on the branches of trees, where it forms pendent bushes, 2–5 ft in diameter. It belongs to the family of *Loranthaceae* (Burkhill, 1985). The plant is widely distributed in tropical African countries such as Ghana, Cameroun and Nigeria. It is a major ingredient used in herbal medicines. It has gained importance as being useful in the treatment of headache, rheumatic pains and pulmonary troubles (Burkhill, 1985). Leaves, fruits and flowers of the plant are used to treat hypertension, while the roots are employed for other therapeutic uses such as ulcer and cancer treatment (Burkhill, 1985). There have been reports on the phytochemical and antimicrobial properties of mistletoe (Osadebe et al., 2004). Folk lore has it that *G. braunii* burns fat when taken with local gin, thus may reduce the risk of cardiovascular disease. However, information is scanty on the mechanism by which the plant brings about this effect.

This study seeks to report that *G. braunii* may have cardiovascular protective antilipemic/hypocholestermic activities by depressing the level of triglyceride, LDL-cholesterol and increase HDL-cholesterol of the normo and hypercholesteremic albino rats.

2. Materials and methods

2.1. Plant material

The leaves of *G. braunii* were purchased from a local market in Ibadan, Nigeria and identified at Herbarium of the Department of Botany, University of Ibadan, Ibadan, Nigeria. They were air dried and grounded to fine texture with laboratory mill and were

* Corresponding author. Tel.: +234 8062179388 (mobile).
E-mail address: loreks@yahoo.co.uk (O.L. Erukainure).

0940-2993/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.etp.2010.05.007

Table 1
Effect of *Globimetula braunii* on body weight (g) and the relative weight (g/100 g b.w.) of visceral organs in cholesterol-fed rats.

Grouping	Initial weight (g)	Final weight (g)	Weight gained (g)	Absolute kidney weight (g)	Absolute liver weight (g)	Absolute heart weight (g)	Relative weight/body weight (%)		
							Kidney	Liver	Heart
Control	287.50 ± 23.39	317.50 ± 25.97	30.00 ± 2.58	1.4 ± 0.39	8.72 ± 0.78	0.91 ± 0.80	0.45 ± 0.14	2.75 ± 0.08	0.29 ± 0.01
CH	115.50 ± 17.62	238.00 ± 16.43	122.5 ± 1.19[a]	0.90 ± 0.07	6.77 ± 0.80	0.77 ± 0.06	0.38 ± 0.02	2.84 ± 0.19	0.32 ± 0.05
GB	75.00 ± 27.00	205.00 ± 10.00	130.00 ± 17.14[a]	0.88 ± 0.08	7.58 ± 0.23	0.73 ± 0.09	0.43 ± 0.03	3.70 ± 0.14[a]	0.36 ± 0.02
CA	150.50 ± 11.99	203.25 ± 18.57	53.25 ± 6.58[a]	0.93 ± 0.18	7.14 ± 1.13	0.76 ± 0.14	0.46 ± 0.04	3.51 ± 0.20[b]	0.37 ± 0.04
CH–CA	150.00 ± 23.39	205.00 ± 10.00	55.13 ± 13.39	0.88 ± 0.08	5.91 ± 0.58	0.76 ± 0.09	0.43 ± 0.05	2.88 ± 0.22	0.37 ± 0.05
CH–GB	92.50 ± 11.18	199.00 ± 13.30	106.50 ± 2.12[b]	0.85 ± 0.06	6.96 ± 0.37	0.73 ± 0.06	0.43 ± 0.05	3.50 ± 0.09	0.37 ± 0.04

Values are given as mean ± SEM. *Key*: CA = cholestyramine; CH = cholesterol; GB = *G. braunii*.
[a] Significantly different from control ($p < 0.05$).
[b] Significantly different from CH group ($p < 0.05$).

extracted with methanol for 8-10 h, distilled and concentrated using steam bath and then stored for subsequent use.

2.2. Animals

Thirty male albino rats of age 40–60 days old were used for the present investigation. They were reared at the Central Animal House of the Institute of Advance Medical Research and Training (IMRAT), College Hospital, Ibadan, Nigeria.

They were acclimatized for two weeks on normal diet of pelletized 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 = cholesterol
GROUP 3 = *G. braunii* + pellet
GROUP 4 = cholestyramine
GROUP 5 = cholesterol + cholestyramine
GROUP 6 = cholesterol + *G. braunii*

Corn oil served as the vehicle for *G. braunii*, cholestyramine and cholesterol. *G. braunii* was dissolved in small amount of dimethy sulphuroxide (DMSO). Dietary cholesterol was administered orally at a dose of 40 mg/kg body weight. Cholestyramine, a bile acid sequestrant was administered orally as a therapeutic dose of 0.26 g/kg body weight and methanolic extract of *G. braunii* was administered at a dose of 250 mg/kg body weight in 0.3 ml of the vehicle. Cholestyramine, *G. braunii* and cholesterol were administered five times a week for six consecutive weeks. The dose of cholestyramine, dietary cholesterol and the period of treatment were selected on the basis of previous studies by Okoro (2008) on the hypocholesteremic effect of *Cucurbita maxima* in hypercholesteremic rats.

At the end of the sixth week, the rats were fasted overnight and sacrificed by cervical dislocation.

The animals used in the present study were maintained in accordance with the approval of the Animal Ethical Committee, University of Ibadan, Ibadan, Nigeria

2.3. Serum preparation

Blood was collected with a 2 ml syringe and needle by cardiac puncture and was centrifuged at 3000 rpm for 10 min and the serum (supernatant) was analyzed to evaluate some biochemical parameters.

2.4. Preparation of tissue homogenates

The organs (Liver, and Kidneys) were removed, rinsed in the ice-cold 1.15% KCl solution to wash off excess blood, blotted dried with filter paper, and weighed. The organs were homogenized in four parts of homogenizing buffer (i.e. 1 g of organ in 4 ml of buffer) and centrifuged at 10,000 rpm for 15 min in an ultracentrifuge at a of temperature $\leq 2\,^{\circ}C$ to get the mitochondrial fraction. The supernatant (post-mitochondrial fraction) was decanted and stored at $\leq 4\,^{\circ}C$ for subsequent analysis. Each time the supernatant was outside the freezer, it was kept in ice bags.

The protein content of the serum and the tissue fractions of the organs were determined by Lowry's method using bovine serum albumin (BSA) as standard.

Serum total cholesterol, triglyceride and High density Lipoprotein (HDL) were measured by enzymatic colorimetric method using Randox kits. The concentration of low-density lipoprotein (LDL) cholesterol was calculated by the formula of Friedwald et al. (1972). Frozen tissues were analyzed for quantitative estimation. Cholesterol was estimated in liver, heart muscle and adrenal gland by method of Zaltkis et al. (1953). Atherogenic Index was calculated using the formula of Abbott et al. (1988), and Coronary Risk Index (CRI) was obtained by the method of Alladi and Shanmugasundaram (1989).

The extent of lipid peroxidation in the liver fractions was determined by measuring the level of TBARS formed (Varshney and Kale, 1990).

2.5. Statistical analysis and scoring of data

Statistical significance of the difference between experimental groups was calculated using the Student's *t*-test (Fisher and Yates, 1963). A $p < 0.05$ was considered significant. One-way ANOVA was also performed.

3. Results

A significant increase ($p < 0.05$) was observed in the body weight and the relative weight of visceral organs in all the groups (Table 1).

There was a significant decrease in HDL-cholesterol and a significant increase in the levels of LDL, total cholesterol and triglycerides in hypercholesteremic rats when compared to normal rats (Fig. 1). Administration of the methanolic extracts restored the elevated levels of serum lipids to normal.

The methanolic extracts of *G. braunii* produced a significant ($p < 0.05$) decrease in the levels of total cholesterol, triglyceride, LDL-cholesterol of the extract treated group compared to hypercholesteremic control (Figs. 1, 2 and 3).

G. braunii caused a significant ($p < 0.05$) increase in the levels of HDL-cholesterol and protein concentration as compared to hypercholesteremic control (Figs. 5 and 6).

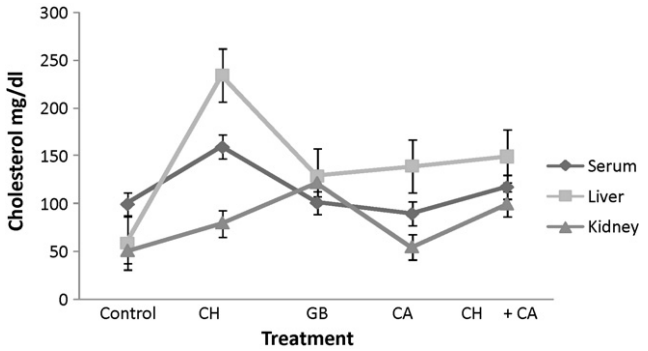

Fig. 1. Effect of *Globimetula braunii* on serum liver and kidney total cholesterol level in cholesterol-fed rats. Values are given as mean ± SEM; ($n = 5$), CA = cholestyramine, CH = cholesterol, GB = *G. braunii*.

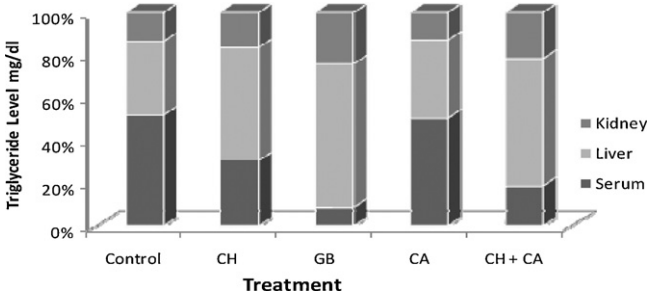

Fig. 2. Effect of *G. braunii* on serum liver and kidney triglyceride level in cholesterol-fed rats. Values are given as mean ± SEM; ($n = 5$), CA = cholestyramine, CH = cholesterol, GB = *G. braunii*.

G. braunii significantly ($p < 0.05$) reduced Lipid peroxidation in cholesterol-fed rats.

A low Atherogenic Index (AI) and Coronary Risk Index (CRI) were observed at a significant difference ($p < 0.05$) in cholesterol-fed rats administered *G. braunii* extracts.

4. Discussion

The process of atherogenesis has been considered by many to consist largely of the accumulation of lipids within the artery wall. High plasma concentrations of cholesterol, in particular those of low-density lipoprotein (LDL) cholesterol, are one of the principal risk factors for atherosclerotic cardiovascular disease (Ross, 1999). In this study we investigated the cardiovascular protective antilipemic/hypocholestermic potentials of *G. braunii* by depressing the level of triglyceride, LDL-cholesterol and increase

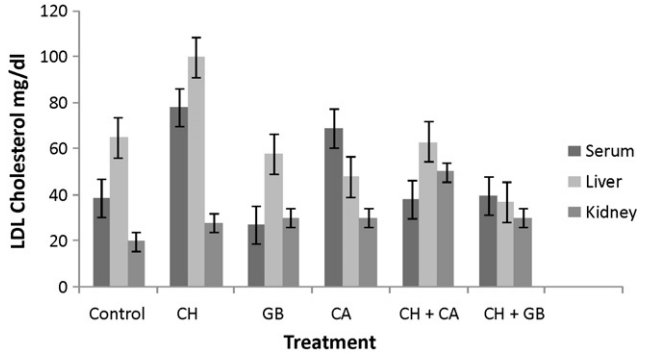

Fig. 3. Effect of *G. braunii* on serum liver and kidney low-density lipoprotein level in cholesterol-fed rats. Values are given as mean ± SEM; ($n = 5$), CA = cholestyramine, CH = cholesterol, GB = *G. braunii*.

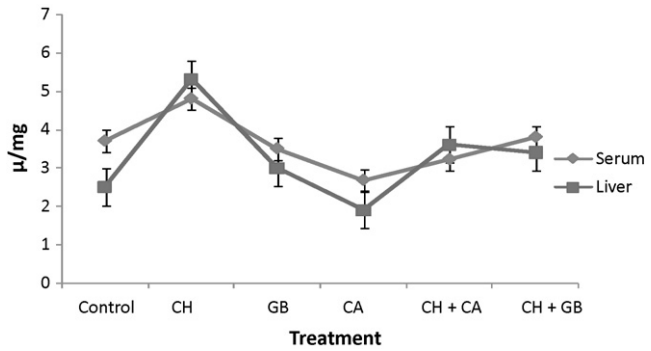

Fig. 4. Effect of *G. braunii* on serum and liver on the lipid peroxidation product level in cholesterol-fed rats. Values are given as mean ± SEM; ($n = 5$), CA = cholestyramine, CH = cholesterol, GB = *G. braunii*.

HDL-cholesterol of the normo and hypercholesteremic albino rats.

There are no data on the mechanism of action of *G. braunii* on the ratio of body weight/weight of tissues.

The relationship between postprandial hyperlipidemia and cardiovascular diseases has been described by many research groups (Przybycien et al., 2000; Hamsten et al., 1998; Hennig et al., 2001). In all of these studies the patients with clinically documented atherosclerosis were found to have an elevated postprandial triglyceride (TG) response to a fatty meal. *G. braunii* caused a significant ($p < 0.05$) reduction in TG and total cholesterol levels of the tissues of normo rats. A higher reduction was observed when *G. braunii* was administered to rats fed cholesterol. This may be as a result of its hemicellulose content as observed by Chahlia (2009) after *Capparis decidua* administration to diabetic rats.

The value and safety of lowering plasma LDL-cholesterol (LDL-C) in the treatment of cardiovascular disease has been established unequivocally (Root et al., 2002). Elevated low-density lipoprotein cholesterol (LDL-C) has been reported to be a causal risk factor for the development of coronary artery disease (CAD). In both its native and oxidized forms, LDL-C causes direct endothelial cell injury and dysfunction, predisposing to an inflammatory response in the artery wall that promotes the development of an atherosclerotic plaque (Ross, 1999). Clinical studies have shown that decreasing plasma LDL-C significantly reduces the coronary heart disease morbidity and mortality, and decreases the progression and increases regression of atherosclerotic lesions (LaRosa, 1989; Probstfield and Rifkind, 1991; Moghadasian et al., 2000). *G. braunii* significantly ($p < 0.05$) reduced serum and tissue LDL-C.

Lipid peroxidation (LPO) is a marker of oxidative stress (Ohkawa et al., 1979; Onyema et al., 2005). Dietary cholesterol generated the reactive oxygen species (ROS) that caused LPO. Lipid peroxidation

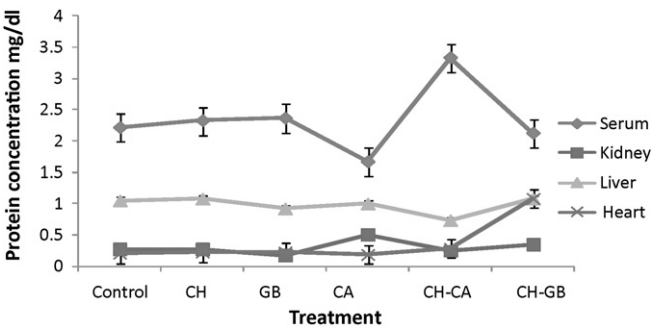

Fig. 5. Effect of cholesterol and *G. braunii* on protein concentration in cholesterol-fed rats. Values are given as mean ± SEM; ($n = 5$), CA = cholestyramine, CH = cholesterol, GB = *G. braunii*.

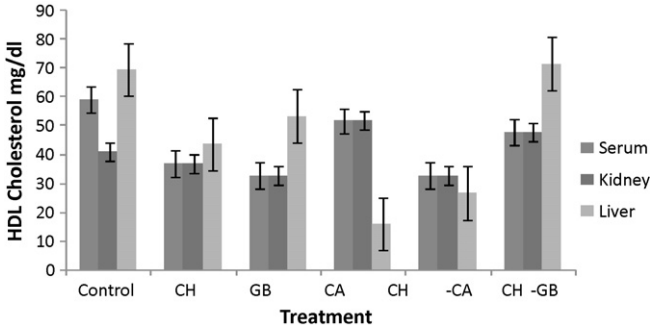

Fig. 6. Effect of *G. braunii* on high density lipoprotein cholesterol levels in cholesterol-fed rats. Values are given as mean ± SEM; ($n = 5$), CA = cholestyramine, CH = cholesterol, GB = *G. braunii*.

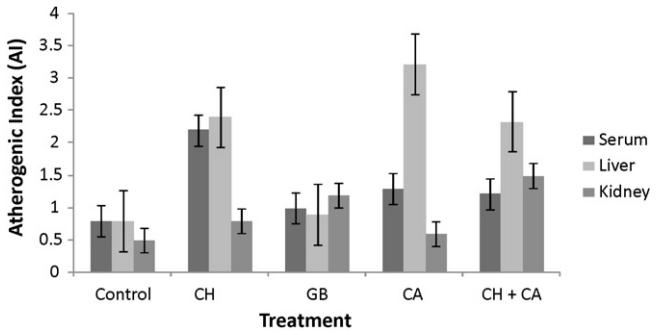

Fig. 7. Effect of *G. braunii* on Atherogenic Index (AI) in cholesterol-fed rats. Values are given as mean ± SEM; ($n = 5$), CA = cholestyramine, CH = cholesterol, GB = *G. braunii*.

has been implicated in the pathogenesis of increased membrane rigidity, reduced erythrocyte survival and perturbation in lipid fluidity (Ochoa et al., 2009). *G. braunii* significantly ($p < 0.05$) reduced LPO (Fig. 4) compared to treatment with cholestyramine. This results correlates with the report of Wegwu et al. (2005) on the hepato-protective antioxidant effect of crude methanolic extract of *Cassia alata* pods on rats treated with carbon tetrachloride.

HDL functions in the transport of cholesterol away from the peripheral tissues to the liver, thus preventing the genesis of atherosclerosis (Chahlia, 2009). The observed significant ($p < 0.05$) increase in the level of HDL further points to the cardiac protective potentials of the *G. braunii*.

Favorable Antherogenic and Coronary risk indexes (Figs. 7 and 8) observed in rats fed *G. braunii*, may indicate its cardio-protective potentials. This is due to the fact that the process of atherogenesis is considered to consist largely of accumulated lipids within the artery wall. A favorable antherogenic and coronary risk indexes upsets

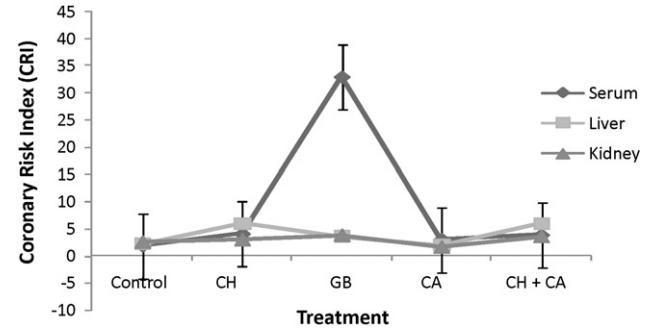

Fig. 8. Effect of *G. braunii* on Coronary Risk Index (CRI) in cholesterol-fed rats. Values are given as mean ± SEM; ($n = 5$), CA = cholestyramine, CH = cholesterol, GB = *G. braunii*.

the onset, thus protecting against atherosclerotic cardiovascular disease.

5. Conclusion

The results obtained from this study are indications of the antilipemic and hypocholestermic activities of *G. braunii* as well as its cardio-protective potential in normo and hypercholesteremia conditions, as it compared favorably to cholestyramine. *G. braunii* can be complimented with cholestyramine to archive effective results. This methanolic extract activity also merits clinical trials, to ensure that this affordable treatment is not left alone to the local inhabitants where the plant is in abundance.

Acknowledgement

We sincerely appreciate the effort of Late Professor Michael A. Fafunso towards the supervision of our MSc project from which this paper was extracted. He never lived to see its publication.

References

Abbott RD, Wilson PW, Kannel WB, Castelli WP. High density lipoprotein-cholesterol, total cholesterol screening and myocardial infarction. The Framingham Study. Arteriosclerosis 1988;8:207–11.
Alladi S, Shanmugasundaram KR. Induction of hypercholesterolemia by supplementing soy protein with acetate generating amino-acids. Nutr Rep Int 1989;40(5):893–9.
Burkhill HM. Useful plants of West Africa. vol. 1: Families A–D, 2nd ed. Kew: Royal Botanic Gardens; 1985.
Chahlia N. Effect of *Capparis decidua* on hypolipidemic activity in rats. J Med Plant Res 2009;3(6):481–4.
Edijala JK, Asagba SO, Eriyamremu GE, Atomatofa U. Comparative effects of garden egg fruit, oat and apple on serum lipid profile in rats fed on a high cholesterol diet. Pak J Nutr 2005;4:245–9.
Fisher RA, Yates F. Statistical tables for biological, agricultural and medical research. 6th ed. Edinburgh: Oliver and Boyd; 1963.
Friedwald WT, Levy RT, Frederickson DS. Estimation of the concentration of low lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. Clin Chem 1972;18:499–802.
Hamsten A, Bjorkengren J, Boquist S, Nilsson L, Ruotolo G, Eriksson P, Silveira A, Karpe F. Postprandial lipaemia and coronary heart disease. In: Jacotot B, Mathé D, Fruchart J-C, editors. Atherosclerosis XI. New York: Elsevier Science; 1998. p. 141–9.
Hennig B, Toborek M, McClain CJ. High-energy diet, fatty acids and endothelial cell function: implications for atherosclerosis. J Am Coll Nutr 2001;20: 97–105.
Jensen T, Stender S, Deckert T. Abnormalities in plasma concentrations of lipoprotein and fibrinogen in type 1 (insulin-dependent) diabetic patients with increased urinary albumin excretion. Diabetologia 1988;31:142–5.
LaRosa J. Review of clinical studies of bile acid sequestrants for lowering plasma lipid levels. Cardiology 1989;76(1):55–64.
Maghrani M, Lemhadri A, Zeggwagh NA, El Amraoui A, Haloui M, Jouad H, Eddouks M. Effect of *Retama raetam* on lipid metabolism in normal and recent-onset diabetic rats. J Ethnopharmacol 2004;90:323–9.
Moghadasian MH, Mancini GB, Frohlich JJ. Pharmacotherapy of hypercholesterolaemia: statins in clinical practice. Expert Opin Pharmacother 2000;1:683–95.
Motta M, Giugno I, Bosco S, Pistone G, Ruello P, Maugeri D, Malaguarnera M. Serum lipoprotein(a) changes in acute myocardial infraction. Panminevra Med 2001;43:77–80.
Ochoa JJ, Vilchez MJ, Palacios MA, Garcia JJ, Reiter RJ, Munoz-Hoyos A. Melatonin protects against lipid peroxidation and membrane rigidity in erythrocyte from patients undergoing cardiopulmonary bypass surgery. J Pineal Res 2009;35:104–8.
Ohkawa H, Ohisi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.
Okoro EE. Hypocholesteremic effect of *Cucurbita maxima* hypercholesteremic rat. MSc thesis, Biochemistry department, University of Ibadan, Ibadan, Nigeria; 2008.
Onyema OO, Farombi EO, Emerole GO, Ukoha AI, Onyeze GO. Effect of vitamin E on monosodium glutamate induced hepatoxicity and oxidative stress in rats. Ind J Biochem Biophys 2005;43:20–4.
Osadebe PO, Okide GB, Akabogu IC. Study of anti-diabetic activities of crude methanolic extracts of *Loranthus micranthus* (Linn.) sourced from five different host trees. J Ethnopharmacol 2004;95:133–8.
Probstfield JL, Rifkind BM. The lipid clinics coronary primary prevention trial: design, results, and implications. Eur J Clin Pharmacol 1991;40(1):S69–75.
Przybycien K, Kornacewicz-Jach Z, Torbus-Lisiecka B, Naruszewicz M. Is abnormal postprandial lipemia a familial risk factor for coronary artery disease in indi-

viduals with normal fasting concentrations of triglycerides and cholesterol? Coronary Artery Dis 2000;11:377–81.
Root C, Chari D, Smith CD, Sundseth SS, Pink HM, Wilson JG, Lewis MC. Ileal bile acid transporter inhibition, CYP7A1 induction, and antilipemic action of 264W94. J Lipid Res 2002;43:1320–30.
Ross R. The pathogenesis of atherosclerosis. New Eng J Med 1986;314:488–500.
Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
Varshney R, Kale RK. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int J Radiat Biol 1990;58:733–43.
Wegwu MO, Ayalogu EO, Sule OJ. Antioxidant protective effects of *Cassica alata* exposed to carbon tetrachloride. J Appl Sci Environ Manage 2005;9: 73–80.
Zaltkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Clin Med 1953;41:486–92.