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Effects of *Garcinia kola* biflavonoid fractions on serum lipid profile and kidney function parameters in hyperlipidemic rats

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Abstract

Background: *Garcinia kola* is used in traditional medicine in various parts of Africa including Nigeria for the amelioration of hypertension, cough, diabetes, sickle cell anemia, bacterial and fungal infections amongst others. The prophylactic and therapeutic efficacy of *Garcinia kola* biflavonoid fractions (GKBF); root bark (RBBF), stem bark (SBBF) and seed (SBF)) on the lipid profile and kidney function of Poloxamer 407 (P407) induced hyperlipidemic rats were determined.

Methods: Hyperlipidemia was induced by a single intraperitoneal dose of P407 after 19 days in the prophylactic group and after every 48 h in therapeutic group for 21 days. Atorvastatin (standard drug) and the GKBF were administered to different subgroups before induction in Prophylactic group and after induction in therapeutic group. Experimental animals were euthanized after day 21 of the study and blood samples collected for the evaluation of lipid levels and kidney function.

Results: All Prophylactic and therapeutic group treated sub-groups had significantly ($p < 0.05$) lower serum concentrations of total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-c) when compared to hyperlipidemic control. The RBBF and SBBF increased the levels of high density lipoprotein cholesterol (HDL-c). SBBF and SBF significantly ($p < 0.05$) increased HDL-c/TC ratio. The LDL-c/HDL-c and log (TG/HDL-c) level were lowered by the extracts except in the therapeutic group where only the SBF reduced log (TG/HDL-c). Kidney function test results in all groups showed no significant ($p > 0.05$) change in urea and creatinine concentrations.

Conclusion: This study shows that *Garcinia kola* biflavonoid fraction has potential prophylactic and therapeutic efficacy in the management of hyperlipidemia.

Keywords: Biflavonoid, *Garcinia kola*, Hyperlipidemia, Poloxamer 407, Prophylactic, Therapeutic

Background

Plants appear to be the major source of drugs for the majority of the world's population, with substances derived from higher plants constituting about a quarter of all prescribed medicines [1]. Several herbal medicines have advanced to clinical use in modern times. *Garcinia kola* a dicotyledonous plant, species of flowering plant in the *Clusiaceae* or *Guttiferae* family is one of such plants. It is a medium-sized tree mostly about 12 m high and sometimes up to 28 m in height. The bark is thick and brownish, yielding a yellow juice. The leaves are broadly elliptic, acute or shortly acuminate at the apex. The fruits are reddish yellow globular berries exhaling an apricot scent and about 6 cm in diameter with 2 to 4 brown seeds embedded in an orange coloured pulp. The seeds are bitter and chewed like kola nuts. Its natural habitat is subtropical or tropical moist lowland forests found in west and central Africa in countries like Benin, Cameroon, Democratic Republic of the Congo, Ivory Coast, Gabon, Ghana, Liberia, Nigeria (South Western and South Eastern parts), Senegal and Sierra Leone.

In Nigeria, it is commonly known as bitter kola due to its bitter taste, male kola due to reported aphrodisiac properties, Orogbo (Yoruba), Aku ilu (Igbo) and Namijin goro (Hausa). It is also called a "wonder plant" because all of its parts have been found to be of medicinal importance [2].

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Biological activity reported for different plant part extracts include; antidiabetic activity, antihepatotoxic activity, antimicrobial activity, antioxidant activity [3–6]. Most of *Garcinia kola* biological activities have been attributed to the presence of a biflavonoid complex.

Poloxamer 407 (P407), a nonionic surfactant is a triblock copolymer comprising of polyoxyethylene and polyoxypropylene units. It is known for its biocompatibility and potential to deliver different drugs for a variety of disease states [7] and as a barrier in preventing postsurgical adhesions [8]. It has an unusual thermoreversible properties, it is liquid at room temperature while it self-assembles into micelles then aggregate into a gel at body temperature. These temperature-dependent micellization and gelation properties have led to the widespread use of P407 in personal care products such as mouthwashes, deodorants, and skin care products and also as an excipient in a variety of pharmaceutical preparations. Johnston et al. [7] showed that one intramuscular or intraperitoneal injection of P407 causes dose-dependent hyperlipidemia in rats, increasing plasma triglyceride (TG) more than 60 fold and cholesterol 8 fold and since then has been a growing model in different hyperlipidemic studies.

Hyperlipidemia is one of the greatest risk factors contributing to the prevalence and severity of cardiovascular disease and cardiovascular disease is regarded as a leading cause of death worldwide [9]. Cardiovascular disease covers a wide array of disorders, including diseases of the cardiac muscle and of the vascular system supplying the heart, brain, and other vital organs [10].

Hyperlipidemia is a lipid disorder hence alters lipid profile. It is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels [11] though may be asymptomatic. Hyperlipidemia causes atherosclerotic cardiovascular disease which eventually affects organs such as the kidney, leading to glomerular injury, interstitial fibrosis and tubular atrophy, ischemic nephropathy and End Stage Renal Disease [12]. Increase in body weight and certain organs such as liver and spleen are also associated with hyperlipidemia due to increase in cholesterol and triglycerides and infiltration of these lipids to the organs respectively. This study was therefore carried out to explore the possible efficacy of prophylactic and therapeutic administration of *G. kola* (root bark, stem bark and seed) biflavonoid fractions on hyperlipidemic rats in relation to kidney function, body and organ weight change.

Methods

Collection of plant material and identification

Root bark, stem bark, and seed of *Garcinia kola* were collected from Abak, Akwa Ibom state, Nigeria, in the month of August, 2012. The plant was identified and authenticated by Mr. Umar Shehu Gallah, a plant taxonomist at the herbarium unit of Biological Sciences Department, Ahmadu Bello University, Zaria, where voucher specimen number 1783 was given and deposited for future reference.

Plant preparation and extraction

The root bark were washed and sliced, the seeds peeled and sliced and both separately pulverized with an electric blender and air-dried in the laboratory at room temperature alongside the stem bark which was coarsely ground after drying. Biflavonoid fractions of the coarsely ground root bark, stem bark and seed were respectively extracted by the method of Iwu et al. [3].

Animals

A total of 60 apparently healthy Wistar albino rats of both sexes weighing between 150 and 200 g obtained from the National Institute for Trypanosomiasis Research, Kaduna, Nigeria were acclimatized for a period of 2 weeks and used for the experiment. The rats were fed with water and pelleted growers' mash (Vital feed ®, Nigeria) ad libitum. The animals were properly handled according to the guidelines of the Animal Ethical Committee of the University (Ahmadu Bello University, Zaria, Nigeria) which is in compliance with NIH Guide for Care and Use of Laboratory Animals (pub. No. 85–23, Revised 1985).

Acute toxicity study (LD_{50})

The mean lethal dose (LD_{50}) of *Garcinia kola* (root bark, stem bark and seed) biflavonoid fractions were determined by a method described by Lorke [13].

Preparation of standard drug

Atorvastatin (Pfizer Ireland pharmaceuticals, Ireland) was purchased in a tablet form at strength 20 mg. Tablets were crushed into powder, dissolved in distilled water and administered orally.

Induction of hyperlipidemia

P407 (Lutrol F127; BASF, Ludwigshafen, Germany) was used as the inducing agent. Prior to the administration, P407 was dissolved in distilled water and refrigerated overnight to facilitate its dissolution. Needles and syringes to be used for administration were also cooled to prevent gelation within the syringe during injection [14]. The induction of hyperlipidemia was confirmed by collection of blood samples from the induced animals 2 h post-P 407 administration for assay of total cholesterol and triglyceride concentrations. Rats with total cholesterol and triglyceride levels above200mg/dl and 160 mg/dl respectively were considered hyperlipidemic [15].

Animal grouping

The rats were randomly divided into 2 major groups (Prophylactic and Therapeutic); with a total of 10 sub-groups comprising of 6 rats each as previously described by Ameh et al. [16]. Briefly, sub-groups 3–6 were administered atorvastatin, root bark, stem bark and seed biflavonoid fractions respectively for 19 days and on the 19th day, injected with P407 (500 mg/kg b. wt) and sacrificed 48 h after (Prophylactic study) while sub-groups 7–10 were administered P407 (500 mg/kg b. wt) at 48 h interval for 21 days; treatment commenced 2 h after induction (Therapeutic Study).

Prophylactic study (Group one)

- Group I: fed normal chow and distilled water only for 21 days (NC).
- Group II: induced using P407 without treatment (HC).
- Group III: treated with Atorvastatin (ATV) at 10 mg/kg body weight/day for 19 days and then induced for 2 days
- Group IV: treated with root bark biflavonoid fraction (RBBF) at 200 mg/kg body weight/day for 19 days and then induced for 2 days
- Group V: treated with stem bark biflavonoid fraction (SBBF) at 200 mg/kg body weight/day for 19 days and then induced for 2 days
- Group VI: treated with seed biflavonoid fraction (SBF) at 200 mg/kg body weight/day for 19 days and then induced for 2 days

Therapeutic study (Group two)

- Group I: fed normal chow and distilled water only for 21 days (NC).
- Group II: induced using P407 without treatment (HC).
- Group VII: induced and treated with ATV at 10 mg/kg body weight/day for 21 days.
- Group VIII: induced and treated with RBBF at 200 mg/kg body weight/day for 21 days.
- Group IX: induced and treated with SBBF at 200 mg/kg body weight/day for 21 days
- Group X: induced and treated with SBF at 200 mg/kg body weight/day for 21 days

The rats were routinely weighed on a weekly basis to facilitate administration of the correct dose of P407, *G. kola* biflavonoid fractions and standard drug (Atorvastatin) and to monitor relative weight change. All drugs were administered Per Os once daily with the exception of P407 which was administered intraperitoneally. The rats were also monitored for clinical signs and death.

Collection and preparation of sera samples

All experimental animals were anesthetized at the end of the 21-day experimental period using the chloroform-inhalation technique and bled by cardiac puncture. Serum was harvested into plain bottles from the coagulated blood by centrifugation at 3000 rpm for 15 min. The organs (heart, liver, kidney and spleen) were also harvested and weighed.

Determination of parameters

Determination of serum lipid profiles and kidney function parameters

Total cholesterol (TC) and Triglycerides (TG) levels were determined using the Randox® kit (Randox Laboratories Limited UK), high-density lipoprotein-cholesterol (HDL-c) level was determined using ELITech® kit (ELITech Clinical Systems, France), low-density lipoprotein cholesterol (LDL-c) was determined by the protocol of Friedewald [17] using the equation: LDL-c (mg/dl) = TGL/5 – HDL-c, and atherogenic risk factor was calculated using formula of Dobiasova and Frohlich [18]. Serum creatinine and urea concentrations were evaluated using commercial kits: Randox® kit (Randox Laboratories Limited UK).

Body weight change and organ weight

The body and organ weights were measured using sensitive weighing balance to monitor the change in body weight and the percentage organ weight.

$$\%\,\text{Organ weight} = \frac{\text{Organ weight}}{\text{Animal weight}} \times 100$$

Data analysis

Data are expressed as mean ± standard deviation (SD) and were analyzed by the analysis of variance (ANOVA). The difference between the various biflavonoid fractions and animal groups were compared using the Duncan Multiple Range Test. P value less than 0.05 was considered significant ($p < 0.05$).

Results

Changes in lipid profile

The Prophylactic effect of *Garcinia kola* biflavonoid fractions (root bark, stem bark and seed) on lipid profile showed significant ($p < 0.05$) decrease in serum concentrations of total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-c) in all treated groups compared to hyperlipidemic control with the seed having the most significant ($p < 0.05$) reduction in TC (79 %) and TG (67 %) levels compared to other biflavonoid fractions. However, only the root bark and stem

bark fractions significantly ($p < 0.05$) increased high density lipoprotein cholesterol (HDL-c) (Table 1).

Therapeutic study (Table 2) showed all treatments significantly ($p < 0.05$) reduced serum TC, TG and LDL-c concentrations when compared to hyperlipidemic control with the root bark and stem bark having the most reduction in TC (77 and 78 % respectively) when compared to all other induced treated groups. HDL-c concentration was significantly ($p < 0.05$) increased by only the seed fraction.

Atherogenic risk predictor indices

Serum atherogenic risk predictor indices of the prophylactic study (Table 1) showed only biflavonoid fractions significantly ($p < 0.05$) increased HDL-c/TC ratio and significantly ($p < 0.05$) reduced log (TG/HDL-c) ratio when compared to atorvastatin and hyperlipidemic control while all treatments (atorvastatin and biflavonoid fractions) significantly ($p < 0.05$) reduced LDL-c/HDL-c ratio.

The Therapeutic effect of oral administration of *Garcinia kola* biflavonoid fractions on serum atherogenic risk predictor indices of P407 induced hyperlipidemic rats (Table 2) showed all treatments significantly ($p < 0.05$) increased HDL/TC ratio except the root biflavonoid fraction when compared to hyperlipidemic control. LDL-c/HDL-c ratio of all treated groups was significantly ($p < 0.05$) lower than that of hyperlipidemic control but only the seed and atorvastatin treated groups had significantly ($p < 0.05$) lower log (TG/HDL-c) when compared to other groups.

Kidney function test

No significant ($p > 0.05$) difference was observed in serum creatinine and urea concentrations of all the groups in both prophylactic and therapeutic studies (Tables 3 and 4).

Change in body weight

In the prophylactic study there was a significant ($p > 0.05$) increase in the body weight for root bark biflavonoid treated group compared to all other groups (Table 5) while in the therapeutic study, the seed biflavonoid fraction significantly ($p > 0.05$) reduced body weight compared to all the groups except stem bark fraction (Table 6).

Percentage organ weights

In the prophylactic study, liver and spleen weights were significantly ($p < 0.05$) reduced by atorvastatin and all biflavonoid fractions. However, there was no significant ($p > 0.05$) change in kidney weight of all the groups (Table 7).

The therapeutic study showed no significant ($p > 0.05$) change in heart and kidney weights of all the groups (Table 8).

Discussion

Poloxamer 407, a nonionic surfactant is well known to induce dose dependant hyperlipidemia [19] by inhibiting capillary (heparin releasable) lipoprotein lipase (LPL), the major enzyme responsible for the hydrolysis of plasma lipoprotein triglycerides (TG) and indirectly stimulating the activity of 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, the rate limiting enzyme in cholesterol synthesis, thereby leading to hypertriglyceridemia and hypercholesterolemia respectively. It is nontoxic and safe for chronic administration and long term studies [20]. The significant ($P < 0.05$) increase in TC (25 fold), TG (11 fold) and LDL-c (23 fold) seen in hyperlipidemic models administered intraperitoneally 500 mg/kg body weight of P407 [21] indicates successful induction of hyperlipidemia.

Hyperlipidemia is responsible for the onset and progression of atherosclerosis, a major risk factor in the development of coronary heart diseases (CHDs) such as ischemic heart disease, myocardial infarction and stroke

Table 1 Prophylactic effect of *Garcinia kola* biflavonoid fractions on lipid profile and atherogenic risk predictor indices of P407 induced hyperlipidemic albino rats

Group ($n = 6$)	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	HDL-c/TC	LDL-c/HDL-c	Log(TG/HDL-c)
NC	59.36 ± 7.20^{a}	37.73 ± 8.45^{a}	34.23 ± 5.33^{a}	17.9 ± 5.55^{a}	0.56 ± 0.06^{d}	0.53 ± 0.18^{a}	-0.32 ± 0.16^{a}
P407	642.25 ± 50.29^{e}	946.13 ± 21.09^{f}	46.33 ± 13.92^{a}	406.69 ± 33.35^{c}	0.07 ± 0.02^{a}	9.16 ± 1.89^{b}	1.00 ± 0.07^{d}
ATV + P407	166.62 ± 15.60^{b}	625.33 ± 82.03^{e}	25.39 ± 5.22^{a}	16.17 ± 5.12^{a}	0.15 ± 0.02^{a}	0.68 ± 0.23^{a}	0.98 ± 0.09^{d}
RBBF + P407	234.17 ± 40.00^{c}	420.00 ± 28.00^{c}	103.05 ± 13.49^{b}	47.12 ± 15.70^{a}	0.44 ± 0.02^{c}	0.45 ± 0.10^{a}	0.25 ± 0.03^{b}
SBBF + P407	292.72 ± 7.80^{d}	508.67 ± 29.14^{d}	91.90 ± 12.58^{b}	99.08 ± 11.76^{b}	0.31 ± 0.04^{b}	1.10 ± 0.29^{a}	0.37 ± 0.04^{b}
SBF + P407	135.10 ± 27.02^{b}	312.67 ± 77.11^{b}	37.26 ± 18.13^{a}	35.30 ± 13.13^{a}	0.27 ± 0.10^{b}	1.19 ± 0.88^{a}	0.59 ± 0.15^{c}

Values are means ± Standard deviation

Values with different superscripts down the column differ significantly ($P < 0.05$)

NC Normal Control Rats, *P407* Poloxamer 407 Induced Rats, *ATV + P407* 10 mg of Atorvastatin + P407 Induced Hyperlipidemic Rats, *RBBF + P407* 200 mg of Root bark Biflavonoid Fraction + P407 Induced Hyperlipidemic Rats, *SBBF + P407* 200 mg of Stem bark Biflavonoid Fraction + P407 Induced Hyperlipidemic Rats, *SBF + P407* 200 mg of Seed Biflavonoid Fraction + P407 Induced Hyperlipidemic Rats, *TC* Total cholesterol, *TG* Triglycerides, *HDL-c* High density lipoprotein cholesterol, *LDL-c* Low density lipoprotein cholesterol

Table 2 Therapeutic effect of *Garcinia kola* biflavonoid fractions on lipid profile and atherogenic risk predictor indices of P407 induced hyperlipidemic albino rats

Group ($n = 6$)	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	HDL-c/TC	LDL-c/HDL-c	Log(TG/HDL-c)
NC	59.36 ± 7.20^{a}	37.73 ± 8.45^{a}	34.23 ± 5.33^{abc}	17.59 ± 5.55^{ab}	0.56 ± 0.06^{e}	0.53 ± 0.18^{a}	-0.32 ± 0.16^{a}
P407	642.25 ± 50.29^{d}	946.13 ± 21.09^{e}	46.33 ± 13.92^{bc}	406.69 ± 33.35^{c}	0.07 ± 0.02^{a}	9.16 ± 1.89^{b}	1.00 ± 0.07^{d}
P407+ ATV	243.18 ± 27.02^{c}	737.33 ± 106.93^{d}	53.91 ± 7.94^{cd}	41.81 ± 4.36^{b}	0.22 ± 0.01^{cd}	0.79 ± 0.16^{a}	0.78 ± 0.02^{c}
P407 + RBBF	149.78 ± 11.80^{b}	611.33 ± 21.39^{bc}	17.58 ± 8.42^{a}	9.94 ± 0.77^{a}	0.12 ± 0.05^{ab}	0.66 ± 0.30^{a}	0.99 ± 0.02^{d}
P407 + SBBF	139.60 ± 15.60^{b}	532.00 ± 85.16^{b}	23.29 ± 6.80^{ab}	9.91 ± 4.84^{a}	0.17 ± 0.07^{bc}	0.50 ± 0.42^{a}	0.95 ± 0.12^{d}
P407 + SBF	239.68 ± 41.27^{c}	686.00 ± 77.95^{cd}	74.92 ± 29.98^{d}	26.56 ± 4.60^{ab}	0.31 ± 0.07^{d}	0.41 ± 0.19^{a}	0.56 ± 0.08^{b}

Values are means ± Standard deviation
Values with different superscripts down the column differ significantly ($P < 0.05$)
NC Normal Control Rats, *P407* Poloxamer 407 Induced Rats, *P407 + ATV* P407 Induced Hyperlipidemic Rats + 10 mg of Atorvastatin, *P407 + RBBF* P407 Induced Hyperlipidemic Rats + 200 mg of Root bark Biflavonoid Fraction, *P407+ SBBF* P407 Induced Hyperlipidemic Rats + 200 mg of Stem bark Biflavonoid Fraction, *P407 + SBF* P407 Induced Hyperlipidemic Rats + 200 mg of Seed Biflavonoid Fraction, *TC* Total cholesterol, *TG* Triglycerides, *HDL-c* High density lipoprotein cholesterol, *LDL-c* Low density lipoprotein cholesterol

[22]. CHDs are responsible for about 17 million deaths in the world [23].

In clinical practice, effective and intensive lipid-lowering is important in order to reduce and prevent [24] CHDs. *Garcinia kola* (root bark, stem bark and seed) biflavonoid fractions significantly ($p < 0.05$) reduced TC, TG and LDL-c concentrations in both studies. These reductions in TC, TG and LDL levels suggest the ameliorative effect of *Garcinia kola* fractions in hyperlipidemia (Tables 1 and 2).

The elevation of TC concentration in this study was achieved by the indirect stimulation of HMG CoA reductase following an intraperitoneal (i.p) injection of P407 [19]. Hence the possible TC lowering effects of *Garcinia kola* (root bark, stem bark and seed) biflavonoid fractions could be attributed to decreased activity of hepatic HMG CoA reductase and/or stimulation of cholesterol-7-alpha-hydroxylase, which converts cholesterol into bile acids. Besides, the standard drug (Atorvastatin) used in this study inhibits HMG CoA reductase, a rate limiting enzyme in the biosynthesis of cholesterol. The results obtained in this work conform to earlier report by Patel et al. [25] that flavonoids possess antilipidemic activity.

Increase in TG concentration following P407 i.p. injection results primarily from an inhibition of TG degradation, P407 directly inhibits capillary lipoprotein lipase (LPL) responsible for plasma TG hydrolysis [19]. Although the standard drug might not decrease TG concentrations by activating lipoprotein lipase, the biflavonoid fractions from *Garcinia kola* could have reduced TG levels by either activating endothelium bound lipoprotein lipase which hydrolyses the triglyceride into fatty acid hence decreasing triglyceride levels as seen in a report by Sikarwar and Patil [26].

LDL (low density lipoprotein) is responsible for transporting cholesterol to the body cells. It transports about 60–70 % of total cholesterol. Therefore, an increase in TC level consequently increases LDL-c. The increased

Table 3 Prophylactic effect of *Garcinia kola* biflavonoid fractions on kidney function parameters of P407 induced hyperlipidemic albino rats

Group ($n = 6$)	Creatinine (mg/dl)	Urea (mg/dl)
NC	0.54 ± 0.17^{a}	31.86 ± 7.20^{a}
P407	0.59 ± 0.19^{a}	33.75 ± 5.15^{a}
ATV + P407	0.51 ± 0.20^{a}	35.90 ± 2.53^{a}
RBBF + P407	0.51 ± 0.34^{a}	44.18 ± 7.47^{a}
SBBF + P407	0.50 ± 0.17^{a}	36.72 ± 4.79^{a}
SBF + P407	0.56 ± 0.17^{a}	34.51 ± 4.17^{a}

Values are means ± Standard deviations
Values with different superscripts down the column differ significantly ($p < 0.05$)
NC Normal Control Rats, *P407* Poloxamer 407 Induced Rats (500 mg/kg), *ATV + P407* Atorvastatin (10 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg), *RBBF + P407* Root bark Biflavonoid Fraction (200 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg), *SBBF + P407* Stem bark Biflavonoid Fraction (200 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg), *SBF + P407* Seed Biflavonoid Fraction (200 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg)

Table 4 Therapeutic effect of *Garcinia kola* biflavonoid fractions on kidney function parameters of P407 induced hyperlipidemic albino rats

Group ($n = 6$)	Creatinine (mg/dl)	Urea (mg/dl)
NC	0.54 ± 0.17^{a}	31.86 ± 7.20^{a}
P407	0.59 ± 0.19^{a}	33.75 ± 5.15^{a}
P407 + ATV	0.51 ± 0.34^{a}	33.67 ± 7.52^{a}
P407 + RBBF	0.43 ± 0.17^{a}	36.26 ± 2.32^{a}
P407 + SBBF	0.57 ± 0.39^{a}	35.62 ± 3.31^{a}
P407 + SBF	0.48 ± 0.19^{a}	36.88 ± 1.88^{a}

Values are means ± Standard deviation
Values with different superscripts down the column differ significantly ($p < 0.05$)
NC Normal Control Rats, *P407* Poloxamer 407 Induced Rats (500 mg/kg), *P407 + ATV* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Atorvastatin (10 mg/kg), *P407 + RBBF* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Root bark Biflavonoid Fraction (200 mg/kg), *P407 + SBBF* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Stem bark Biflavonoid Fraction (200 mg/kg), *P407 + SBF* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Seed Biflavonoid Fraction (200 mg/kg)

Table 5 Prophylactic effect of *Garcinia kola* biflavonoid fractions on body weight of P407 induced hyperlipidemic albino rats

Group	Mean daily feed intake (g/100 g/day)			Mean initial body weight (g)	Mean final body weight (g)	Change in body weight (g)
	Week 1	Week 2	Week 3			
NC	70.56 ± 2.40[a]	68.80 ± 1.44 [a]	69.32 ± 3.06 [a]	207.50	235.75	28.50 ± 14.52[a]
P407	66.92 ± 3.50 [a]	68.68 ± 1.40 [a]	68.12 ± 0.61 [a]	164.00	193.33	29.33 ± 13.87[a]
ATV + P407	70.80 ± 0.80 [a]	68.00 ± 2.00 [a]	70.00 ± 2.00 [a]	189.75	216.00	26.25 ± 9.21[a]
RBBF + P407	69.20 ± 1.20 [a]	67.60 ± 2.12 [a]	68.92 ± 0.61 [a]	181.75	229.75	48.00 ± 9.02[b]
SBBF + P407	67.32 ± 1.20 [a]	66.68 ± 3.06 [a]	67.72 ± 2.05 [a]	184.00	213.50	29.50 ± 11.70[a]
SBF + P407	65.88 ± 1.70 [a]	67.48 ± 1.67 [a]	68.40 ± 1.44 [a]	193.80	221.60	27.80 ± 10.66[a]

Values are means ± Standard deviations
Values with different superscripts down the column differ significantly ($p < 0.05$)
NC Normal Control Rats, *P407* Poloxamer 407 Induced Rats (500 mg/kg), *ATV + P407* Atorvastatin (10 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg), *RBBF + P407* Root bark Biflavonoid Fraction (200 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg), *SBBF + P407* Stem bark Biflavonoid Fraction (200 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg), *SBF + P407* Seed Biflavonoid Fraction (200 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg)

LDL-c which was not removed in the process of lipid metabolism is likely to flow into the subendothelial space, and subsequently undergo oxidation. The oxidized LDL is phagocytized by the scavengers of macrophages and the fat-laden macrophage is left with the lipid core filled with cholesterol after necrocytosis and then arteriosclerosis is initiated [27]. This work shows significant ($P < 0.05$) reduction in LDL-c levels by all *Garcinia kola* biflavonoid fractions (Tables 1 and 2), the biflavonoid fractions may have increased LDL-c receptors densities in the liver binding to apolipoprotein B thereby making liver cells more efficient to remove LDL-c from blood as reported by Baum et al. [28].

HDL-c act as cholesterol scavengers, they pick up excess cholesterol and cholesterol esters from the blood and peripheral tissues to the liver where it is broken down to bile acids. It plays an important role in reducing blood and peripheral cholesterol concentrations and inhibits formation of atherosclerotic plaque in the aorta [29], therefore known as the protective cholesterol. The present studies show significant ($P < 0.05$) increase in HDL-c by root bark and stem bark biflavonoid fractions (Table 1) and significant ($P < 0.05$) increase in HDL-c by seed biflavonoid fraction (Table 2). This could possibly be due to increasing activity of lecithin-cholesterol acyl transferase (LCAT), an enzyme responsible for incorporating free cholesterol into HDL-c as suggested by Geetha et al. [30], there by promoting reverse cholesterol transport and competitively inhibiting the uptake of LDL-c by endothelial cells and preventing the generation of oxidized LDL-c.

Atherogenic risk predictor indices (HDL-c/TC, LDL-c/HDL-c and log (TG/HDL-c)) are mathematical relationships between TC, TG, LDL-c and HDL-c that have been successfully used as markers of assessing atherosclerosis development [31] and extent of CHDs. HDL-c/TC ratio greater than 0.3 and LDL-c/HDL-c ratio less than 2.3 indicate a reduced risk of peripheral arterial disease [32]. However, log (TG/HDL-c) has been considered the most accurate in determining the extent of atherosclerosis and the risk of myocardial infarction Dobiasova et al. [33]. It has been suggested that log (TG/HDL-c) values of -0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high cardiovascular disease risk [34]. According to these ranges provided by Ojiakor and Nwanjo [32] for HDL-c/TC and LDL-c/HDL-c ratios and Dobiasova [34] for log (TG/HDL-c), the most important atherogenic risk predictor

Table 6 Therapeutic effect of *Garcinia kola* biflavonoid fractions on body weight of P407 induced hyperlipidemic albino rats

Group	Mean Daily Feed Intake (g/100 g/day)			Mean initial body weight (g)	Mean final body weight (g)	Change in body weight (g)
	Week 1	Week 2	Week 3			
NC	70.56 ± 2.40 [a]	68.80 ± 1.44 [a]	69.32 ± 3.06 [a]	207.50	235.75	28.50 ± 14.52[b]
P407	66.92 ± 3.50 [a]	68.68 ± 1.40 [a]	68.12 ± 0.61 [a]	164.00	193.33	29.33 ± 13.87[b]
P407 + ATV	68.12 ± 2.34 [a]	68.12 ± 2.28 [a]	68.52 ± 1.29 [a]	191.33	221.00	29.67 ± 10.01[b]
P407 + RBBF	68.40 ± 1.44 [a]	67.32 ± 2.36 [a]	67.72 ± 2.05 [a]	189.50	215.00	25.50 ± 12.23[b]
P407 + SBBF	67.72 ± 2.41 [a]	66.92 ± 1.66 [a]	69.60 ± 1.44 [a]	197.00	216.25	16.75 ± 5.12[ab]
P407 + SBF	66.67 ± 1.40 [a]	67.02 ± 1.66 [a]	67.48 ± 2.20 [a]	194.67	200.67	6.00 ± 2.64[a]

Values are means ± Standard deviations
Values with different superscripts down the column differ significantly ($p < 0.05$)
NC Normal Control Rats, *P407* Poloxamer 407 Induced Rats (500 mg/kg), *P407 + ATV* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Atorvastatin (10 mg/kg), *P407 + RBBF* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Root bark Biflavonoid Fraction (200 mg/kg), *P407 + SBBF* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Stem bark Biflavonoid Fraction (200 mg/kg), *P407 + SBF* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Seed Biflavonoid Fraction (200 mg/kg)

Table 7 Prophylactic effect of *Garcinia kola* biflavonoid fractions on percentage organ weight of P407 induced hyperlipidemic albino rats

Group ($n = 6$)	Heart (g)	Kidney (g)	Liver (g)	Spleen (g)
NC	0.29 ± 0.03^{ab}	0.55 ± 0.04^{a}	3.25 ± 0.44^{ab}	0.42 ± 0.09^{a}
P407	0.31 ± 0.04^{b}	0.61 ± 0.08^{a}	4.66 ± 0.88^{c}	0.87 ± 0.17^{b}
ATV + P407	0.29 ± 0.02^{ab}	0.55 ± 0.04^{a}	3.57 ± 0.39^{b}	0.44 ± 0.97^{a}
RBBF + P407	0.26 ± 0.02^{a}	0.51 ± 0.08^{a}	2.94 ± 0.12^{a}	0.38 ± 0.08^{a}
SBBF + P407	0.26 ± 0.01^{a}	0.54 ± 0.03^{a}	3.25 ± 0.28^{ab}	0.45 ± 0.06^{a}
SBF + P407	0.28 ± 0.01^{ab}	0.52 ± 0.05^{a}	3.13 ± 0.24^{ab}	0.38 ± 0.05^{a}

Values are means ± Standard deviations
Values with different superscripts down the column differ significantly ($p < 0.05$)
NC Normal Control Rats, *P407* Poloxamer 407 Induced Rats (500 mg/kg), *ATV + P407* Atorvastatin (10 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg), *RBBF + P407* Root bark Biflavonoid Fraction (200 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg), *SBBF + P407* Stem bark Biflavonoid Fraction (200 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg), *SBF + P407* Seed Biflavonoid Fraction (200 mg/kg) + P407 Induced Hyperlipidemic Rats (500 mg/kg)

index, all induced animals in both studies are at high cardiovascular disease risk after intra peritoneal administration of 500 mg/kg body weight of P407 with log (TG/HDL-c) > 0.24 but the biflavonoid fractions did significantly ($p < 0.05$) reduce this risk (Tables 1 and 2) suggesting anti-atherogenic abilities of *Garcinia kola* (root bark, stem bark and seeds) biflavonoid fractions, hence reduction in development of cardiovascular disease.

Kidney helps in maintaining homeostasis of the body by reabsorbing important material and excreting waste products [35]. Its function is usually assessed by the levels of urea and creatinine in the blood; creatinine being the most specific. Urea is the main end product of protein catabolism; it varies directly with protein intake and inversely with the rate of excretion. Renal diseases which diminish the glomerular filteration lead to urea retention and decrease in urea is seen in severe liver disease with destruction of cells leadings to impairment of the urea cycle [36]. Creatinine is a waste product formed in muscle by creatine metabolism. Creatinine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle. Its retention in the blood is evidence of kidney impairment. There was no significant ($p > 0.05$) change in urea and creatinine levels of all the groups in both studies (Tables 3 and 4) indicating no impairment of kidney function by the administration of P407 and *Garcinia kola* biflavonoid fractions did not also significantly ($p > 0.05$) exert any effect.

Table 8 Therapeutic effect of *Garcinia kola* Biflavonoid fractions on percentage organ weight of P407 induced hyperlipidemic albino rats

Group ($n = 6$)	Heart (g)	Kidney (g)	Liver (g)	Spleen (g)
NC	0.29 ± 0.03^{a}	0.55 ± 0.04^{a}	3.25 ± 0.44^{a}	0.42 ± 0.09^{a}
P407	0.31 ± 0.04^{a}	0.61 ± 0.08^{a}	4.66 ± 0.88^{b}	0.87 ± 0.17^{c}
P407 + ATV	0.29 ± 0.03^{a}	0.59 ± 0.03^{a}	4.37 ± 1.23^{ab}	0.63 ± 0.07^{b}
P407 + RBBF	0.28 ± 0.03^{a}	0.56 ± 0.02^{a}	4.07 ± 0.78^{ab}	0.52 ± 0.05^{ab}
P407 + SBBF	0.27 ± 0.04^{a}	0.56 ± 0.03^{a}	4.19 ± 0.86^{ab}	0.63 ± 0.11^{b}
P407 + SBF	0.30 ± 0.02^{a}	0.57 ± 0.05^{a}	4.33 ± 0.84^{ab}	0.72 ± 0.10^{b}

Values are means ± Standard deviations
Values with different superscripts down the column differ significantly ($p < 0.05$)
NC Normal Control Rats, *P407* Poloxamer 407 Induced Rats (500 mg/kg), *P407 + ATV* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Atorvastatin (10 mg/kg), *P407 + RBBF* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Root bark Biflavonoid Fraction (200 mg/kg), *P407 + SBBF* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Stem bark Biflavonoid Fraction (200 mg/kg), *P407 + SBF* P407 Induced Hyperlipidemic Rats (500 mg/kg) + Seed Biflavonoid Fraction (200 mg/kg)

This work showed no significant ($p > 0.05$) difference in feed intake and body weight of the hyperlipidemic control compared to the normal control (Tables 5 and 6). A result agreeing with earlier report by Johnston et al. [37] that P407 does not significantly ($p > 0.05$) affect body weight of animals. The non-appreciable rate of weight gain between the initial and final weights observed in the seed biflavonoid fraction treated group (Table 6) is in agreement with earlier reports which attributed it to the anti-atherogenic effect of *Garcinia kola* seed biflavonoid fraction and its anti-adipogenic effect inhibiting accumulation of lipid droplets in fat cells [38–40].

The significantly ($p < 0.05$) increased liver and spleen weights in the hyperlipidemic control (Tables 7 and 8) could be as a result of fatty infiltration and increased blood cells in the spleen as suggested by Sheyla et al. [41]. However, all biflavonoid fractions reduced the liver and spleen weights (Tables 7 and 8). These reductions in organ weights show the protective or restoring potentials of the plant biflavonoid fractions on the organs (liver and spleen).

Conclusion

This study showed *Garcinia kola* (root bark, stem bark and seed) biflavonoid fractions can be used in the management of hyperlipidemia as the use has no deleterious effect on kidney function, body and organ weights.

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Authors' contributions

EBA performed all of the experiments in the laboratory. Article was written by EBA. Critical revision of the article was done by DAA, DBJ, OAO and USN. EBA made the necessary corrections in the write up. Conception, experiment design, overall monitoring and final approval of the article was done by EBA, DAA and DBJ. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication
All authors take full responsibility for the content of the paper.

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