

Anti-hypercholesterolemic effect of unripe *Musa paradisiaca* products on hypercholesterolemia-induced rats

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ABSTRACT

Hypercholesterolemia is a metabolic disorder caused by an increase in the concentrations level of plasma low-density lipoprotein (LDL) cholesterol. It has been implicated as a primary risk factor related to the pathogenesis of atherosclerosis or coronary heart disease, ischemic heart disease or cardiovascular disease, including myocardial infarction. *Musa paradisiaca* (*M. paradisiaca*) is a remarkable medicinal plant. Its potential in the management of diabetes mellitus as well as in nephropathy and myocardial infarction in animal models has been reported. This present study aimed at examining the effects of unripe plantain (*M. paradisiaca*) products (elastic pastry and roasted plantain) commonly known as *amala* and *boli*, respectively, in Nigeria on hypercholesterolemia-induced rats. The anti-hypercholesterolemic activity of these products was studied in 1% cholesterol-induced rats. Thirty-six rats were randomly divided into six groups and fed for 21 days with different plantain-supplemented diets. The hypercholesterolemic potential of the products was evaluated by measuring biochemical parameters, such as plasma lipid peroxidation (LPO), plasma lipid profiles, and plasma liver biomarkers. Results revealed that the inclusion of “amala” and “boli” in hypercholesterolemic rat diets not only significantly decreased the high levels of plasma LPO, total cholesterol, triglyceride, LDL cholesterol, and plasma liver biomarkers but also increased the activities of high-density lipoprotein cholesterol in the plasma of treated animals as compared with the control. This study, therefore, suggests that unripe plantain products *amala* and *boli* confer protective effects against various biochemical changes in experimentally-induced hypercholesterolemic animal models.

INTRODUCTION

Cholesterol is a waxy, fat-like substance found in the blood and body cells of all humans and animals. It falls under one of the three major groups of lipids which are manufactured and utilized to build membranes in all kinds of animal cells. It also serves as a precursor for the production of steroid hormones, vitamin D and bile acids, it is the main of sterol in the tissues of all animals (Vazhacharickal *et al.*, 2017). Cholesterol is amphipathic in nature; consisting of a polar head group (the hydroxyl group at C₃) and a nonpolar hydrocarbon body (the hydrocarbon

side chain at C₁₇ and the steroid nucleus) which may be as long as a 16-carbon fatty acid in its elongated conformation (Nelson and Cox, 2008). The administration of cholesterol in rats has been shown to enhance hepatic lipid metabolism and triglyceride levels (Wang *et al.*, 2010). Thus, the increase in total serum cholesterol stands as a major cause of impairment in triglyceride metabolism which leads to the accumulation/deposition of free fatty acids in the liver, generating a disorder known as fatty liver (Wang *et al.*, 2010). Expansion in the liver fatty acid pool leads to an increase in peroxisomal and mitochondrial β -oxidation, which results in the production of reactive oxygen species, which may, in turn, stimulate the generation of a local proinflammatory state that causes a progression in the liver injury (Schwimmer *et al.*, 2008).

The adverse effects of an increase in cholesterol levels in the body have been linked to several life threatening diseases, such as hypertension, atherosclerosis, cardiovascular diseases, metabolic syndrome, obesity, hypercholesterolemia, as well as

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diabetes (Murray *et al.*, 2003; Colpo, 2005; Medhat *et al.*, 2017). Hypercholesterolemia refers to a metabolic disorder which is caused by an elevated level in the concentrations of plasma low-density lipoprotein (LDL) cholesterol (Mu *et al.*, 2017). It has been implicated as a primary risk factor for the pathogenesis of cardiovascular and many other related diseases due to the presence of high levels of cholesterol in the blood (Nelson and Cox, 2008). The disorder is typically caused by obesity, dietary intake, and other environmental and genetic factors or a combination of both (Bhatnagar *et al.*, 2008). Type-2-diabetes mellitus, alcohol, dialysis, monoclonal gammopathy, hypothyroidism, anorexia nervosa, nephrotic syndrome, Cushing's syndrome, obstructive jaundice, and medications such as; thiazide diuretics, glucocorticoids, ciclosporin, and retinoic acid beta blockers are some of the secondary causes of hypercholesterolemia (Bhatnagar *et al.*, 2008; Borch *et al.*, 2016; Schwingshackl *et al.*, 2017). Therefore, as part of combined activities to reduce hypercholesterolemia, several studies on the role/effects of traditional plants on hypocholesterolemia have been performed over the years.

In recent times, there has been clear-cut evidence that most developing countries have resorted to the administration of plants in the treatment of ailments and diseases; this may be due to the unavailability or the expensiveness of orthodox drugs and health care as well as the fact that many countries now jettison the use of synthetic drugs for natural sources. *M. paradisiaca*, commonly known as plantain, is one of the major foods in tropical equatorial Africa and Andean regions of the world and has been named as the 10th most common staple food consumed in the world of today. In Africa, its consumption provides more than 25% of required carbohydrates for no less than 70 million people (Randy *et al.*, 2007); due to its minute fatty content and greater starch concentration. It has been adopted as potential and alternative foods for geriatric and gastric ulcer patients, respectively and is also consumed for the management of coeliac disease and colitis (Ojewole and Adewumi, 2003). Historically, distinctive parts of the plant; ripe or unripe, such as; the rootstocks and fruit are served as sources of food and are steamed, boiled, grilled, baked, or fried depending on the country and culture. In Nigeria and other central and West African countries, unripe plantain is conventionally processed into flour for an elastic pastry (amala). This is a traditional dish usually eaten in the Yoruba part of Nigeria with vegetable soup depending on the consumer's choice (Oyesile, 1987).

It has been reported that plantain possess antioxidant activities due to its phenolic contents which help in scavenging free radicals in the body by chelating metallic catalyts, reducing tocopherol radicals, activating antioxidant enzymes, and inhibiting oxidases (Amic *et al.*, 2003; Revadigar *et al.*, 2017). It has also been shown that the presence of phenolic activities in certain diets leads to the reduction of chronic diseases (Liu, 2004; Revadigar *et al.*, 2017). In recent time, several studies have shown the hypoglycemic effects or antihyperglycemic and antidiabetic activity of *M. paradisiaca* (Arun *et al.*, 2017; Ajiboye *et al.*, 2018; Sarma and Goswami, 2018). The histochemistry evaluation of *M. paradisiaca* on testis and testosterone levels of male Wistar rats has shown that the fruit enhances the reproductive potential when consumed moderately, but this beneficiary effect may not be related

to testosterone levels (Alabi *et al.*, 2017). More so, the peels of *M. paradisiaca* has been employed as an adsorbent in the removal of heavy metal ions from heavy metal contaminated water through the agro-waste process (Ibisi and Asoluka, 2018). The starch from *M. paradisiaca* Linn. has also been isolated and evaluated as a binder in a tablet (Sandhan *et al.*, 2017). Traditionally, plantain is used in the treatment and management of several diseases due to its anti-ulcerogenic, hypoglycemic, and analgesic activities, some of which include diabetes, ulcers, and wound healing (Ojewole and Adewumi, 2003). Therefore, this present study focused on the anti-hypercholesterolemic effect of unripe plantain (*M. paradisiaca*) products on the hypercholesterolemia-induced rats.

MATERIALS AND METHODS

Laboratory animals

Laboratory albino male and female rats weighing between 150 and 180 g were obtained from the University of Ibadan. They were maintained under standard laboratory conditions where clean water and correct feeds were provided, so as to adapt to their new environment and to nullify the effect of changes in their general metabolism (acclimatization). At random, the animals were assigned to different groups depending on their weight. All animals received basic human care and all experiments were carried out according to Adekunle Ajasin University Approved Protocol and Guidelines (AAUAPG) for Animal Experimentation with approval number (AAUAPG/SCI/1008).

Collection of samples

Unripe plantain (*M. paradisiaca*) was obtained from Ago Panu market in Owo local government Area of Ondo State, Nigeria and the plant was identified in the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State.

Chemical and reagent preparation

All chemicals used in this study for sensitive biochemical assays were from Randox and were of the good analytical grade. Distilled water was used in all biochemical assays.

Preparation of elastic pastry of unripe plantain (Amala)

In preparing the unripe plantain flour, the method described by Akubor and Ukwuru (2003) was adopted. This started by hand peeling the matured unripe plantain fruits, which were washed in tap water. Thereafter, the edible portion (pulp) was sliced into 2.5 cm thick and sundried. Afterward, the sundried fruits product was milled into flour which was then passed through a sieve of 0.45 mm mesh-sized and stored in an air-tight container for future use. Then, the sieved unripe plantain flour was used to prepare elastic pastry (amala) by boiling the flour in hot water for 3–5 minutes on heat cooker. 10 g of sundried elastic pastry (amala) and roasted (boli) of unripe plantain was collected and weighed into 100 ml of distilled water to produce 1% extraction supplement.

Hypercholesterolemia rat model; High cholesterol-fed bioassay

Two weeks after acclimatization of the rats, they were randomly subdivided into six groups based on their sex

containing six animals each. Group 1 received only basal diet, Group 2 (hypercholesterolemic control group) received a basal diet containing 1% cholesterol (adapting the method described by Oboh *et al.*, 2015). Groups 3, 4, 5, and 6 were fed with diets containing various formulations as described below. After 21 days of the experiment, the animals were sacrificed by cervical dislocation after an overnight fast. The blood was collected rapidly by puncturing the heart of sacrificed rats, thereafter, the plasma sample was prepared. Subsequently, the high-density lipoprotein (HDL)-cholesterol, triglyceride, total cholesterol, and LDL-cholesterol were evaluated using kits which are commercially available. Additionally, plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) contents were determined using the same commercially available kits from the same manufacturer. In order to determine the content of plasma malondialdehyde (MDA), the method described by Ohkawa *et al.* (1979) was employed.

Feed preparation and treatment groups

The method of Oboh (2005) was employed to prepare the basal and fresh diets, and these prepared diets were kept in closed containers and stored at 4°C for further use.

- Group 1 (normal control rats)—received a basal diet containing; 44% skimmed milk, 10% groundnut oil, 42% corn starch, and 4% vitamin & mineral premix);
- Group 2 (hypercholesterolemic rats group)—received a basal diet containing 1% cholesterol (i.e., 1% cholesterol and 99% basal diet);
- Group 3 rats—received a basal diet supplemented with 10% elastic pastry of unripe plantain (amala) plus 1% cholesterol (i.e., 1% cholesterol, 10% amala, and 89% basal diet);
- Group 4 rats—received a basal diet supplemented with 20% elastic pastry of unripe plantain (amala) plus 1% cholesterol (i.e., 1% cholesterol, 20% amala, and 79% basal diet);
- Group 5 rats—received a basal diet supplemented containing 10% roasted unripe plantain (boli) plus 1% cholesterol (i.e., 1% cholesterol, 10% boli, and 89% basal diet);
- Group 6 rats—received a diet supplemented containing 20% roasted unripe plantain (boli) plus 1% cholesterol (i.e., 1% cholesterol, 20% boli, and 79% basal diet).

Note: Skimmed milk = 36% protein; 1 g of the mineral and vitamin premix contains 600 i.u vitamin D₃, 3,200 i.u vitamin A, 2.8 mg vitamin E, 0.8 mg vitamin B₁, 0.6 mg vitamin K₃, 6 mg niacin, 1 mg vitamin B₂, 2.2 mg pantothenic acid, 0.004 mg vitamin B₁₂, 0.8 mg vitamin B₆, 0.2 mg folic acid, 70 mg choline chloride, 0.1 mg biotin H₂, 0.08 mg cobalt, 8.4 mg iron, 1.2 mg copper, 0.4 mg iodine, 16 mg manganese, 12.4 mg zinc, 0.08 mg selenium, and 0.5 mg antioxidant.

Preparation of plasma

The blood of the different sacrificed rats was collected at the end of each feeding trial into Ethylenediaminetetraacetic acid (EDTA) bottles. Thereafter, blood samples collected were centrifuged at 800 × g for 10 minutes in order to separate the plasma. This separated plasma was then transferred into plain sample bottles and kept in a refrigerator for further analysis.

Evaluation of plasma triglyceride concentration

The concentration of plasma triglyceride was evaluated using the colorimetric method illustrated by Tietz (1990). This involved the quick mixing of 10 µl of the sample with 1 ml of Pipes reagent (5.5 mM 4-chlorophenol, 40 mM phosphate buffer, and 17.5 mM Mg²⁺) and enzyme reagent (adenosine triphosphate, 4-aminophenazone, glycerol-3-phosphate oxidase, lipase, peroxidase, and glycerol kinase). Subsequently, the mixture was incubated at 37°C for 5 minutes and the absorbance was taken at 546 nm within 60 minutes against the reagent blank. The concentration of triglyceride was then calculated against the standard.

Evaluation of plasma total cholesterol concentration

Cholesterol concentration was examined after enzymatic hydrolysis and oxidation according to the principle described by Allain *et al.* (1974). The indicator quinonemine was generated from 4-aminoantipyrine and hydrogen peroxide in the presence of peroxidase and phenol. 1 ml of the reacting mixture consisting of 4-aminoantipyrine, cholesterol esterase, phenol, cholesterol oxidase, peroxidase, and 80 mM Pipes buffer at pH 6.8 was combined with 10 µl of plasma. Subsequently, the mixture was incubated at 37°C for 5 minutes and the absorbance was taken at 546 nm within 60 minutes against the reagent blank, thereafter, the cholesterol concentration in the sample was calculated against a standard.

Assessment of plasma HDL-cholesterol concentration

According to the technique of Lopes-Virella *et al.* (1977), precipitation was carried out as illustrated by the kit's manufacturer (Randox Laboratories, UK). 200 µl of plasma was briefly mixed with 500 µl of the precipitant (25 mM magnesium chloride and 0.55 mM phosphotungstic acid) and the mixture was incubated at room temperature for 10 minutes, thereafter, the mixture was centrifuged at 800 × g for 10 minutes, then the pure supernatant was removed and subjected to the same procedure for the evaluation of cholesterol.

Evaluation of plasma LDL-cholesterol concentration

The equation described by Friedewald *et al.* (1972) was used to determine the concentration of LDL-cholesterol in the plasma samples;

$$\text{LDL - Cholesterol (mg / dl)} = \frac{\text{Total Cholesterol - Triglycerides}}{5 - \text{HDL Cholesterol}}$$

Investigation of tissue lipid peroxidation

The method described by Ohkawa *et al.* (1979) was employed in carrying out lipid peroxidation (LPO) assay. 300 µl of tissue homogenate, Thiobarbituric acid (TBA), 500 µl of acetic acid/HCl (pH = 3.40), and 300 µl of 8.1% Sodium dodecyl sulphate were briefly added together. The mixture was incubated for 1 hour at 100°C, then the TBA reactive species produced was ascertained at 532 nm and calculated as MDA equivalent.

Evaluation of plasma aspartate aminotransferase (AST) activity

This was achieved by using [Reitman and Frankel \(1957\)](#) methods following the manufacturer's guide (Randox Laboratories, UK). The mixture of 100 μ l of the test sample and 500 μ l of buffer (100 mM phosphate buffer pH 7.4, 2 mM α -oxoglutarate, and 100 mM L-aspartate) was generated. This mixture was subsequently incubated at 37°C for 30 minutes, afterward 500 μ l of 2 mM 2,4 dinitrophenylhydrazine was added to the reaction mixture and incubated at 25°C (room temperature) for 20 minutes. 500 μ l of 0.4 mM NaOH was added and vigorously mixed together, then the absorbance was measured after 5 minutes at 546 nm against a reagent blank and the AST activity evaluated.

Evaluation of plasma alanine aminotransferase (ALT) activity

[Reitman and Frankel \(1957\)](#) methods following the manufacturer's guide (Randox Laboratories, UK) was used to determine the activity of ALT. The mixture of 100 μ l of the test sample and 500 μ l of buffer (100 mM phosphate buffer at pH 7.4, 2 mM α -oxoglutarate, and 200 mM L-alanine) was produced, subsequently, the mixture was incubated at 37°C for 30 minutes. Moreover, 500 μ l of 2 mM 2,4 dinitrophenylhydrazine was added into the mixture and the samples were left at room temperature (25°C) for 20 minutes. 500 μ l of 0.4 mM NaOH was later added and vigorously mixed together, then, the absorbance was measured at 546 nm for 5 minutes against a reagent blank and the activity of ALT was ascertained.

Determination of plasma alkaline phosphatase (ALP) activity

This was achieved by using the colorimetric method by Deutsche Gesellschaft für Klinische Chemie, [DGKC \(1972\)](#). The mixture of 20 μ l of the test sample and 1 ml of reacting mixture (1 M Diethanolamine buffer pH 9.8, 10 mM *p*-nitrophenyl phosphate, and 0.5 mM MgCl₂) was produced briefly. The absorbance was

measured between 1 minute intervals for 3 minutes at 405 nm and the activity of ALP was evaluated.

Statistical analysis

The results were carried out in replicates and merged as well as expressed as a mean \pm standard deviation. A one-way analysis of variance and the minimum significance difference were determined. Significance was accepted at $P \leq 0.05$.

RESULTS

The results of triglyceride, total cholesterol, HDL, and LDL are presented in [Figures 1–4](#). The figures reveal that the administration of elastic pastry (amala) and roasted (boli) unripe plantain 10% and 20% supplemented diets greatly reduces the concentration of plasma lipid profile of hypercholesterolemic rats with a significant difference when compared with the control group ($P < 0.05$). Although, the supplemented diets of 10% and 20% elastic pastry (amala) and roasted (boli) of unripe plantain, respectively, increases the concentration levels of HDL of hypercholesterolemic rats with a significant difference when compared with the control group ($p < 0.05$).

The inclusion of 1% cholesterol (i.e., 1% cholesterol and 99% basal diet) caused a remarkable increase ($p < 0.05$) in the level of plasma MDA concentration as shown in [Figure 5](#). Thus, the supplementation of 10% and 20% elastic pastry (amala) and roasted (boli) of unripe plantain inhibited the production of MDA in the liver with 10% elastic pastry (amala) having the highest significant difference.

The results of AST, ALT, and ALP are presented in [Table 1](#). The contents of plasma liver biomarker enzymes increase in the control group which in turn results in the malfunction of the enzymes. The supplemented diets of 10% and 20% elastic pastry (amala) and roasted unripe plantain (boli), respectively, caused a significant decrease in the concentration levels of plasma AST, ALT, and ALP when compared with the control ($P < 0.05$).

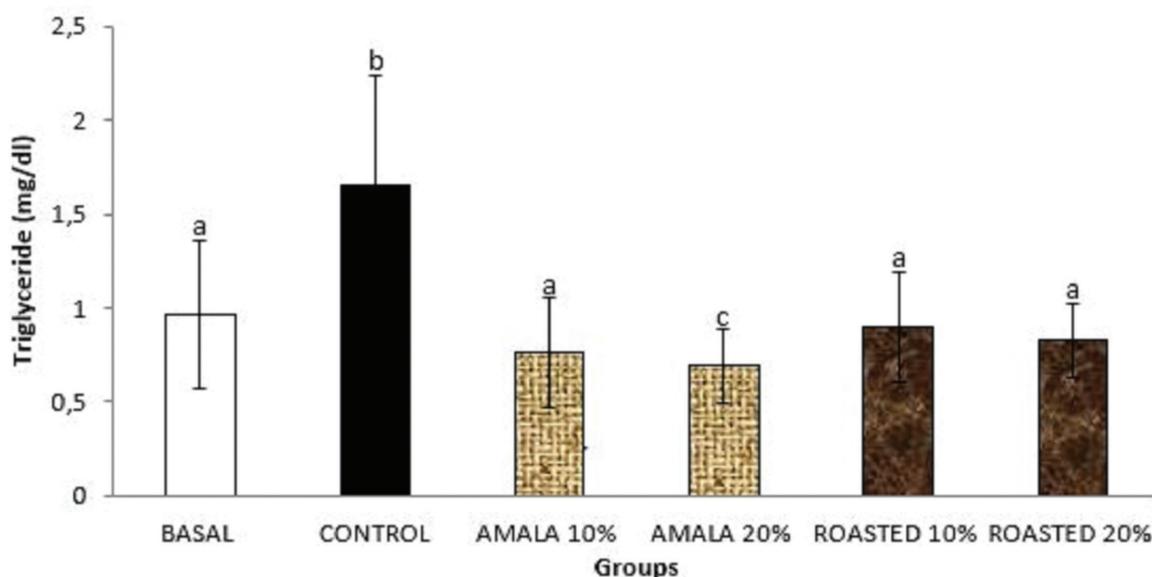


Figure 1. Showing the effects of elastic pastry (amala) and roasted (boli) of unripe plantain diet supplements on triglyceride of hypercholesterolemic rats. Bars with the same annotation are not significantly ($p < 0.05$) different.

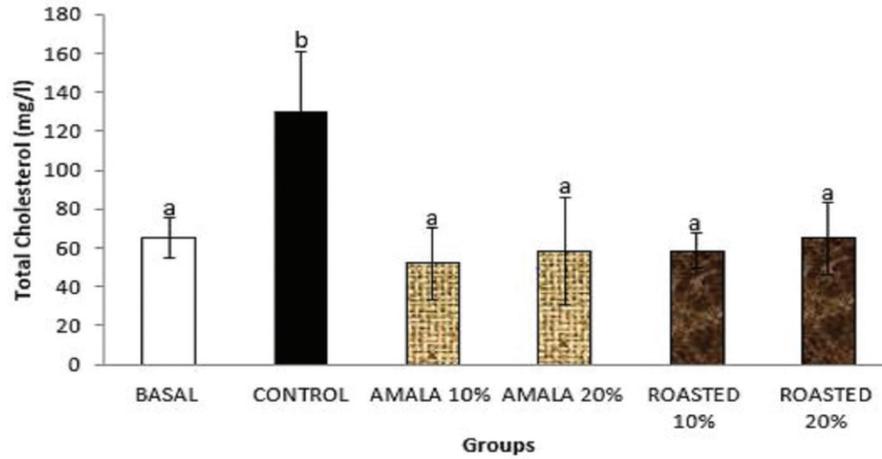


Figure 2. Showing the effects of elastic pastry (amala) and roasted (boli) of unripe plantain diet supplements on total cholesterol of hypercholesterolemic rats. Bars with the same annotation are not significantly ($p < 0.05$) different.

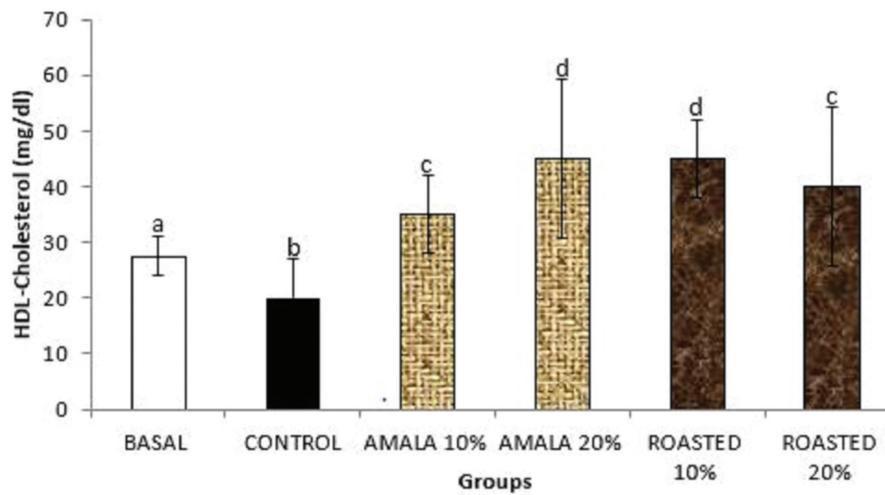


Figure 3. Showing the effects of elastic pastry (amala) and roasted (boli) of unripe plantain diet supplements on HDL of hypercholesterolemic rats. Bars with the same annotation are not significantly ($p < 0.05$) different.

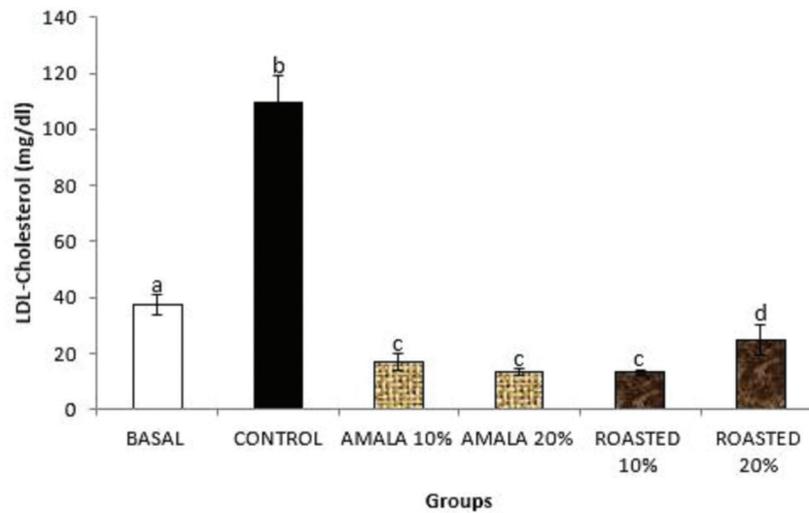


Figure 4. Showing the effects of elastic pastry (amala) and roasted (boli) of unripe plantain diet supplements on LDL of hypercholesterolemic rats. Bars with the same annotation are not significantly ($p < 0.05$) different.

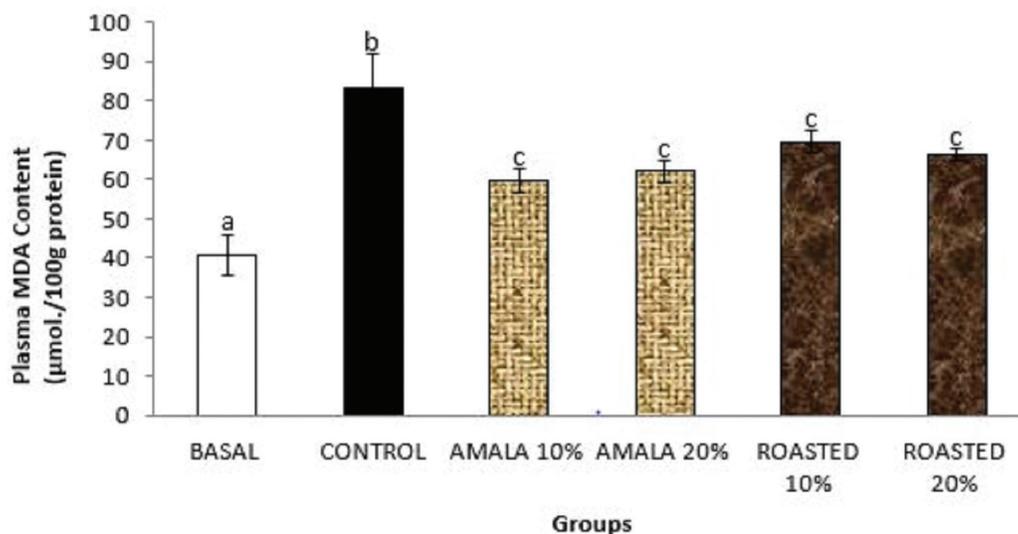


Figure 5. Showing the effects of elastic pastry (amala) and roasted (boli) of unripe plantain diet supplements on plasma MDA of hypercholesterolemic rats. Bars with the same annotation are not significantly ($p < 0.05$) different.

Table 1. Effects of elastic pastry (amala) and roasted (boli) of unripe plantain diet supplements on AST, ALT, and ALP contents of hypercholesterolemic rats.

	Basal	Control	Amala 10%	Amala 20%	Roasted 10%	Roasted 20%
AST(U/I)	160.7664 ± 6.183424 ^a	188.2045 ± 10.93099 ^b	163.7300 ± 1.992299 ^a	170.4890 ± 3.702557 ^c	173.074 ± 3.604967 ^c	169.1229 ± 5.634441 ^c
ALT(U/I)	3.2 ± 1.131371 ^a	15.2 ± 2.828427 ^b	8.2 ± 1.414214 ^c	7.2 ± 1.414214 ^c	3.2 ± 0.282843 ^a	3.0 ± 0.282843 ^a
ALP(U/I)	13.80 ± 3.903229 ^a	42.78 ± 5.854844 ^b	20.70 ± 5.854844 ^c	22.08 ± 7.806459 ^c	15.18 ± 1.951615 ^a	23.46 ± 1.951615 ^c

Values indicate mean ± standard deviation. Values with the same superscript alphabet on the same row are not significantly ($p < 0.05$) different

DISCUSSION

Green plantain (*M. paradisiaca*) when consumed, has been shown by several studies to have distinct advantages such that they are administered in the management and treatment of chronic diseases (Liu, 2004). Other research studies have highlighted the hypoglycemic, analgesic, and anti-ulcerogenic properties of plantain, all of which make the plant useful for the management of diseases like; diabetes, wound healing, and ulcers (Ojewole and Adewumi, 2003). In addition to these qualities, it also possesses antioxidant activities which make it capable of eliminating or scavenging free radicals by chelating metallic catalysts, reducing tocopherol radicals, activating antioxidant enzymes, and inhibiting oxidases (Amic *et al.*, 2003).

Results from a pilot study conducted prior to the commencement of this study show that *M. paradisiaca* incorporated diet had beneficial effect on the body weight gain in experimental animal, because there was reduction in body weight gained by rats fed on *M. paradisiaca* incorporated diet for 21 days when compared with rats fed with standard rat pellets (Ladokun Feeds, Nigeria). This could be attributed to the ability of the *M. paradisiaca* to reduce hyperglycemia (Iroaganachi *et al.*, 2015; Nwozo *et al.*, 2015a, b). On the other hand, dietary administration of *M. paradisiaca* and 1% cholesterol did not cause any noticeable alteration in the feeding pattern and the quantity of feed intake in the experimental rats. Fecal matter examination, particularly urine sample was not carried out and blood glucose changes were not monitored in this study.

An increase in plasma atherogenic lipids has been observed through a number of studies done in rats fed with high

cholesterol at 1% dietary inclusion (hypercholesterolemic rats) (Zhang *et al.*, 2002; Jang *et al.*, 2007), where it was ascertained that an increase in the level of cholesterol diets (1% diet supplement) resulted in high triglyceride and total plasma cholesterol levels in rats. However, a decline in these plasma atherogenic lipids in rats fed the elastic pastry (amala) and roasted (boli) unripe plantain in this present study is in accordance with other previous studies which have shown plants that possess cholesterol-lowering agents (Endo, 1992; Kim *et al.*, 2006). Earlier reports by Oboh and Erema, (2010) that, there is a presence of high total phenolic and flavonoids, as well as the fiber content in the unripe plantain products may have enhanced its favorable cholesterol metabolism. It has been studied and understood that; the cholesterol biosynthesis, absorption of dietary cholesterol, cholesterol removal from the circulatory system, and its excretion through bile and feces are being regulated by the concentration of the plasma cholesterol (Kim *et al.*, 2006).

In this study, Figures 1–3 reveals that the administration of elastic pastry (amala) and roasted (boli) unripe plantain supplemented diets greatly reduces the concentration of plasma lipid profile of hypercholesterolemic rats with a significant difference when compared with the control group ($P < 0.05$). Studies have revealed that the presence of phytochemicals such as phenols in plants inhibits the actions of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase); the rate-limiting enzyme in the biosynthesis of cholesterol in the liver (Endo, 1992). Moreover, phenols also possess inhibitory capacity on intestinal acyl CoA: cholesterol acyltransferase; which plays a significant role in the absorption of cholesterol via the process of esterification to cholesterol absorption (Zhang *et al.*, 2002). At several stages of

the gastrointestinal tract (GIT), polyphenols are released which possess advantageous effects on cholesterol metabolism; this may suggest that the polyphenolic content of elastic pastry and roasted unripe plantain diets released in GIT, partially responsible for the hypocholesterolemic effect of unripe plantain products through the inhibition and synthesis of absorption of dietary cholesterol.

Hypercholesterolemia is a chief risk factor in the development of cardiovascular diseases (such as; cerebro-vascular diseases, atherosclerosis, heart attacks, and myocardial infarction), which are the major causes of death globally (Law *et al.*, 2003). However, lowering the concentration levels of plasma cholesterol has been documented to lessen the risk of these diseases (Barter and Rye, 1996). Although, the supplemented diets of 10% and 20% elastic pastry (amala) and roasted (boli) of unripe plantain, respectively, as shown in Figure 4, increases the concentration levels of HDL of hypercholesterolemic rats with a significant difference when compared with the control group ($p < 0.05$). Hence, this observable increase in the levels of HDL-cholesterol plasma concentration suggests that the elastic pastry (amala) and roasted unripe plantain (boli) could enhance the homeostasis of cholesterol in the body. The presence of HDL-cholesterol in the body is regarded as “good cholesterol” (Stein and Stein, 1999) which helps in the transportation of cholesterol from peripheral cells to the liver where it is metabolized into bile acids (Jang *et al.*, 2007). This could then enhance positive regulation and control of cholesterol in the maintenance of cholesterol homeostasis between blood and peripheral tissues.

It has been reported that hypercholesterolemia enhanced the production of oxidative stress and increased LPO (Cox and Cohen, 1996). Studies have shown that a diet rich in high cholesterol concentration results in an increase in the levels of LPO by free radicals and aggravates hypercholesterolemia (Lee *et al.*, 2006). The increase in cholesterol diet also caused a marked elevation in the levels of plasma MDA; an initial outcome of LPO. However, an observable decrease in the levels of plasma MDA of hypercholesterolemic rats treated with the “amala” and “boli” supplemented diets (Fig. 5) clearly indicates a great significant regulation of cholesterol metabolism by lowering the MDA level. Therefore, unripe plantain products (amala and boli) supplemented diets can be considered as important supplementary therapeutic diet in the hypercholesterolemic state; due to their great significant regulatory effect in the plasma cholesterol concentration by lowering the plasma MDA which in turn results in the inhibition of oxidative stress.

Furthermore, an increase in liver biomarkers such as AST, ALT, and ALP in the plasma of rats fed with high cholesterol diet (1% dietary supplement) could be an indication of liver damage resulting in the injury of hepatocytes which may have caused a leakage of cytosolic enzymes (AST, ALT, and ALP) from the cell into circulation, thus, leading to an increase in the levels of these enzymes in the plasma (Pratt and Kaplan, 2000). Table 1, on the other hand, shows a reduction in the function of liver biomarker enzymes due to the increase in AST, ALT, and ALP levels as compared to the basal. Supplementing the diets with elastic pastry (amala) and roasted unripe plantain (boli) caused a significant decrease in plasma AST, ALT, and ALP levels when compared with the control ($P < 0.05$). Generally, hypercholesterolemia is considered to be an increase in both the abnormal hepatic and serum cholesterol and triglyceride levels (Wang *et al.*, 2010). The administration of dietary cholesterol has been shown to influence hepatic lipid metabolism in

rats (Wang *et al.*, 2010). Also, an increase in serum total cholesterol may result in impairment of triglyceride metabolism which causes deposition/accumulation of free fatty acids in the liver, thereby leading to a condition otherwise known as fatty liver (Wang *et al.*, 2010). This expanded liver fatty acid pool results in an increase in peroxisomal and mitochondrial β -oxidation which leads to the formation of reactive oxygen species. This may, in turn, result in the progression of liver injury via the process of a local proinflammatory state (Schwimmer *et al.*, 2008). Hence, as shown in Table 1, the supplemented diets of “amala” and “boli” may be able to protect the liver from oxidative damage due to its phenolic contents.

CONCLUSION

The outcome of this present study suggests that elastic pastry (amala) and roasted (boli) of unripe plantain may be able to protect the liver from oxidative damage. It also revealed that the treatment of hypercholesterolemic rats with elastic pastry “amala” and roasted “boli” from unripe plantain inhibited the generation of MDA in the plasma, which in turn resulted in the formation of LPO. Additionally, the scavenging activities and the hypocholesterolemic effects of these plantain products after the administration of high cholesterol (hypercholesterolemia) were also established by the study.

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