

The Influence of Unripe Plantain Processing on The Lipid Profile and Liver Biomarkers in Hypercholesterolemic Rats

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ABSTRACT

Hypercholesterolemia is considered as a condition that leads to the development of coronary heart disease and other related liver problems. Laboratory trials of hypercholesterolemia have been demonstrated towards its management. Unripe plantain (*Musa paradisiaca*) is one of the most important global food commodities that serves as a good source of carbohydrate with a least contribution to body fat content. This study sought to investigate the influence of unripe plantain products supplemented diet in hypercholesterolemia induced experimental rats. Hypercholesterolemia was induced by using 1% cholesterol while the hypercholesterolemic rats were treated with diets supplemented with 20-40% of raw plantain flour (RAPF) and roasted plantain flour (ROPF) respectively for four weeks. The influence of the diets on the lipid profile, liver function biomarkers and lipid peroxidation were examined. A significant ($p < 0.05$) increase in weight, total cholesterol, triglyceride, LDL-c values with a corresponding reduction of HDL-c in hypercholesterolemic rats were observed in hypercholesterolemic rats. However, these parameters were significantly ($p < 0.05$) reduced after the rats fed on RAPF and ROPF for four weeks. ROPF diet groups exhibited the overall highest anti-hypercholesterolemic effect. The exerted anti-hypercholesterolemic effect could be attributed to the activities of bioactive phytoconstituents that abound in unripe plantain earlier studied *in vitro*.

Keywords

Plantain, Hypercholesterolemia, Lipid profile, Lipid peroxidation, Liver biomarkers.

Introduction

Hypercholesterolemia is regarded as an important factor of consideration in the development of coronary heart disease. Ischemic stress of the heart is a major cause of mortality in both the developed and under-developed countries alike. Chronic elevation of serum cholesterol forms part of atheromatous plaques in the arteries as it constitutes one of the highlighted risk factors that develops into cardiovascular diseases (CVDs) and lipid metabolism malfunctions [1]. A sustained occlusion of a coronary artery results in a myocardial infarction while occlusion of an artery conveying blood to the brain can lead to stroke [2]. Apart from this, hypercholesterolemia is also regarded as the permissive factor that opens door to other risk factors such as, high blood pressure, low level of high-density lipoprotein (HDL) cholesterol, elevated level of low density lipoprotein (LDL) cholesterol, cigarette smoking

and increased age [3]. Hypercholesterolemia can relatively be secondary to other complications such as liver dysfunction by altering the serum lipids and lipoproteins concentrations in the liver [4].

The regular consumption of high amounts of fat has been directly linked to hypercholesterolemia in humans and clinical trials have also demonstrated that intensive reduction of plasma LDL-Cholesterol levels could reverse atherosclerosis and decrease the incidents of cardiovascular diseases. Consequently, experiments in laboratory animals has formed part of adopted researches explored to get a better knowledge of series of disorders in cholesterol metabolism and to test for the possible treatments of regulating the circulating cholesterol level [5].

Plantain (*Musa paradisiaca*) fruit has been classified as the fourth most important global food commodity after rice, wheat and maize that is grown in many Countries in the world. Plantain cultivation produces an all year-round fruiting capacity to bridge the hunger

gap between harvesting periods. Hence, contributing immensely to the food and income security of farmers that are engaged in its production and trade, especially in a developing country like Nigeria. According to FAO [6], plantain cultivation has been recorded in 52 countries with world production of 33 million metric tons. Coming down to Africa, only eight Countries were tagged the top ten world producers of plantain in which Nigeria was ranked the fifth. Plantain fruits are taken in their unripe form adopting different kinds of processing methods such as roasting, steaming, baking or grilling [7].

In Nigeria the plantain pulp can be roasted either in their ripe or unripe form on hot charcoal and consumed with traditional delicacies. This practice is also popular in other plantain producing countries in Africa. Unripe plantain in particular can also be processed into flour as a means of preservation which can be mixed with boiling water to prepare an elastic pastry (a brown bolus that is fondly called 'Oka' among 'Yorubas' in western side of Nigeria) to be consumed with melon soup and as 'Fufu' in Cameroun is also eaten with various sauces [8]. Primary processing of unripe plantain involves the exposure of the plantain fruit by cutting it open. Normally, enzymes and substrates are understood to be located in separate cellular compartments but can interact with each other after the membranes have become permeable [9].

The analysis of plantain nutrient contents reveals it to a good source of energy in the form of carbohydrate with a least contribution to body fat content. However, as this process proceeds, the fruits are observed to show strong enzymatic browning reactions. This physical change has invariably been attributed to the oxidation of phenolic compounds [10] while further heat processing darkened the fruit through the generation of non-enzymatic browning that leads to the accumulation of melanoidins [11].

In the recent time, phenolics and non-enzymatic browning (melanoidins) had been reported to have health promoting potentials [12]. The relevance of these compounds has been demonstrated to exert health augmenting activity and certain therapeutic potentials [13]. Nevertheless, reports on the use of plants have also been demonstrated to protect the liver from hepatic injury [14].

This research was aimed at determining the influence of unripe plantain flour and roasted unripe plantain on the lipid profile (total cholesterol, triglyceride, LDL and HDL), plasma malondialdehyde (MDA) and liver function biomarkers [aspartate transferase (AST), alanine transferase (ALT) and alanine phosphatase (ALP)] activities of hypercholesterolemic rats.

Materials and Methods

Sample collection

Fresh 'False horn' mature unripe plantains were collected at Owena-Ondo community, Ondo State. The unripe plantain was authenticated at the Department of Biology, Federal University of Technology, Akure, Nigeria. All chemicals and reagents used were purchased from Sigma chemical Co. (St. Louis, MO), while the distilled water used was provided by the Chemistry Department,

Federal University of Technology, Akure. Ondo State. Nigeria.

Sample Preparation

Raw plantain flour (RAPF)

The unripe plantain fruits were washed and divided into two portions for sample preparations. The first portion was cut open to reveal the pulp. At the time of peeling, there was an observed change of colour from cream to brown. The pulps were sliced and sun-dried for about 3 weeks to dryness and later ground into flour. The flour was passed through a sieve and later kept in an air tight container prior to analysis.

Roasted plantain Flour (ROPF)

The other portion was peeled and the pulp was roasted over a wire gauze placed on red hot charcoal to simulate the traditional processing method. The pulp was turned continuously to maintain even browning during the roasting process which lasted for 15 min. Thereafter, the roasted pulp was hand-crushed and dried for 3 weeks to constant weight, milled into powder and kept in an air tight container prior to analysis.

Animals

The handling and the use of the animals were in accordance with NIH Guide for the care and use of laboratory animals. Adult male Wistar albino rats weighing <200 g used for this experiment were purchased from the breeding colony of the Department of Biochemistry, University of Ilorin, Nigeria. The rats were maintained at 25°C on a 12:12 h light/dark cycle with free access to food and water ad libitum. They were acclimatized under these conditions for two weeks prior to the commencement of the experiment. The experimental study was approved by the institutional animal ethical committee of the Federal University of Technology, Akure, Nigeria.

Experimental design

Preparation of experimental rat model for high cholesterol-fed bioassay

After acclimatization, the rats were divided into six (6) groups of six (6) experimental rats each (n=6). The animal feed preparation was done according to the method described by Matos et al. [15] with modifications. Group I consisting the normal rats received basal diet only (44% skimmed milk, 10% groundnut oil, 42% corn starch, and 4% vitamin and mineral premix), Group II- (as control) received basal diet plus 1% cholesterol powder for induction of HPC, Group III-IV received basal diet plus 1% cholesterol supplemented with 20% and 40% raw plantain flour (RAPF) respectively and Group V-VI received basal diet plus 1% cholesterol supplemented with 20% and 40% roasted plantain flour (ROPF) respectively. The rats were maintained in their respective cages and the experiment lasted for 4 weeks.

Biochemical investigation

The blood sample of different sacrificed rats was collected at the end of each feeding trial and quickly centrifuged at 3000 rpm (using refrigerated SIGMA 2-16KL centrifuge) for 10 min to collect the plasma. The produced supernatant plasma was quickly

removed and kept in a refrigerator for biochemical analyses. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-c) and triglycerides (TG) were determined in the plasma of the rats by adopting the protocol outlined in the manufacturer's assay kit from Randox Laboratories Ltd, Ardmore, Co. Antrim, UK. LDL-c was calculated using the formula that was described by Friedewald et al. [16]:

$$\text{LDL-c} = \frac{\text{Total cholesterol} - \text{Triglyceride} - \text{HDL-c}}{5}$$

The Atherogenic index (AI) was calculated according to Harnafi et al. [17] formula:

$$\text{Atherogenic index (AI)} = \frac{\text{Total cholesterol} - \text{HDL-c}}{\text{HDL-c}}$$

The Liver of each rat (0.5 g) was quickly excised and homogenized in 5 ml saline solution using Teflon homogenizer. The clear homogenate will be used to test for AST, ALT and ALP contents using commercially available kits (Randox Laboratories UK). Thiobarbituric acid reactive substances (TBARS) which indicates lipid peroxidation was determined by the thiobarbituric acid reaction in the tissue homogenate according to the method of Okhawa [18].

Data analysis

The results of replicate readings were pooled and expressed as mean \pm standard deviation. Appropriate analysis of variance was used to analyze the means and Duncan's multiple test was used for the post-hoc treatment of mean [19]. Significance was accepted as ($p < 0.05$).

Results

The effect of plantain products (RAPF and ROPF) on weight of hypercholesterolemic rats

Figure 1 summarizes the average weight of experimental rats on the first and fourth week and average feed intake of the experimental animals (Figure 1). The experimental rats coped with the formulated diets provided and they survived the duration of the experiments as well. The average feed intake of all the experimental rats recorded no significant ($p < 0.05$) difference. The weight of the rats also displayed no significant difference ($p < 0.05$) in the first week. Group II (hypercholesterolemic rats) however recorded a dramatic weight increase in the fourth week (186.2-212.5 g). A steady weight decline was reported for unripe plantain products diet treated rats (Group III-VI) by the end of the fourth week. However, the ROPF treated rats (Group V and VI) had the least reported weight. There was no significant change in the parameters of the normal rats in Group I.

Effect of plantain products (RAPF and ROPF) on the atherogenic plasma lipid profile of hypercholesterolemic rats

The results of TG, TC (Figure 2) and LDL-c (Figure 3) revealed the effect of diet supplemented with unripe plantain products on the lipid profile of the experimental rats. There was a significant ($p < 0.05$) increase in the plasma levels of TG (165.05 mg/dl), TC (110.50 mg/dl) and LDL-c (95.67 mg/dl) in Group II (hypercholesterolemic rats) when compared with normal rats in Group I (46.32, 62.67

and 20.31 mg/dl) respectively. However, diet treated rats with 20 and 40% inclusion of RAPF (Group III and IV) and ROPF (Group V and VI) displayed significant decreased values of TG, TC and LDL-c with Group V and VI exhibiting the least values. On the contrary, the estimated HDL-c level (Figure 3) of the Group II rats was observably lower compared to normal group while the 40% ROPF treated rats (Group VI) exhibited the highest value. In other words, the inclusion of RAPF and ROPF favoured the boosting of HDL-c level in the unripe plantain products diet treated rats. A similar profile after calculating the atherogenic index was reported in RAPF (Group III and VI) and ROPF (Group V and VI) treated rats with least values in comparison to the normal rats while the Group II had the highest value (Table 1). The TBARS result also revealed a marked increase in hypercholesterolemic rats (Group II) compared to the normal rats in Group I. The least significant ($p < 0.05$) reduction was reported in 20% ROPF (Group V) treated rats (Figure 4).

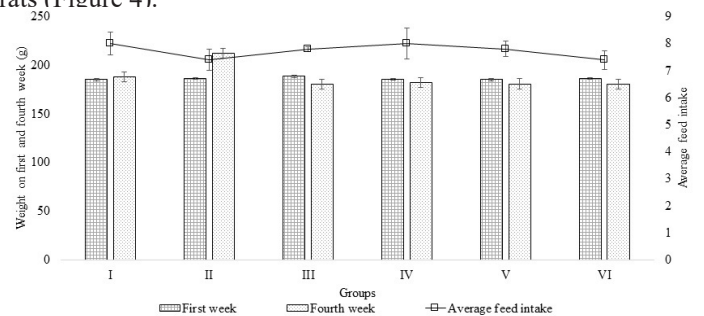


Figure 1: Representation of weight on 1st and 4th week, Average weights (g) and average feed intake of experimental rats.

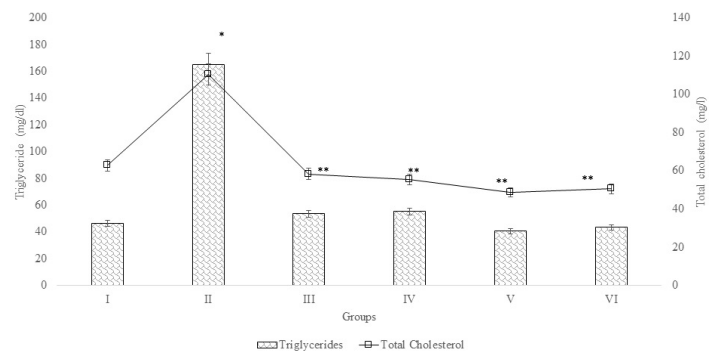


Figure 2: Effect of RAPF and ROPF supplemented diets on plasma Total cholesterol and Triglyceride levels in hypercholesterolemic rats. *Values are significantly ($p < 0.05$) different from basal group. **Values are significantly ($p < 0.05$) different from control group.

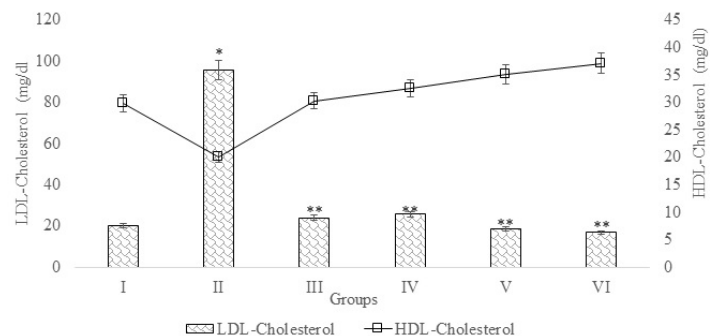


Figure 3: Effect of RAPF and ROPF supplemented diets on plasma LDL-cholesterol and HDL- Cholesterol levels in hypercholesterolemic rats. Please refer Figure 2 for * (asterisks) meanings.

The estimated hepatic enzyme activities of ALP, AST and ALT also revealed the condition of the liver in all the experimental rats. The results revealed highest values of the hepatic enzyme activities in hypercholesterolemic rats (Group II) compared to the normal rats in Group I while the unripe plantain diet exerted a beneficial influence on the activities of the hepatic enzymes by lowering their concentrations for rats in Group III-VI (Figure 4-5). However, there was no significant difference in the level of reported reduction observed in Group V and VI.

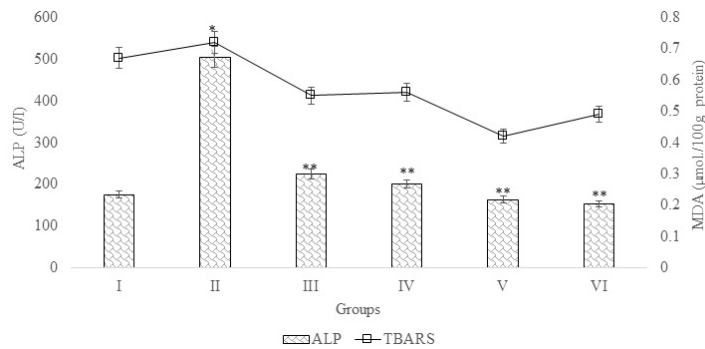


Figure 4: Effect of RAPF and ROPF supplemented diets on plasma ALP activity and plasma Malondialdehyde (MDA) level in hypercholesterolemic rats. Please refer Figure 2 for * (asterisks) meanings.

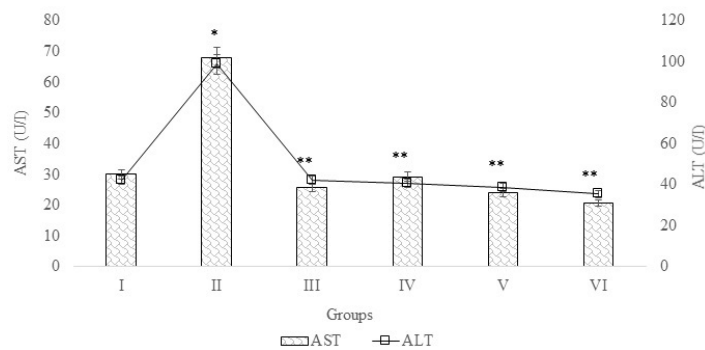


Figure 5: Effect of UPF and RUP supplemented diets on plasma AST and ALT activities in hypercholesterolemic rats. Please refer Figure 2 for * (asterisks) meanings.

Groups	Atherogenic Index (AI)
I	1.10 ± 0.02b
II	4.51 ± 0.05a
III	0.93 ± 0.00c
IV	0.71 ± 0.07d
V	0.38 ± 0.02e
VI	0.36 ± 0.02e

Table 1: Atherogenic index of experimental rats. Values with the same superscript number on the same column are not significantly ($p < 0.05$) different.

Discussion

The continual ingestion of saturated fatty acids had been demonstrated to increase the concentrations of plasma LDL-cholesterol and triglyceride which in turn inactivates the activities of lipoprotein lipase and LDL receptors that are responsible for the metabolism and transport of LDL-cholesterol [20]. Hence, eventual LDL-c increase acts as an exacerbating factor that leads to cholesterol dysfunction. In this present study, these could be related to observed condition of significant increase in the weight, plasma triglyceride, total cholesterol and LDL-cholesterol values of hypercholesterolemic rats in Group II. According to Kim et al. [21], plasma cholesterol concentration can be regulated by cholesterol biosynthesis, cholesterol removal from the circulatory system, absorption of dietary cholesterol and cholesterol excretion via bile. Apart from these, contributory role of LDL-c has been linked to the increase in the level of secretory group IIa phospholipase A2 which in turn partakes in atherosclerosis to infiltrate the arterial wall lipid moiety to be oxidized by reactive oxygen species to ox-LDL. The ox-LDL in turn promotes the release of phospholipids, activation of endothelial cells, initiate an inflammatory cascade that subsequently leads to formation of foam cells and fatty streaks. The findings of this present study that revealed a marked significant decrease to the concentrations of total cholesterol, triglyceride and LDL-c in diet treat rats (Group III-VI) in comparison to hypercholesterolemic rats (Group II) corroborates the earlier studies that revealed the impact of HDL in the management of LDL activities [22]. The presence of Apolipoprotein A-1 (Apo A-1) on HDL-C particles has also been researched to exert an anti-atherogenic function by preventing LDL from oxidation [23].

The two-edged incidence of malondialdehyde production which results from lipoxygenase-mediated oxidation of arachidonic acid can also alter LDL into a form that upregulates macrophage scavenger receptors that allows cholesterol esterification in foam cells [24]. The marked increase in plasma malondialdehyde (MDA) level in Group II was reduced on the ingestion of the RAPF and ROPF (Figure 4). Literature review has also revealed that cholesterol-rich diet increases lipid peroxidation by free radicals and exacerbates hypercholesterolemia [25].

Hepatoprotective functionality of plants may be investigated by estimating the concentration of some hepatic enzymes released into the blood [26]. The liver endures many oxidative insults because of its dynamic roles in xenobiotic metabolism [27]. To ensure the liver protection, it is of utmost importance to estimate the concentration of some hepatic enzymes released into the blood. The presence of AST and ALP in liver is normally high in concentration but when there is an aggravation due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are leaked from the cells into the blood and their levels increases [28]. The concentrations of ALT, AST and ALP were estimated in this study and there was significant ($p < 0.05$) reduction in their levels in the diet supplemented groups when compared with the hypercholesterolemic rats (Figure 4-5). These however fall in line with the earlier work of Grespan et al. [29].

The abnormal condition of lipid metabolism coupled with increased hepatic enzymes concentrations and lipid peroxidation in hypercholesterolemic rats (Group II) was reduced with the inclusion of varied concentration of RAPF and ROPF (Group III-VI). This positive impact can be linked to the presence of some phytoconstituents that exerted their antioxidant activity as emphasized through our earlier in vitro research on characterized phenolic compounds in unripe plantain products [30]. Phenolics had been widely studied and accredited with the antioxidant and medicinal properties. They play key roles in the inhibition of the destructive impart of free radicals to biomolecules. Damaged biomolecules induce diseases due to lipid peroxidation. This is also in agreement with another earlier report where phenolic contents with high antioxidant activities could function against exacerbating factors that could lead to cholesterol malfunction [31].

A suggestion of compounds like melanoidins (data not shown) generated from application of dry heat has been reported to potentiate improved health condition [12]. According to literatures, maillard reactions products contain hydroxyl groups which act as hydrogen donators therefore, demonstrating the ability to scavenge free radicals [32]. Their antioxidant activities have also been attributed to the presence of reductone-like structure and catecholic moieties [33]. Apart from the fact that roasting improves the aroma of food, an earlier research was conducted to establish the preparation of melanoidins standard from glucose and glycine, adopted for use in the study of different aspects of melanoidins as a continual exploration into the antioxidant activities and their protective effect against reactive oxygen species (ROS) that participate in glycosylation of macromolecules [34]. Dry-processed unripe food samples also contain apparent increase in nutrient contents due to removal of moisture [35]. Earlier researched works had reported low fat content as well as high ash and fibre contents of unripe plantain products as they are not affected by processing treatments [36] and characterized low fat content [36,37]. Based on the improved status of lipid profile and liver function in diet treated rats in comparison to the hypercholesterolemic rats, it could therefore be inferred that unripe plantain products inclusion in diet could significantly reverse the abnormal condition of lipid profile and liver function to normalcy with special reference to roasted plantain flour. The revealed information of this research could be harnessed for future purpose to prevent or manage the condition of hypercholesterolemia.

Conclusion

The present study demonstrated the anti-hypercholesterolemic properties of unripe plantain products (RAPF and ROPF) supplemented diets in experimental rats. There was an observed reduction in the values reported for weight, total cholesterol, LDL-cholesterol and plasma MDA in diet treated Groups. The reduction of these parameters led to a concomitant improvement in their HDL-cholesterol concentration. The ingestion of the unripe plantain diet also normalized the condition of liver biomarker enzymes by reducing their concentrations. Of note is the overall significant influence of roasted plantain flour. Our

earlier experiments hitherto made show that phenolic compounds abound in unripe plantain products therefore associating it with their display of anti-hypercholesterolemic effects. In the light of this, healthy diet modifications through the use of unripe plantain products can be adopted in meals to prevent and manage lipid metabolism especially in individuals with hypercholesterolemic condition in order to forestall its long-term complications.

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