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Traditional Fermented Condiments Modulate Biochemical Indices in High Cholesterol Diet-Fed Rats

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ABSTRACT: Hypercholesterolemia is implicated in cardiovascular diseases (CVD) and is identified as a common cause of mortality in this degenerative condition. Hence, CVD management strategy should include control of hypercholesterolemia. This study describes the effect of some legume condiments on biochemical indices in hypercholesterolemia rat model. Adult male Wistar rats were used for this study and hypercholesterolemia was induced by inclusion of 1% cholesterol in the rat feed. The animals were divided into five groups containing six animals each and were fed with diets supplemented with 16% fermented soybean, bambara groundnut and African locust bean. The study lasted for 30 days after which plasma was analyzed for the lipid profile and liver function marker enzymes and the liver tissue analyzed for malondialdehyde (MDA) content. Elevated plasma total cholesterol, triglyceride, LDL-cholesterol, alanine aminotransferase, aspartate transaminase, alkaline phosphatase and MDA content showed significant ($p<0.05$) reduction in the rats fed with fermented legume condiment-supplemented diets, with a concomitant increase in plasma HDL-cholesterol as compared with the hypercholesterolemia control rats. This study revealed that fermented legume condiment-supplemented diets attenuate hypercholesterolemia and protect the liver of the experimental rats from oxidative damage, with African locust bean condiment displaying the best biological potential.

Keywords: Condiments; hypercholesterolemia; lipid profile; liver marker enzymes

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INTRODUCTION

Cholesterol is a waxy, fat-like substance found in the blood and the body cells of humans and animals as the major sterol in animal tissues (Nelson and Cox, 2008). Plant and animal foods contain sterols, with cholesterol present only in animal foods. The liver synthesizes most needed cholesterol while the rest are gotten from dietary source. Cholesterol administration has been reported to influence hepatic lipid metabolism in rats, the high dietary cholesterol was concerned with the increasing concentrations of serum and hepatic total cholesterol (TC), especially the level of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) (Wang *et al.,* 2010). Hence, it has been declared a culprit behind various life threatening diseases like hypercholesterolemia and cardiovascular diseases (Colpo, 2005; Nelson and Cox, 2008).

Hypercholesterolemia is usually characterized by both abnormal serum and hepatic triglyceride and cholesterol levels which are implicated in the accumulation of free fatty acids in the liver (Wang *et al.,* 2010). This expanded liver fatty acid pool leads to increased mitochondrial and peroxisomal â-oxidation, which produces reactive oxygen species (ROS) and exacerbate oxidative stress.

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Oxidative stress occurs when the production of harmful ROS and other free radical molecules overwhelms the protective capability of the innate antioxidant defense mechanism (Alia *et al.,* 2003). Free radicals are chemicals containing atoms with an unpaired electron in their outer orbit; they are short lived, unstable and react with other molecules to achieve stability (Nadia *et al.,* 2004). However, dietary phenolic compounds have been known to counteract the destructive effect of oxidative stress. These phenolic compounds which are derived majorly from plant foods exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic cardioprotective and vasodilatory effects (Manach *et al.,* 2005).

Legumes refer to the edible seeds of leguminous plants belonging to the family *Leguminosae* which include beans and pulses (Lambein *et al.,* 2008; Oboh *et al.,* 2009). Legume grains/seeds occupy important place in human nutrition, especially in the dietary pattern of low income earners of the developing countries (Tharanathan and Mahadevamma, 2003), who consume them as major sources of proteins because they are cheaper than animal proteins. It has long been recognized that legumes are functional foods that both promote good health and have therapeutic properties as they are a good source of numerous phytochemicals such as phenolics, flavonoids and isoflavonoids (Romani *et al.,* 2004; Ademiluyi *et al.,* 2014) which are chemopreventive agents with hypocholesterolemic properties (Anderson et al. 1999). Legumes are subjected to various processing methods like roasting, drying, fermentation etc before consumption. However, fermentation of legumes is the biochemical conversion of the complex molecules to simpler molecules via the action of microbes (Achi, 2005). This process improves the nutritional composition of foods through the biosynthesis of vitamins, essential amino acids and proteins, by improving protein quality and fiber digestibility. Thus, aiding the degradation of anti-nutritional factors and enhances micronutrient bioavailability (Raimbault, 1998).

Underutilized legumes abound in tropical Africa where they are regularly consumed in various forms. These legumes are fermented into traditional local condiments which have been the pride of culinary industries for several centuries. Furthermore, studies have shown that fermentation increases the phenolic content of the legume seeds (Oboh *et al.,* 2009) hence, improvement in their help promoting properties. Therefore, this study sought to investigate the effect of some fermented legume condiments on critical biomarkers of hepatic function and lipid metabolism in high cholesterol-fed rats.

Bambara groundnut (Vigna subterranean L. Verdc) **Soybean *(Glycine max l. Merrill)*** **African locust bean *(Parkia biglobosa G. Don)***

Plate 1: Fermented legume condiments

MATERIALS AND METHODS

Sample collection

Dried legume seeds; Bamabara groundnut (*Vigna subterranea* L. verdc), Locust bean (*Parkia biglobosa*) and Soybean (*Glycine max* (L.) Merrill) were purchased at the Erekesan market in Akure metropolis, Nigeria. Authentication of the samples was carried out at the Department of Crop, Soil and Pest Management (CSP), Federal University of Technology, Akure, Nigeria. The samples were sorted out to remove stones and dirt before they were made ready for condiment preparation.

Preparation of the fermented legume condiments

The legumes were soaked overnight and boiled until soften to facilitate dehulling which was done to the testa. Large volume of water was then added to separate the hull from the beans. The dehulled beans were washed again under running water to remove dirt. Thereafter, the bean were further cooked for 1 hr and drained. The drained beans were later spread into a clean basket covered with banana leaves and fermented for 3 days (72 hrs) to produce the condiments (Odunfa, 1985).

Animal: Fermented legume condiments

Adult male Wistar rats weighing 140–200 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 h light/dark cycle with free access to food and water. They were acclimatized under these conditions for two weeks prior to the commencement of the experiments. The animals received humane treatment according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by EU Directive 2010/63/EU for animal experiments. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments. The experiment was carried out at the Functional Food, Nutraceuticals and Phytomedicine Laboratory, Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria.

Experimental design and treatment groups

After two weeks of acclimatization, the rats were randomly divided into five groups of six animals each. Group I was fed basal diet (44% skimmed milk, 42% corn starch, 4% mineral & vitamin premix and 10% groundnut oil), Group II was fed basal diet containing 1% cholesterol, Group III was fed basal diet containing 1% cholesterol and 16% inclusion of fermented soybean condiment, Group IV was fed basal diet containing 1% cholesterol and 16% inclusion of fermented bambara groundnut condiment while Group V was fed basal diet containing 1% cholesterol and 16% inclusion of fermented locust bean condiment. The experiment lasted for 30 days and the animals were decapitated after an overnight-fast following cervical dislocation. The blood was rapidly collected by direct heart puncture and the plasma was prepared.

Note: *Skimmed milk = 36% protein; 1g of the mineral and vitamin premix contains; 3200i.u vitamin A, 600i.u vitamin D3, 2.8mg vitamin E, 0.6mg vitamin K3, 0.8mg vitamin B1, 1mg vitamin B2, 6mg niacin, 2.2mg pantothenic acid, 0.8mg vitamin B6, 0.004mg vitamin B12, 0.2mg folic acid, 0.1mg biotin H2, 70mg choline chloride, 0.08mg cobalt, 1.2mg copper, 0.4mg iodine, 8.4mg iron, 16mg manganese, 0.08mg selenium, 12.4mg zinc, 0.5mg antioxidant. Supplementation of both cholesterol and the fermented legume condiments were done on equal-weight basis.*

Preparation of plasma and tissue homogenates

At the end of the feeding trial, whole blood of the sacrificed rats was collected into EDTA bottles and centrifuged at 800 × g for 10 min to separate the plasma. The plasma was then decanted into plain sample bottle and stored overnight (12 hrs) at 4 °C in a refrigerator prior to analysis. The liver was rapidly isolated placed on ice and weighed. This tissue was rinsed in cold 0.9 % normal saline (1:3, w/v), subsequently

homogenized in sodium phosphate buffer (pH 6.9) and the homogenates centrifuged at 5,000 × g. The clear supernatants obtained were used for various biochemical assays (Belle *et al.,* 2004).

Determination of plasma triglyceride concentration

The triglyceride concentration was determined using colorimetric method as described by Tietz (1982). Briefly, 10 ìl of the sample was mixed with 1 ml of Pipes reagent (40 mM phosphate buffer, 5.5 mM 4-chlorophenol and 17.5 mM Mg^{2+}) and enzyme reagent (4- aminophenazone, adenosinetriphosphate, lipase, glycerolkinase, glycerol-3-phosphate oxidase and peroxidase). Thereafter the mixture was incubated for 5 min at 37°C and the absorbance at 546 nm was taken against reagent blank within 60 min. The triglyceride concentration was subsequently calculated against the standard.

Determination of plasma total cholesterol concentration

One millilitre of the reacting mixture containing 4-aminoantipyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase and 80 mM Pipes buffer pH 6.8 was mixed with 10 ìl of plasma and incubated for 5 min at 37°C. The absorbance at 546 was then taken against the reagent blank within 60 min. The concentration of cholesterol in the sample was subsequently calculated against a standard (Tietz, 1982).

Determination of plasma HDL-cholesterol concentration

The precipitation was carried out according to the method of Lopes-Virella et al., (1977) as described in the kit's manufacturer (Randox Laboratories Ltd) manual. Briefly, 200 ìl of plasma was mixed with 500 ìl of the precipitant (0.55 mM phosphotungstic acid and 25 mM magnesium chloride) and allowed to sit for 10 min at room temperature. Then, the mixture was centrifuged for 10 min at 800 × *g*. Thereafter, the clear supernatant was separated off and subjected to the same procedure for the determination of cholesterol described above.

Determination of plasma LDL-cholesterol concentration

The LDL-cholesterol concentration of the plasma samples was determined according to the equation of Friedewald et al., (1972).

LDL Cholesterol (mg/dl) = Total Cholesterol – Triglycerides/5 – HDL Cholesterol

Determination of plasma aspartate aminotransferase (AST) activity

This was carried out according to the method of Reitman and Frankel, (1957) as described in the Manufacturer's manual (Randox Laboratories Ltd). Briefly, 100 ìl of test sample was mixed with 500 ìl of buffer (containing 100 mM phosphate buffer pH 7.4, 100 mM L- aspartate, and 2 mM á-oxoglutarate) and the mixture was incubated for 30 min at 37°C. Thereafter, 500 ìl of 2 mM 2,4 dinitrophenylhydrazine was added to the reaction mixture and allowed to stand for 20 min at 25°C. Then, 500 ìl of 0.4 mM NaOH was added and thoroughly mixed; the absorbance was read after 5 min at 546 nm against a reagent blank and the AST activity determined.

Determination of plasma alanine aminotransferase (ALT) activity

This was carried out according to the method of Reitman and Frankel, (1957) as described in the Manufacturer's manual (Randox Laboratories Ltd). Briefly, 100 ìl of test sample was mixed with 500 ìl of buffer (containing 100 mM phosphate buffer pH 7.4, 200 mM L- alanine and 2 mM á-oxoglutarate) and the mixture was incubated for 30 min at 37°C. Thereafter, 500 ìl of 2 mM 2,4 dinitrophenylhydrazine was added to the reaction mixture and allowed to stand for 20 min at 25°C. Then, 500 ìl of 0.4 mM NaOH was added and thoroughly mixed; the absorbance was read after 5 min at 546 nm against a reagent blank and the AST activity determined.

Determination of plasma alkaline phosphatase (ALP) activity

The plasma ALP concentration was determined using spectrophotometric method according to the recommendations of Deutsche Gesellschaft fur Klinische Chemie, (1972). Briefly, 20 ìl of the test sample was mixed with 1 ml of reacting mixture (containing 1 M Diethanolamine buffer pH 9.8, 0.5 mM $MgCl_2$ and 10 mM *p*-nitrophenylphosphate). The absorbance was then read at 1 min interval for 3 min at 405 nm and the ALP activity was subsequently calculated.

Determination of tissue lipid peroxidation (Malondialdehyde content)

The lipid peroxidation assay was carried out using the modified method of Okhawa et al., (1979). Briefly 300 ìl of tissue homogenate, 300 ìl of 8.1% SDS (Sodium dodecyl sulphate), 500 ìl of Acetic acid/HCl (PH = 3.4) and TBA (Thiobarbituric acid) were added, and the mixture was incubated at 100°C for 1 hr. Thereafter, the thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm and calculated as Malondialdehyde (MDA) equivalent.

Data analysis

The results of replicate readings were pooled and expressed as mean ± standard deviation with the level of significance taken at $P<0.05$. One way analysis of variance was used to analyze the results and Duncan multiple test was used for the post-hoc (Zar, 1984). Statistical package for Social Science (SPSS) 10.0 for Windows was used for the analysis.

RESULTS AND DISCUSSION

The effect of the condiments on biochemical indices in high cholesterol-fed (Hyper-cholesterolemia rat model) rats was investigated. The average feed intake (Table 1) of all the experimental animals were calculated after the 30 days of study and it was discovered that, there was no significant ($P>0.05$) difference in the average feed intakes for all groups. However, there was a significant ($P<0.05$) weight gain in Group II (hypercholesterolemic control) rats compared to both Group I (normal control) and Groups III – V (treated rats); furthermore, all the treated rats exhibited significant ($P<0.05$) weight loss compared with the Group I and Group II rats (Table 1).

Also, there was a significant ($P<0.05$) increase in the plasma level of atherogenic lipids such as triglyceride, total cholesterol and LDL-

Table 1: Average feed intake (g/rat/day) and weights (g/rat) of rats fed diets supplemented with 1% cholesterol and fermented legume condiments

Groups	Average feed intake (g/rat/day)	Weight (g/rat)		Weight gain/loss (%)
		1st day	30th day	
I	9.0 ± 2.8^{a}	150.2 ± 12.1	160.7 ± 10.3	9.8^{d}
II	7.2 ± 1.5^{a}	145.7 ± 9.9	175.2 ± 20.0	20.3^{e}
III	8.6 ± 2.2^{a}	153.3 ± 13.1	103.1 ± 9.7	-32.6^{a}
IV	8.0 ± 1.5^{a}	149.9 ± 4.5	132.0 ± 2.0	-13.4^{b}
V	7.9 ± 0.9^{a}	140.0 ± 10.4	132.1 ± 8.8	-5.7^{c}

Values represent mean ± standard deviation (n = 6). Values with the same superscript letter down the same column are not significantly ($P<0.05$) different. Group I – normal control rats, fed with basal diet; Group II – rats fed with basal diet plus 1% cholesterol (hypercholesterolemic rats); Group III – rats fed diet supplemented with 16% fermented Soybean condiment plus 1% cholesterol; Group IV – rats fed diet supplemented with 16% fermented Bambara groundnut condiment plus 1% cholesterol; Group V – rats fed diet supplemented with 16% fermented African locust bean condiment plus 1% cholesterol.

Table 2: Effect of fermented legume condiments supplemented diets on the atherogenic index in rats fed diets containing 1% Cholesterol

Groups	TC/HDL	LDL/HDL
I	3.9^{c}	2.3^{c}
II	13.4^{d}	5.7^{d}
III	1.1^{a}	0.1^{a}
IV	1.6^{a}	0.5^{a}
V	2.7^{b}	1.0^{b}

Values represent mean ± standard deviation (n = 6). Values with the same superscript letter on the same column are not significantly (P<0.05) different. Group I – normal control rats, fed with basal diet; Group II – rats fed with basal diet plus 1% cholesterol (hypercholesterolemic rats); Group III – rats fed diet supplemented with 16% fermented Soybean condiment plus 1% cholesterol; Group IV – rats fed diet supplemented with 16% fermented Bambara groundnut condiment plus 1% cholesterol; Group V – rats fed diet supplemented with 16% fermented African locust bean condiment plus 1% cholesterol.

cholesterol in the Group II (hypercholesterolemic control) rats compared with the normal control rats as shown in Table 2. However, treatment of rats with diets supplemented with 16% legume condiments caused a significant (P<0.05) decrease in the elevated plasma atherogenic lipids level. Likewise, the Group II animals have significantly (P<0.05) higher atherogenic index (TC/HDL and LDL/HDL) when compared to both Group I and the treated groups (III – V); with the treated groups having significantly (P<0.05) lower atherogenic index compared to both normal (Group I) and hypercholesterolemic control (Group II) groups.

Furthermore, significant increase (P<0.05) in the plasma HDL-cholesterol was observed in all the fermented legume fed rats (Groups III – V) as against those of the normal and hypercholesterolemic control rats groups (Figure 1). Conversely, a significant decrease (P<0.05) in the plasma LDL-cholesterol, triglycerides and total cholesterol in all the fermented legume fed rats as against those of the normal control and the hypercholesterolemic rats groups was observed as shown in Figure 1. As observed in Figure 2 and 3 showing the plasma activity levels of liver function marker enzymes AST, ALT and ALP as well as MDA content respectively, it revealed that hypercholesterolemic control rats have significantly (P<0.05) elevated plasma MDA content and activity levels of the liver function marker enzymes compared to normal control rats. And this trend was significantly reversed in fermented legume condiment supplemented diets fed groups.

Over the years, plant materials have been employed in the treatment/management of many degenerative diseases and its attendant complications in many African countries with legume products widely used as a result of their low glycemic index and hypocholesterolemic effect (Madar and Stark, 2002). Furthermore, epidemiological studies have established lower incidences of cardiovascular disease among legume consumers (Hu, 2003) and recent findings have shown that health promoting effects of legumes may be associated with their phenolic constituents (Vadivel *et al.,* 2012; Ademiluyi *et al.,* 2014). Fermented legume seeds with increased phenolic contents may therefore come with an added advantage therapeutically. The observed increase in the plasma atherogenic lipids in the hypercholesterolemic rats (rats fed high cholesterol at 1% dietary inclusion) is in agreement with earlier findings of Zhang et al., (2002) and Jang et al., (2007) where high cholesterol diets (1% diet inclusion) caused elevated plasma total cholesterol and triglyceride levels in rats. However, the decrease in these plasma atherogenic lipids, in rats fed fermented legume condiments supplemented diets is in agreement with earlier reports where legumes were shown to possess some

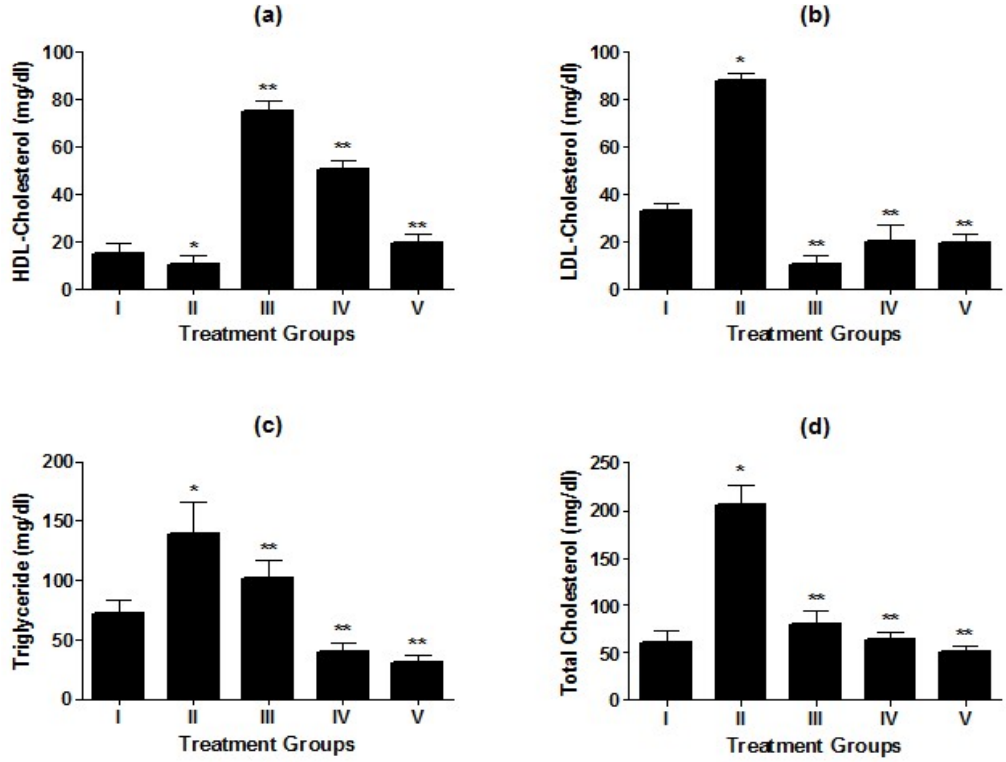

Figure 1: Effect of fermented legume condiments supplemented diets on plasma (a) HDL-Cholesterol, (b) LDL-Cholesterol, (c) Triglyceride and (d) Total Cholesterol levels in rats fed diets containing 1% Cholesterol

Values represent mean ± standard deviation (n = 6). * value is significantly ($P < 0.05$) different from Group I while, ** values are significantly ($P < 0.05$) different from Group II. Group I – normal control rats, fed with basal diet; Group II – rats fed with basal diet plus 1% cholesterol (hypercholesterolemic rats); Group III – rats fed diet supplemented with 16% fermented Soybean condiment plus 1% cholesterol; Group IV – rats fed diet supplemented with 16% fermented Bambara groundnut condiment plus 1% cholesterol; Group V – rats fed diet supplemented with 16% fermented African locust bean condiment plus 1% cholesterol.

cholesterol lowering agents (Troszyñska *et al.,* 2002). The mechanism by which fermented legume condiments lower plasma cholesterol remains unclear; nevertheless, the high total phenolic content of these legume products as previously reported by Ademiluyi and Oboh, (2012) may have influenced this favourable cholesterol metabolism. It is well known that plasma cholesterol concentration can be regulated by cholesterol biosynthesis, cholesterol removal from the circulatory system, absorption of dietary cholesterol, and its excretion via bile and faeces (Kim *et al.,* 2006). Studies have shown that phytochemicals such as phenolics inhibit the activities of 3-hydroxyl-3-methylglutaryl CoA reductase (HMG-CoA reductase); the rate limiting enzyme in the cholesterol biosynthesis in the liver (Endo, 1992; Ademosun et al., 2015) and also intestinal acyl CoA: cholesterol acyltransferase (ACAT) which plays a key role in the absorption of cholesterol by esterification of cholesterol prior to absorption (Zhang *et al.,* 2002); suggesting that inhibition of absorption of dietary cholesterol and synthesis of cholesterol may at least be partly responsible for the hypocholesterolemic effect of the fermented legume condiment diets. Polyphenols are released at various points of the gastrointestinal tract (GIT); here, they exert their beneficial effects (Oboh *et al.,* 2007).

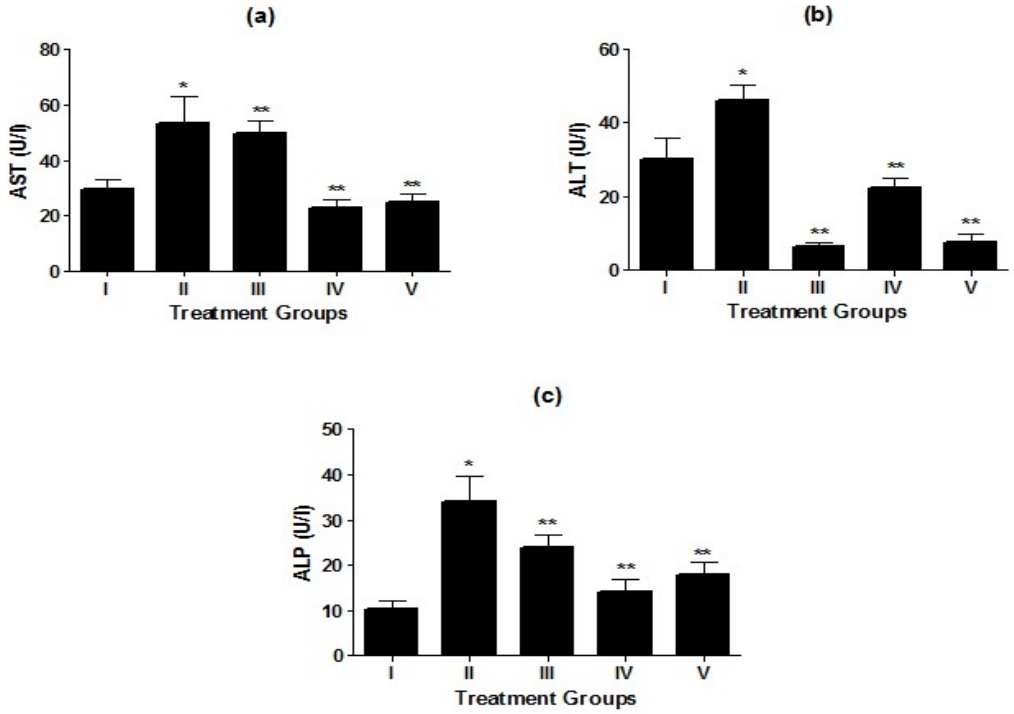

Figure 2: Effect of fermented legume condiments supplemented diets on plasma (a) aspartate aminotransferase (AST), (b) alanine aminotransferase (ALT) and (c) alkaline phosphatase activities in rats fed diets containing 1% Cholesterol.

Values represent mean ± standard deviation (n = 6). * value is significantly ($P < 0.05$) different from Group I while, ** values are significantly ($P < 0.05$) different from Group II. Group I – normal control rats, fed with basal diet; Group II – rats fed with basal diet plus 1% cholesterol (hypercholesterolemic rats); Group III – rats fed diet supplemented with 16% fermented Soybean condiment plus 1% cholesterol; Group IV – rats fed diet supplemented with 16% fermented Bambara groundnut condiment plus 1% cholesterol; Group V – rats fed diet supplemented with 16% fermented African locust bean condiment plus 1% cholesterol.

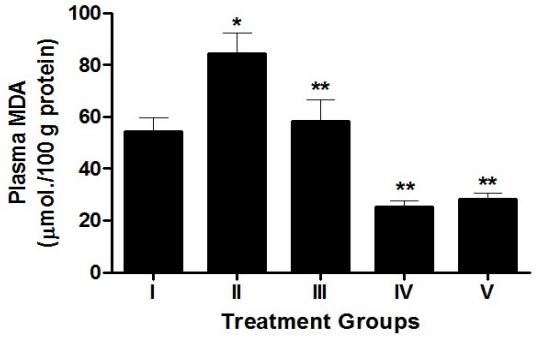

Figure 3: Effect of fermented legume condiments supplemented diets on liver malondialdehyde (MDA) content in rats fed diets containing 1% Cholesterol.

Values represent mean ± standard deviation (n = 6). * value is significantly ($P < 0.05$) different from Group I while, ** values are significantly ($P < 0.05$) different from Group II. Group I – normal control rats, fed with basal diet; Group II – rats fed with basal diet plus 1% cholesterol (hypercholesterolemic rats); Group III – rats fed diet supplemented with 16% fermented Soybean condiment plus 1% cholesterol; Group IV – rats fed diet supplemented with 16% fermented Bambara groundnut condiment plus 1% cholesterol; Group V – rats fed diet supplemented with 16% fermented African locust bean condiment plus 1% cholesterol.

Hypercholesterolemia is a major risk factor in the development of cardiovascular diseases such as atherosclerosis, myocardial infarction, heart attacks and cerebrovascular diseases which are leading causes of death in many countries of the world (Wald and Law, 1995; Félix-Redondo et al., 2013). Lowering plasma cholesterol level has been documented to reduce the risk of these diseases (Barter and Rye, 1996). It was observed that fermented legume condiments supplemented diets counteracted hypercholesterolemia by improving plasma lipid profile. This was evident by the marked increase in the plasma HDL-cholesterol levels (Figure 1) and suggests that fermented legume condiments could improve the body cholesterol homeostasis. HDL-cholesterol which is regarded as "good cholesterol" (Stein and Stein, 1999) is responsible for the transporting of cholesterol from peripheral cells to the liver where the cholesterol is metabolized into bile acids (Jang *et al.,* 2007). This pathway is crucial for maintaining cholesterol homeostasis between blood and peripheral tissues.

Furthermore, the increase in the liver function enzyme markers; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the plasma of rats placed on high cholesterol diet (1% dietary supplement) could be an indication of liver damage. Injury to the hepatocytes causes these cytosolic enzymes (ALT, AST and ALP) to leak out of cell into the circulation, raising plasma levels (Pratt and Kaplan, 2000; Green and Flamm, 2002). Cholesterol administration has been reported to influence hepatic lipid metabolism in rats and hypercholesterolemia is usually characterized by both abnormal serum and hepatic triglyceride and cholesterol levels (Wang *et al.,* 2010). Increased serum total cholesterol may have caused impairment in the triglyceride metabolism leading to the accumulation/deposition of free fatty acids in the liver, thus triggering a condition known as fatty liver (Wang *et al.,* 2010). This expanded liver fatty acid pool leads to increased mitochondrial and peroxisomal â-oxidation, which produces reactive oxygen species. This may, in turn, promote a local pro-inflammatory state leading to progressive liver injury (Schwimmer *et al.,* 2008).

However, supplementing the diets with fermented legume condiments caused significant decrease in the plasma ALT, AST and ALP suggesting that the condiments were able to protect the liver from oxidative damage. The hepatoprotective properties of the fermented legume condiments could be due to their high total phenolic content previously reported. Polyphenols are strong antioxidants and therefore could prevent the hepatocytes from oxidative damage induced by hypercholesterolemia. The disturbed balance between free radical generation and antioxidative process plays an important role in the pathogenesis of cardiovascular diseases (Griendling and FitzGerald, 2003). The high cholesterol diet also caused a marked increase in liver malondialdehyde (MDA) level (a primary product of lipid peroxidation). Studies have shown that cholesterol-rich diet increases lipid peroxidation by free radicals and exacerbate hypercholesterolemia (Lee *et al.,* 2006), the later is reported to be related to enhanced oxidative stress and increased lipid peroxidation (Cox and Cohen, 1996).

Nevertheless, the reduction in the plasma MDA level of hypercholesterolemic rats treated with the legume condiments supplemented diets clearly indicates marked improvement in the *in vivo* antioxidant status with supplementation of the diets with fermented legume condiments. This fermented legume condiments are rich in bioactive substances like phenolic compounds which exhibit strong antioxidant properties as recently reported by Ademiluyi and Oboh, (2015). Therefore, inhibiting oxidative stress in hypercholesterolemic state is considered to be an important therapeutic approach.

CONCLUSION

The fermentation of selected legume grains/ seeds to condiments led to increase in their phenolic content with a concomitant increase in their antioxidant properties. Feeding of these phenolic-rich fermented legume condiments to experimental animals improved their body antioxidant status, lowered their atherogenic lipids and displayed hypocholesterolemic effect. Therefore, consumption of phenolic-rich fermented legume condiments could be employed as a practical dietary means for the management of hypercholesterolemia and its attendant cardiovascular complications. On the overall scale, fermented African locust bean condiment appeared to be the most promising.

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