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The effects of *Afzelia africana* seed processed with different fermentation periods on lipid profile of alloxan-induced diabetic adult male Wistar rats

Ifeanyi-Uche U. P.[1], Okoroigwe F. C[2]., Agu H. O.[3]., Ejezie V. N.[4], Okoye C. J.[5] and Ihekwoaba C. C.[6]

[1]Department of Home Economics, Federal College of Education (Technical), Umunze, Anambra State, Nigeria.
[2]Natural Science Unit, School of General Studies University of Nigeria, Enugu Campus. Department of Nutrition and Dietetics University of Nigeria, Nsukka Enugu State Nigeria
[3]Department of Food Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[4]Department of Chemistry, Federal College of Education (Technical), Umunze.
[5]Natural Science Unit, School of General Studies University of Nigeria, Enugu Campus
[6]Federal Polytechnic Uggep, Cross River State Nigeria.
*Corresponding author e-mail: ho.agu@unizik.edu.ng

ABSTRACT

The study sought to assess the impact of unfermented and fermented Afzelia africana seed flour on alloxan-induced diabetic rats on their triglyceride and cholesterol levels. 50 rats were involved in this research, and they were placed in five groups, ten rats per group. One group was kept normal, and four groups were made diabetic by injecting a freshly prepared 5% aqueous solution of alloxan monohydrate, given in a single dose of 130 to 170 mg/kg body weight. The composition of the experimental diet was formulated on the basis of 10g dietary fiber obtained from the seeds. Lipid profile levels were measured on day 1(prior to acclimatization), day 7 (48h after induction), and day 21 (following experiment).The result of this finding showed a significant decrease ($p < 0.05$) in the body weight of rats that were fed fermented Afzelia africana seed flour for 24h and 48h compared with rats fed unfermented seed flour. Rats fed with fermented Afzelia africana showed a significant decrease ($p<0.05$) in total cholesterol, LDL, and triglyceride levels compared to those fed with unfermented samples, which, on the other hand, had an increase ($p<0.05$) in total cholesterol and LDL levels. Additionally, the rat groups fed with fermented Afzelia africana showed an increase in high-density lipoprotein (HDL) ($p<0.05$). By contrast, the groups fed with unfermented Afzelia africana seed flour showed a reduction in HDL. The study discovered that 48h and 24h fermented Afzelia africana display anti-diabetic and hypocholesterolemic activity in diabetic rats relatively.
Keywords: cholesterol, Afzelia africana, alloxan, fermentation, lipoprotein, rats

INTRODUCTION

Dietary fibers are intricate compounds that can be defined as any non-digestible carbohydrates and lignins that remain in the upper gut [1]. The fibers are the indigestible part of plant foods, which enables food to move through the digestive system, taking in water, and making defecation smoother. Essentially, dietary fibers can be categorized into two forms based on their solubility: water-soluble and insoluble. Soluble dietary fiber consists of pectin, beta-glucans, fructans, oligosaccharides, certain hemicelluloses, guar, and other gums. The nature of this type of fiber is that it dissolves very readily. Soluble fiber easily dissolves in water and is accountable for lowering

cholesterol levels in the body and decreasing the risk of heart disease, arterial disease, and atherosclerosis to a significant level. It also aids in weight management by reducing the risk of obesity, normalizing blood sugar, and reducing sugar levels [2]. Some foods that are high in soluble dietary fiber are legumes, psyllium, nuts, beans, apples, oranges, apricots, dates, raisins, carrots, strawberries, citrus fruits, flaxseed, and beets [3]. Insoluble dietary fiber consists of hemicelluloses, cellulose, and lignin. As indicated by its name, insoluble fiber is unable to dissolve in water. The primary role of insoluble fiber is water absorption, hence increasing bulk to stools in the large intestine. In addition to preventing constipation and hemorrhoids, it reduces the risk of colon cancer through the promotion of the transit of food in the digestive system. The abundant sources of insoluble fibres include vegetables such as green beans, dark green leafy vegetables, whole-wheat products wheat oat, corn bran and nuts [4].The metabolic effects of fibre (reducing blood cholesterol and postprandial glucose peaks) can be attributed wholly to soluble fibre, more specifically to viscous fibre [4]. Since it is adequately hydrated, such fibre can thicken the contents of the intestine, thus slowing down the absorption rate and diluting the nutrient load over time. Such effect, combined with physical binding of bile acid to the fibre induces progressive faecal bile acid loss, the primary mechanism whereby fibre can lower serum cholesterol [5]. An investigation [6] reported the effect of guar gum in Wistar rats even though there was an elevation of all the lipidemic parameters (total cholesterol, triacylglycerol, HDL and LDL cholesterol) due to diabetes, the rats fed guar gum diets had lower serum cholesterol, triacylglycerol and LDL-cholesterol concentrations, compared to the rats fed 0% guar diet. The significant increase in HDL cholesterol levels in rats fed a diet supplemented with guar gum pointed to the beneficial actions of the intake of this fiber. References [7] and [8] reported the reduction of triglycerides in rats fed dietary fiber from fermented mung beans and rats fed pumpkin powder, respectively. Yet, the exact mechanisms of the lowering of circulating and tissue cholesterol are not yet known. A few theories tries to explain the involvement of the soluble fibre fraction with an unstirred water layer present along luminal surface of mucosa intestinal cells, increases the thickness of that layer forming a physical barrier to the cholesterol absorption [9].The early studies of [10] demonstrated variable effects of supplementing rat diets with different forms of dietary fibre. The researchers showed that SNSP such as pectin, guar gum, and locust bean gum and carrageen in the diet reduced cholesterol levels, and insoluble dietary fibre does not generally show hypocholesterolaemic activity. The effect of dietary fibre to reduce cholesterol levels is probably a result of its ability to reduce availability of fatty acids and cholesterol for absorption in the proximal intestine. This was in contrast to [11] who had a lower mean body weight and increase in cholesterol of rats on unfermented Afzelia than in the rats on the control diet. Afzelia africana is a tree crop and belongs to the family caesalpiniaceae. Some of the names applied to this tree include counter wood tree (English) and akparata (Igbo). It is a widespread species with an open fairly broad crown and massive branches, most readily recognized by the conspicuous hard blackish fruits, the open pods persisting for a long period on the ground [12] in [13]. Afzelia africana is traditionally processed by roasting, dehulling, and grinding into a fine powder that is used as a thickening agent in sauces and soups. Chemical analysis has indicated that the seeds contain 20.9% protein and 37.4% total dietary fiber, of which 24.9% is soluble fiber and 12.5% is insoluble dietary fiber [14]. As Afzelia africana seeds flour contains high dietary fiber, the researcher wanted to investigate the effect of Afzelia africana seeds subjected to various fermentation times on the lipid profile of alloxan-induced diabetic adult male Wistar rats.

MATERIALS AND METHODS

Source of materials

Four kilogrammes (4kg) of *Afzelia africana* were purchased from Ogige market in Nsukka, Enugu State, Nigeria. The seeds were identified and characterized at the Department of Plant Science, University of Nigeria, Nsukka. Adult male Wistar rats were procured from Department of Physiology, Nnamdi Azikiwe University Awka, Anambra State Nigeria.

Production of fermented and unfermented *Alfzelia africana* seed flour

The seeds were roasted in a wide aluminium steel panwith continuous stirring as is traditionally done using gas cooker at 145°C. The roasting lasted for 20- 25min. After roasting, the seeds were dehulled and cracked into small pieces. The dehulled seeds were divided into four equal portions; one portion for unfermented and three portions for fermented. The three portions of the dehulled seeds were fermented separately for 24h, 48h and 72h and oven dried (Include make and model) at 60°C for 24h. The unfermented *Afzelia africana* was not soaked nor dried in the oven. The samples were ground with hammer mill (Include make and model) and sieved separately using cheese cloth (0.4mm diameter) to obtain fine flour. The flour was stored in an air tight container and put in the refrigerator (Include make and model) at 4°C until used for analysis.

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Formulation of diet

The formulation of experimental diets was based on dietary fibre content of the seeds flour of Unfermented and fermented *Afzelia africana*. The test diets were formulated to provide approximately 10g total dietary fibre per 100g diet [11].

Induction of diabetes

The diabetes mellitus was induced in the rats in group 2-4 with alloxanon the 5th day after acclimatization (after overnight fasting). Freshly prepared 5% aqueous solution of alloxan monohydrate (120-150mg/ml concentration of alloxan dissolved in normal saline) was intraperitoneally injected to the rats at a single dose range of 130-170mg/kg body weight of rat. The rats were allowed free access to 5% glucose solution for 48h to avoid hypoglycaemia. After 48h of induction, the blood glucose level of the rats was determined using accu-check glucometer and its test strips. The rats that blood sugar recorded up to 150mg/dl of blood glucose and above were considered diabetic [13].

Feeding trial

Rats that are diabetic were grouped into 4 and treated with the flour samples. The control group received rat chow with distilled water while the other groups received rat chow, 10g of test diets and distilled water. Unfermented and fermented *Afzelia africana*(*Aa*) samples was administered using a cannula. The test groups continued with the rat chow and feeding trial for the duration of 21days. The administration of the diets was oral route (through the mouth). The individual weights of the rats were taken at the beginning of the experiments and at the end of the experiment to determine weight gain. Blood samples were taken from the rats at day 4, 7 and 21 of the administration of the samples to obtain the values of the blood constituents.

Parameter for analysis

Cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL) and triglyceride were analyzed.

Blood samples

At the end of each experiment, rats were subjected to overnight fast and anesthetized using chloroform. Blood samples were collected from the retro-orbital plexus using heparinized needle and syringe as anticoagulant. The blood was centrifuged at 3500 rpm for 15 min. The serum collected was separated from the cells and kept in sealed aliquot tubes at a temperature of -20°C until biochemical analysis. [15]

Serum cholesterol determination

The method of [16] was adopted.

Principle

Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxidase and 4-aminoantipyrine in the presence of phenol and peroxidase

Calculation: The concentration of cholesterol was calculated as follows:

$$\frac{\text{Absorbance of sample}}{Absorbance\ of\ standard} x \frac{\text{Standard concentration (mg/dl)}}{1}$$

Test procedure

Three (3) test tubes was set up in a test tube rack and labeled blank, standard and sample respectively. To the blank, was added (10µl) distilled H_2O, 10µl standard specimen to the standard test tube and 10µl sample to the sample test tube. To each of these test tubes was added 1000ul of the cholesterol reagent. It was thoroughly mixed and incubated for 10min at room temperature (20-25°C). The absorbance of the sample (A sample) against the blank was taken within 60 min at 500nm.

Low density lipoprotein (LDL)

Principle:

LDL-C was determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethyleneglycolmonomethyl ether.

Procedure:

The samples were kept at 2-8°C. The precipitant solution (0.1ml) was added to 0.2ml of the sample and mixed thoroughly and allowed to stand for 15 min and centrifuged at 2,000 × g for 15 min. The cholesterol concentration in the supernatant was determined. The concentration of the total cholesterol as described by [17] was adopted.

Calculation:

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LDL-C(mg/dl) = total cholesterol (mg/dl) – 1.5 x supernatant cholesterol (mg/dl).

High density lipoprotein (HDL)

Principle:

LDL and VLDL (low and very low density lipoproteins) were precipitated from by the action of a polysaccharide in the presence of divalent cations. Then, high density lipoproteins (HDL) present in the supernatant was determined.

Procedure:

The precipitant solution 0.1ml was added to 0.3ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuge at 2000 x g for 15 min. The cholesterol concentration in the supernatant was determined. Concentration of the total HDL was determined as described by [17].

Triglyceride

Principle:

The fasting triglycerides levels were determined after enzymatic hydrolysis with lipases [16]. The indicator was a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triacylglycerol} + H_2O \xrightarrow{\text{lipases}} \text{Glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{4 chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + HCl + 4H_2O$$

Method: A quantity, 0.1ml of the sample was pipetted into a clean labeled tube and 1.0 ml of trichloroacetic acid (TCA) was added to it, mixed and centrifuged at 250rpm for 10 minutes. The supernatant was decanted and reserved for use. The mixtures were allowed to stand for 20 min at 25°C. The absorbance of the sample and standards was read against the blank and was taken at 540nm.

Calculation: The concentration of triglyceride was calculated as follows:

$$\frac{\text{Absorbance of sample}}{\textit{Absorbance of standard}} \; x \frac{\text{Standard concentration (mg/dl)}}{1}$$

Statistical analysis

Statistical analysis of data was performed using the Statistical Product and Service Solution (SPSS) version 22.0.The differences among means were tested for significance using one-way analysis of variance (ANOVA) and least significant difference (LSD) was used for post hoc test. T-test was used to compare the baseline and end values of means. The criterion for statistical significance was set at $p < 0.05$. Data were presented as means with ± SD.

RESULTS AND DISCUSSION

Table 1: The total cholesterol (TCH) in rats fed animal feed, unfermented and fermented *Afzelia africana* flour (mg/dl).

Groups	DAY 1	DAY 7 TCH after induction (Baseline)	DAY 21 TCH at the end of experiment	% difference
Diabetic control	$1.98^{a}\pm0.01^{a}$	$1.98^{a}\pm0.23$	$2.01^{a}\pm0.33$	1.51↑
UnFAa	$2.00^{b}\pm0.05$	$3.42^{b}\pm0.31$	$3.40^{b}\pm0.28$	0.58↓
FAa24hrs	$1.98^{a}\pm0.07$	$3.37^{b}\pm0.55$	$2.90^{b}\pm0.54$	13.43↓
FAa48hrs	$1.97^{a}\pm0.12$	$3.43^{b}\pm0.04$	$2.71^{b}\pm0.42$	20.99↓
FAa72hrs	$2.01^{b}\pm0.19$	$4.37^{b}\pm0.04$	$3.82^{b}\pm0.51$	12.58↓

Values are expressed as Mean ± SD. Mean values with different superscripts along the column are significant at $p<0.05$. UnFAa-Unfermented *Afzelia africana*, FAa 24hrs-Fermented *Afzelia africana* for 24h, FAa48hrs - Fermented *Afzelia africana* for 48h, FAa72hrs-Fermented *Afzelia africana* for 72h.

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The total cholesterol (TCH) levels of rats fed fermented and unfermented *Aa*as shown in Table 1 decreased generally. The rats fed with fermented showed significant decrease in total cholesterol, the decrease in TCH for group fed with unfermented was not significant for *Aa*. The findings of this study on the decrease in unfermented *Aa* was supported by [11] who reported a rise in cholesterol of rats fed *Aa* compared to the rats fed the control diet. The high reduction in TCH may be as a result of high content of dietary fibre in the fermented flour fed experimental rats while the low reduction in TCH may be as a result of some toxic substances in the unfermented flour that hinders the bioavailability of nutrients. This is supported by [18] who reported the effectiveness of dietary fibre in reducing cholesterol levels probably lies in its ability to reduce availability of fatty acids and cholesterol for absorption in the upper intestine. This may be seen as increased faecal fat, increased cholesterol and its bacterial metabolites, and reduced bile acid reabsorption, which affects cholesterol esterification.

Table 2: High density lipoprotein (HDL) levels in rats fed animal feed, unfermented and fermented *Afzelia africana* flour(mmol/l).

Groups	DAY 1	DAY 7 HDL after induction (Baseline)	DAY 21 HDL at the end of experiment	% difference
Diabetic control	1.86^{a}±0.02	1.68^{a} ±0.55	1.67^{a}±0.12	0.01↓
UnFAa	1.30^{a}±0.39	1.51^{a}±0.38	1.49^{a}±0.22	1.32↓
FAa24hrs	0.90^{a}±1.05	1.04^{a}±0.95	1.10^{a}±0.55	14.42↑
FAa48hrs	1.15^{a}±0.02	1.26^{a}±0.47	1.61^{a}±0.52	27.78↑
FAa72hrs	1.35^{a}±1.11	1.48^{a}±0.36	1.53^{a}±0.24	3.38↑

Values are expressed as Mean ± SD. Mean values with different superscripts along the column are significant at $p<0.05$. UnFAa- Unfermented *Afzeliaafricana*, FAa24hrs-Fermented A*fzelia africana* for 24h, FAa48hrs-Fermented *Afzelia africana* for 48h, FAa72hrs-Fermented *Afzelia africana* for 72h

The results of this work showed increase in high density lipoprotein (HDL) levels of rats fed fermented *Aa* while rats fed unfermented *Aa* showed a decrease in HDL. Considering this result, fermentation had a lot of effect on the availability of nutrients in the body. It reduces the antinutrients contents of seeds that can make the nutrients unavailable to the body. This shows that fermentation helps to reduce high cholesterol level in blood, strengthened and support digestive system and immune system thereby, helping the bodies to fight off and prevent diseases like cancer [19]. The increased levels of HDL commonly referred to as the good cholesterol obtained in this study showed that fermented *Aa* seed had potential to protect heart diseases in line with the opinion of [20] who stated that in the past decades, scientific evidence has shown the beneficial effects of increasing consumption of plant seeds and derived products on various health benefits, especially in cardiovascular diseases and type 2 diabetes mellitus.

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Table 3: Low density Lipoprotein (LDL) levels in rats fed animal feed, unfermentedand fermented *Afzelia africana* flour(mmol/l).

Groups	DAY 1	DAY 7 LDL after induction (Baseline)	DAY 21 LDL at the end of experiment	% difference
Diabetic Control	$0.80^{a}\pm0.32$	$0.81^{a}\pm0.51$	$0.83^{a}\pm0.18$	3.70↑
UnFAa	$0.89^{b}\pm0.15$	$1.15^{b}\pm0.55$	$1.18^{b}\pm0.31$	2.61↑
FAa24hrs	$0.90^{b}\pm1.51$	$1.62^{b}\pm1.15$	$0.92^{b}\pm0.14$	43.21↓
FAa48hrs	$0.75^{a}\pm0.88$	$1.82^{b}\pm1.30$	$0.83^{a}\pm0.35$	54.40↓
FAa72hrs	$1.44^{b}\pm0.03$	$2.23^{b}\pm1.62$	$1.60^{b}\pm0.22$	28.25↓

Values are expressed as Mean ± SD. Mean values with different superscripts along the same column are significant at $p<0.05$. UnFAa - Unfermented *Afzelia africana*, FAa24hrs -Fermented A*fzelia africana* for 24h, FAa48hrs -Fermented *Afzelia africana* for 48h, FAa72hrs-Fermented *Afzelia africana* for 72h.

There were decreases in the low density lipoprotein (LDL) levels in all groups of rats fed fermented *Aa* seed flour compared to diabetic control. The group of rats fed unfermented *Aa*had a significant increase ($p>0.05$) in LDL levels compared to control. The findings of this work are in conformity with the results of [6] in their study of antihypercemic effects of fermented and unfermented mung bean extracts on alloxan- induced – diabetic mice. These decreases may be an indication that the seeds may possess hypolipidemic effects. The results of the finding also agreed with the report of [8] who noted a significant decrease in blood LDL of diabetic rats fed pumpkin powder. The higher the concentration of LDL, the higher the risk in the body such risk as heart attack, stroke and peripheral artery disease. LDL reduction may be due to an increase in LDL catabolism. Dietary fibre reduces plasma LDL levels by inhibiting the absorption of bile acids and cholesterol and enhancing the activity of LDL receptors.

Table 4: Triglyceride levels in rats fed animal feed, unfermented and fermented *Afzelia Africana* flour (mmol/l).

Groups	DAY 1 TG	DAY 7 after induction (Baseline)	DAY 21 TG at the end of experiment	% difference
Diabetic Control	$0.66^{a}\pm0.01$	$0.67^{a}\pm0.12$	$0.72^{a}\pm0.24$	7.46↑
UnFBAa	$0.62^{a}\pm0.82$	$1.43^{b}\pm0.33$	$1.47^{b}\pm0.11$	2.80↑
FAa24hrs	$0.60^{a}\pm0.21$	$1.15^{b}\pm0.62$	$0.91^{b}\pm0.56$	20.86↓
FAa48hrs	$0.63^{a}\pm0.02$	$1.13^{b}\pm0.05$	$0.82^{b}\pm0.54$	38.35↓
FAa72hrs	$0.58^{a}\pm0.14$	$0.95^{b}\pm0.63$	$0.78^{b}\pm0.58$	17.89↓

Values are expressed as Mean ± SD. Mean values with different superscripts along the column are significant at $p<0.05$. UnFAa - Unfermented *Afzelia africana*, FAa24hrs -Fermented A*fzelia africana* for 24h, FAa48hrs - Fermented *Afzelia africana* for 48h, FAa72hrs -Fermented *Afzelia africana* for 72h

The result of this study showed high decrease in triglyceride levels of rats fed fermented *Aa*seed flour compared to diabetic control. The result of this study agreed with the report of [7] and [8] who reported reduction in triglycerides of rats fed fermented mung beanand rats fed pumpkin powder respectively. The increase in unfermented *Aa* found in this work agreed with the report of [13] who reported an increase in triglyceride levels of the rats fed with unfermented *Aa*. The lipid reducing effect of fermented *Aa* is probably due to its dietary fibres and fermentation because the longer the fermentation time the higher the dietary fibre and the lower the antinutrients that can inhibit the availability of nutrients to the body. Fermentation does not necessarily increase quantity of nutrients but improves quality of nutrients. Furthermore, a fibre rich diet reduces triglyceride levels by suppressing lipogenesis in the liver [21]. The reducing properties of fermented *Aa* may be partly attributed to the pectin present in it.

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CONCLUSION

This study showed that fermentation of *Afzelia africana* does not just have appreciable nutritive values but possess hypoglycemic and hypolipidemic effects. Fermentation for 24h and 48h were the best periods in this study that reduced total cholesterol, low density cholesterol, tryglyceride levels of rats and increased high density cholesterol. Therefore, adopting fermentation method of processing and continuous consumption of these seeds would be beneficial in the management of diabetes, hyperlipidemia and high blood pressure.

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