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Comparative Effects of Selected Underutilized Wild Beans on Plasma Lipid Profile and Liver Function of Rats Fed with High Fats Diet

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Abstract

Dietary fiber content of beans is known to be responsible in the management of metabolic syndrome by delaying the degree of glucose as fuels, changing fat utilization, and controlling appetite through increased satiety, thus lowering the risk of cardiovascular diseases. Hence some selected varieties of underutilized wild beans were evaluated to study their anti-lipidemic effects. Prior to this, attempts were made to ferment the non-digestible fractions of the beans with fermentable micro-organism and the respective gut metabolites were determined. Lipid profile result carried out in the blood showed high density lipoprotein to be significantly ($p \leq 0.05$) high in *Pakala* group with a value of 2.2 ± 0.02 compared to other groups. While for low density lipoprotein (LDL); rats with *Otili* in their diet had the highest LDL with a value of 0.45 ± 0.01. However, the group of rats fed with *Feregede* had the least cholesterol level compared to other groups of rats fed with respective wild beans and the negative control group. *Otili* had the highest ALP with value of 89 ± 1.0. *Otili* group also had a significant lower value of both aspartate amino transferase and alanine amino transferase. The biochemical indices reported in this study vary from one type of wild bean to another.

Keywords

Metabolic Syndrome, Dietary Fibres, Anti-Lipidemic, Alanine Aminotransferase, Aspartate Aminotransferase

1. Introduction

Metabolic syndrome is the group of metabolic conditions associated with the risks of cardiovascular diseases elevated serum triglycerides and LDL cholesterol, low HDL cholesterol, central adiposity, high serum glucose, and high blood pressure [1] [2]. Consumption of beans in the diet is considered beneficial for healthy individuals as well as those preconditioned for metabolic syndrome by lowering serum total cholesterol and LDL cholesterol [3] [4]. Resistant starch and dietary fiber content of beans are mainly responsible in the management of metabolic syndrome by delaying the degree of glucose as fuels, changing fat utilization, and controlling appetite through increased satiety, thus lowering the risk of cardiovascular diseases [5] [6].

Fermentation of soluble fiber (SF) as well as resistant starch (RS) by bacteria in the large intestine results in the generation of specific short chain fatty acids (SCFA), propionate being the dominant, which alters metabolic pathways resulting in reduced serum cholesterol [7] [8]. Thus, increased production of SCFA by fermentation of RS is an underlying reason for the protective benefits by the consumption of dry beans [7] [9]. The cholesterol lowering effect of dietary fiber has been ascribed to its ability to restrain the intestinal absorption of neutral steroids and bile acids and total steroid excretions [10]. Hypocholesterolemic effect can also be achieved by regular ingestion of beans that reduce the need to rely on animal proteins by replacing it through plant proteins [11]. In addition to this, beans α-amylase inhibitor is known to have anti-obesity effect as α-amylase inhibitory action to starch digestion causes energy restriction resulting in mobilization of body fat reserves [12].

Several epidemiological and clinical studies have shown positive effects of beans consumption in lowering the risk of coronary heart diseases and cardiovascular diseases [7] [13]. In an epidemiological trial, men and women consuming four times or more legumes per week decreased their risks of coronary heart diseases and cardiovascular diseases up to 22% and 11%, respectively, as compared to those with once serving per week [14]. A 1% decline in serum total cholesterol reduces the risk of coronary heart disease by 2%, while each 1% decline in serum LDL cholesterol decreases the risk to about 1% [15]. In a clinical trial, Anderson and Moore [16] investigated that utilization of 275 g of navy beans by hyper cholesterolemic patients for three weeks decreased serum cholesterol and LDL cholesterol up to 19% and 24%, respectively; while in second trial, 24% reduction was observed in both total cholesterol and LDL cholesterol under metabolic ward conditions with similar diet. Anderson [1] had previously also investigated that consumption of baked beans by hypercholesterolemic patients for two weeks reduced total cholesterol and LDL cholesterol by 12% and 15%, respectively. Similarly, Han *et al.* [17] investigated that resistant starches of beans reduce the serum cholesterol concentration in rats. In another study, hyper cholesterolemic men were given half a cup daily intake of baked beans for a period of 8 weeks, which resulted in reductions of serum LDL cholesterol and

total cholesterol by 5% and 6%, respectively [13]. Against this backdrop the mechanism of the prevention of the cardiovascular diseases by beans is well studied but not in the perspective of exploring the underutilized wild beans as presented in this study.

2. Materials and Methods

2.1. Collection of Cultivar

As described in our previous study [18] the legumes (beans) used in this work are of two types; Wild-type beans *Sphenostyles stenocarp* (*Otili* African yam bean), *Cajanuscajan* (*Feregede* Pigeon pea), *Phaseolus lunatus* (*Pakala* lima beans) and Edible bean *Phaseolus vulgaris* (Oloyin kidney bean). They are gotten from the farmers in Ado-Ekiti.

2.2. Experimental Animals

Twenty four albino rats were obtained from the college of medicine Animal house, Ekiti State University, Ado Ekiti, Ekiti state. Their weight ranged between 50 to 100 grams. The animals were acclimatized to the environment for 7 days. In feeding, the method of Shimin *et al.*, [19] was adopted with slight modification. The rats were then randomly divided into five groups as shown in **Table 1**, (4 rats per group) according to average body weight, the groups were fed *ad-libitum* with a normal chow diet (NCD), a high dietary fiber diet (HD) containing 8% fiber supplement in total, a high-fat diet (HF) which provided 45% of its energy from fat, or a high-fat and dietary fiber diet (HFD). The main nutritional ingredients in the HD diets were adjusted to levels similar to those of the normal chow diet, and the main nutritional ingredients in the HFD diet were adjusted levels similar to those of the HF diet. The rats were observed every day and weighed every week and the food intakes were recorded for each group throughout the experiment.

2.3. Dissection and Tissue Collection

All of the rats were anesthetized with chloroform and venous blood was collected from the orbital vein for hematology and blood biochemical analyses. Then, the rats were killed via an aortic cut and immediately dissected. The liver was collected, frozen and stored at −80˚C for further analysis.

2.4. Lipid Analysis

2.4.1. Preparation of Cholesterol Fraction

Blood was collected in a tube with no anticoagulant and allowed to clot at room temperature for 30 minutes before centrifugation at 2500× g for 20 minutes. The serum layer was removed and stored on ice. However, extra care was taken to avoid disturbing the white buffy layer and stored at −80˚C prior to performing the assay. Cholesterol level was assessed following Kit's manufacturer instruction (Randox, USA) on the aliquot samples.

Table 1. Experimental design.

s/n	Experimental group	Composition (100 grams)
1	Basal/Positive Control	Chow
2	Negative Control	Chow + High Fat Diet
3	*Feregede*	Chow + HFD + *Feregede*
4	*Otili*	Chow + HFD + *Otili*
5	*Pakala*	Chow + HFD + *Pakala*

2.4.2. Preparation of HDL Fractions

Blood was collected with heparin or citrate to avoid hemolyzes and centrifuge at 2000 × g and 4˚C for 10 minutes. The white buffy layer-plasma was gently removed and store on ice. Aliquot samples for testing and store. Dilutions in 1X assay were performed on aliquot samples after storage at −80˚C. Thereafter, 200 µL of sample was added to 200 µL of the precipitation Reagent (Randox, USA) and mixed well by vortexing. The mixture was allowed to incubate 5 - 10 minutes at room temperature before taking absorbance at 570 nm.

2.4.3. Preparation of LDL/VLDL Fraction

Pellet obtained after removal of the HDL fraction was re-suspended and dissolved in 400 µL of PBS and mixed well to obtain the LDL/VLDL fraction. Assay was carried out immediately by following Random Kit Manufacturer's instruction.

2.4.4. Liver Function Analyses

For the biochemical analyses, the blood samples were maintained 4˚C for 2 h and then centrifuged at 3000 r/min for 20 min at 4˚C. The supernatant was stored at −80˚C total protein (TP), Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), uric acid levels were analyzed following the commercial Random Kit (USA) and absorbance were measured using an automatic Biochemical Analyzer Camspec M106 Spectrophotometer (USA).

3. Results and Discussion

From this experimental rats model, the results of lipid profile as shown in **Table 2** carried out on the blood of experimental animals showed high density lipoprotein (HDL) which is referred to as good cholesterol found to be highest in *Pakala* group with a value of 2.2 ± 0.02 while the positive control had the lowest HDL level with a value of 0.3 ± 0.3. HDL has several potential anti-atherogenic effects and protective effects on endothelial cells [20]. Therefore abnormal and reduced vaso-protective effects of HDL are closely associated with atherogenesis and an increased risk of cardiovascular disease. Oxidative modifications have been proposed for alteration and deterioration of HDL [21]. The oxidation of HDL particles is likely to have important consequences not only for cholesterol homeostasis in peripheral tissues but also for the development of vascular

Table 2. Lipid profile in blood.

GROUP	High Density Lipoprotein (HDL)	Low Density Lipoprotein (LDL)	Total Cholesterol (TC)
1	0.3 ± 0.3	0.35 ± 0.01	1.1 ± 0.06
2	1.5 ± 0.05	0.02 ± 0.02	2.0 ± 0.01
3	0.8 ± 0.06	0.35 ± 0.07	1.6 ± 0.05
4	2.1 ± 0.01	0.45 ± 0.01	2.1 ± 0.01
5	2.2 ± 0.02	0.4 ± 0.02	2.2 ± 0.02
6	1.0 ± 0.02	0.4 ± 0.01	2.4 ± 0.04

diseases associated with oxidative stress [20]. While for low density lipoprotein (LDL); rats laced with *Otili* in their diet had the highest LDL with a value of 0.45 ± 0.01, though not significantly different from other group fed with other wild underutilized beans. Interestingly the negative control significantly lower LDL compared to other groups with a value of 0.2 ± 0.02. LDL particles pose a risk for cardiovascular disease when they invade the endothelium and become oxidized, since the oxidized forms are more easily retained by the proteoglycans. A complex set of biochemical reactions regulates the oxidation of LDL particles, chiefly stimulated by presence of necrotic cell debris and free radicals in the endothelium. Increased concentration of LDL particles is strongly associated with the development of atherosclerosis over time [22]. However, the group of rats fed with *Feregede* had the least cholesterol level compared to other groups of rats fed with respective wild beans and the negative control group. The cholesterol level of other groups of rats fed with *Otili* and *Pakala* are not significantly different from each other and the negative control. High-density lipoprotein (HDL) is good cholesterol and low-density lipoprotein (LDL) is bad cholesterol. High cholesterol in serum is a leading risk factor for human cardiovascular disease such as coronary heart disease and stroke [23]. Against this backdrop it is suffice to declare that *Feregede* could have protection against heart diseases and other health challenges but the physical observations in the experiment would negate this assumption. The group fed with *Feregede* was less agile and sick compare to *Otili* and *Pakala* groups. The feeding pattern of each group shows that *Otili* group had more appetite for the feed and this is clearly seen in their body mass gain over the period of the experiment. There was a general similarity in the behaviour of the rats which have the wild beans in their feed. Unlike the controls and *Otili* other groups did not huddle together but lay separately, often on their backs with their limbs fully extended. The total protein present in Group 1 is higher with a value of 77 ± 0.15 compare to Group 2, 4, 6 and 5 with value of 75 ± 0.10, 70 ± 0.01, 70 ± 0.03 and 69 ± 0.01 respectively while Group 3 had the lowest with a value of 59 ± 0.04, compared to the control. This indicates that many serve as a protective role against hyperlipidemia and hyperglycemia in rat fed with high fats diet [24] [25].

Alkaline phosphatases are classified as tissue-specific and tissue nonspecific types. Alkaline phosphatases found in the intestine, placenta, and germinal tis-

sue are tissue-specific, which means they are found only in the tissues where they are expressed in physiological conditions but may contribute to the circulating pool of serum alkaline phosphatase under specific situations when there is increased stimulation of their production. The tissue-nonspecific alkaline phosphatase forms the majority of the fraction circulating in serum and therefore, is of clinical interest [26]. In this experimental analysis of the liver as shown in **Table 3**, the lowest value of alkaline phosphatase (ALP) was found in positive control with the value 68 ± 1.5 and *Otili* had the highest ALP with value of 89 ± 1.0. Though the value obtained lies within the normal range [32], there is significant ($p \leq 0.05$) increase in the presence of alkaline phosphatase in other experimental groups. The principal clinical value of measuring serum alkaline phosphatase lies in the diagnosis of cholestatic liver disease. Some of the highest elevations in alkaline phosphatases are seen in patients with cholestasis. Usually, four-fold of the upper limit of normal or greater elevation is seen in up to 75% of the patients with cholestasis, either intrahepatic or extra hepatic [26].

AST is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health. However, it is important to keep in mind that the source of AST (and, to a lesser extent, ALT) in blood tests may reflect pathology in organs other than the liver. Larry (21) submitted that when the AST is higher than ALT, a muscle source of these enzymes should be considered. For example, muscle inflammation due to dermatomyositis may cause AST > ALT. This is a good reminder that AST and ALT are not good measures of liver function because they do not reliably reflect the synthetic ability of the liver and they may come from tissues other than liver [27]. Compared to the control, the other groups were found to be lower in Aspartate Aminotransferase. *Otili* group had a significant lower value of 465 ± 1.2 compare to that of the control while the rest experimental group had an increased value of AST compared to the control. Similar result was also found in alanine amino transferase (ALT) where *Otili* group had lower ALT of 1710 ± 4.2 compare to other groups. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are frequently used as markers of hepatocellular injury. AST is expressed in mitochondria of the liver and cytosol of red blood cells and muscles; thus it is not specific for liver injury [28]. Since ALT is less abundant outside of the liver, an increased ALT level is more suggestive of liver disease. Levels of both are markedly elevated (>5 - to

Table 3. Liver function tests.

GROUP	Alkaline Phosphatase	AST	ALT
1	68 ± 1.5	1080 ± 2.5	1980 ± 1.2
2	82 ± 1.2	850 ± 3.8	2640 ± 1.3
3	85 ± 1.4	712 ± 3.4	4635 ± 1.2
4	89 ± 1.0	465 ± 1.2	1710 ± 4.2
5	71 ± 1.3	1050 ± 2.1	3330 ± 1.1

10-fold normal) with hepatocellular injury caused by hepatitis, hepatotoxicity, ischemia, genetic or metabolic liver disorders. Elevation of AST in excess of ALT suggests extra-hepatic source of injury. With acute biliary obstruction, there are initial sharp increases in ALT and AST levels and a rapid decline in 12 - 72 hours as obstruction is relieved. In chronic cholestasis, aminotransferases are usually only mildly elevated. With hepatocellular injury, ALT and AST levels tend to remain more significantly elevated longer. In acute liver failure a rapid decline in ALT and AST levels with worsening coagulopathy is a poor prognostic factor [29] [30] [31] [32].

Generally the pattern in liver protein in the experimental groups-*Feregede*, *Otili* and *Pakala* compare to the two controls may be ascribed to intake of the diet mixed with high fats. Total protein was found to be higher in the liver of the group fed with *feregede* with the value of 84 ± 8.3. Total protein was found to be higher in Group 3 with the value of 84 ± 8.3 compared to Group 1 having 15.4 ± 0.04. Decreased in the control total protein may be as result of decreased synthetic capacity of the rats [33] [34]. It may be hoped that determinations of liver protein in varying metabolic states will be useful in trying to define the part played by the liver in the processes of protein metabolism. The effect on the blood tissue is in consonance with the view that all the differences are responses to change in functions [35].

4. Conclusion

In summary, dry beans played an important clinical role in the human nutrition with reduced risk of chronic disease and could be an exceptionally cost effectual approach for improving health. Rats fed with *Otili* (*Sphenostyles stenocarpa*) and *Feregede* (*cajanus cajan*) expressed higher resistibility ability in the induction of high fat diet but poor resistibility was exhibited or experienced in *Pakala* (*Phaseolus lunatus*) and edible bean.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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