

IMPROVEMENT OF DIABETIC DYSLIPIDEMIA BY LEGUMES IN EXPERIMENTAL RATS

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ABSTRACT

Grain legumes are a valuable source of food proteins; hence, their exploitation is expected to grow in relation to a growing world's food needs. Apart from high level of dietary fibre, their protein composition makes them useful in managing diabetes. This paper reports a study conducted to evaluate the effects of four different non-soy legume-based (*Vigna unguiculata ssp. dekindtiana var dekindtiana*, *Vigna unguiculata ssp. unguiculata*, *Sphenostylis stenocarpa* and *Vigna subterranea*) diets in rats administered with alloxan monohydrate (150 mg/kg bodyweight). Concentration of plasma glucose, triacylglycerol, total cholesterol, HDL-cholesterol and HDL-triacylglycerol as well as hepatic levels of cholesterol and triacylglycerols were determined spectrophotometrically in alloxan-induced diabetic rats fed on these legumes for five weeks. Induction of rats with alloxan monohydrate led to significant ($p < 0.05$) elevation of fasting plasma glucose and reduction in body weight. Consumption of each of the four legumes led to a significant reduction in the fasting plasma glucose concentrations in the diabetic rats ($p < 0.05$) with *V. subterranean* causing about 60% reduction. *Vigna unguiculata ssp. dekindtiana var dekindtiana* and *Sphenostylis stenocarpa* caused a reversal of the diabetes-induced reduction of hepatic cholesterol ($p < 0.05$). Plasma dyslipidemia was observed in the alloxan-induced diabetic rats as significant ($p < 0.05$) increases in total cholesterol, triacylglycerols, HDL-cholesterol, HDL-triacylglycerols and LDL-cholesterol levels were observed. The legumes improved the plasma lipid profile as shown by a significant ($p < 0.05$) reduction in the ratios of total cholesterol/HDL-cholesterol (ranging from 1.25-2.25 for control groups to 1.00-1.35 for the legume-fed groups) and LDL-Cholesterol/HDL-Cholesterol (ranging from 0.50-1.75 for control groups to 0.50-0.75 for the legume fed groups). The results suggest that wild cowpea, white cowpea, african yam bean and bambara groundnut equally reversed diabetes-associated dyslipidemia as indicated by the indexes of cardiovascular disorder. This, therefore, underscores the potential of these underutilized legumes in managing dyslipidemia associated with diabetes in experimental rats. These data should contribute toward enhancing the nutraceutical potential and utility of these legumes.

Key words: Diabetes, lipid profile, rats, legumes

INTRODUCTION

Legumes belong to the family *Leguminosae*, which is probably the second most important source of food next only to the family *Gramineae* – the cereal grains [1]. They are important in human nutrition due to relative high protein content. They contribute, substantially, to daily protein intakes for a significant proportion of the world's population and especially in the Third World countries where the ingestion of proteins has been reported to be below the recommended daily allowance due to high animal protein cost [1]. Interestingly, the observation that diets low in meat and high in legumes are beneficial to health has recently become a topic of scientific interest. The role that legumes may play in the etiology of chronic diseases was highlighted by hypotheses put forward by Burkitt and Trowell, who suggested that dietary fibre levels may be beneficial for preventing and/or managing several diseases that are common in Western societies [2]. Based on observation of diet and disease in Africa compared to North America and Europe, they proposed that the refining of grains and lack of dietary fibre may be important in diseases such as diabetes [2]. Due to changes in dietary patterns and life style, the incidence of diabetes is on the increase in the developing world where most patients will likely be found in 2025 [3,4].

Diabetes is an endocrine-metabolic disease characterised by hyperglycemia associated with impairment in insulin secretion and/or insulin action as well as alteration in intermediary metabolism of carbohydrate, protein and lipid. Studies have shown that hyperglycemia and dyslipidemia, generally, coexist in diabetic patients with poor glucose metabolic control and this interaction of hyperglycemia and dyslipidemia also increase the risk of macro- and micro-vascular complications synergistically [3,4]. For example, hyperglycemia has been shown to increase atherosclerotic lesions in proatherogenic mice with signs of dyslipidemia [3]. Dyslipidemia, which includes not only quantitative but also qualitative abnormalities of lipoproteins, plays a significant role in the proatherogenesis of vascular complications in diabetic subjects and a biomarker for cardiovascular disease [3]. Cardiovascular disease is responsible for approximately 75% of all deaths in diabetic patients, and mortality from coronary heart disease (CHD) is approximately three times higher in diabetic patients than in the general population [3].

Diabetes is a chronic disease but emphasis is on managing short-term as well as long-term associated complications. A scrupulous control is needed to help reduce the risk of long-term complications [3]. The high level of dietary fibre in legumes has long been attributed to their usefulness in managing diabetes [2,3,5]. Apart from their dietary fibre contents, the role of proteins from legumes in the management of diabetes has received considerable attention [6]. Earlier animal studies suggested that the amino acid composition of soy protein, which compared with that of animal protein, played a major role [6]. Taha and Wasif studied the effect of soy flour added to whole-durum meal in alloxan-diabetic hypercholesterolemic rats and reported that the addition of soy flour with or without methionine lowered the elevated plasma glucose, cholesterol and lipid concentration[7]. In another study of BioBreeding rats

prone to type I diabetes, Atkinson *et al.* [8] reported that, compared with a diet containing a mixture of animal and plant proteins, a diet containing only soy protein reduced the frequency of and delayed the onset of diabetes[8]. Although these studies underscore the beneficial influence of legumes in diabetes, they have concentrated attention on soy, which unlike other legumes is not readily consumed [6,7,8].

Therefore, the work reported in this paper explored the influence of the common varieties of legumes on hyperglycemia and associated dyslipidemia in an experimental animal model of diabetes.

MATERIAL AND METHODS

Animals and Diets

Healthy adult albino rats weighing between 100 and 150 g were purchased from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. The rats were housed individually, and partly restricted in metabolic cages to mimic a sedentary lifestyle and feed was given freely, except for the fasting period prior to blood glucose determination. The animal weight and total feed intake were recorded daily. The legumes namely, Wild Cowpea (*Vigna unguiculata* spp. *dekindtiana*), White Cowpea (*Vigna unguiculata* spp. *unguiculata*), African Yam Bean (*Sphenostylis stenocarpa*) and Bambara Groundnut (*Vigna subterranean*) were purchased from Lafenwa market, Abeokuta, Ogun State, Nigeria and prepared to contain 35% (w/w) legume flours (Table 1), as earlier described [4].

Induction of Diabetes

Diabetes mellitus was induced in overnight-fasted rats by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg bodyweight) dissolved in normal saline [4]. The animals in the control group received single intraperitoneal injection of normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h after injection. The animals with blood glucose concentration more than 200 mg/dL were used for the study.

Experimental Design

In the experiment, 42 rats were divided into 6 groups of 7 replicates each, after the induction of diabetes and grouped as follows:

Group 1: Normal control (untreated rats); Group 2: Diabetic control rats; Group 3: Diabetic rats fed on *V. unguiculata* spp *dekindtiana*-based diet; Group 4: Diabetic rats fed on *V. unguiculata* spp *unguiculata*-based diet; Group 5: Diabetic rats fed on *S. stenocarpa*-based diet, and Group 6: Diabetic rats fed on *V. subterranean*-based diet.

Blood samples were collected from the tails of the rats for the determination of baseline (before induction of diabetes) and initial (after induction of diabetes) glucose concentrations. After five weeks of feeding the animals, the diets, the rats were sacrificed and blood collected by cardiac puncture into a tube containing fluoride

oxalate as anticoagulant. These were used for the determination of post treatment (final) plasma glucose concentration and other biochemical analysis. The blood samples were collected between 7.00 am and 10.00 am after the rats were fasted overnight. The plasma was separated from the erythrocyte by centrifuging the whole blood at 400 x g for 10 minutes. The erythrocyte was washed thrice with normal saline to remove white blood cells. The erythrocyte and plasma were frozen at -20°C until required for analysis. The liver was excised, weighed and 10% tissue homogenate was prepared in 0.1M phosphate buffer (pH 7.0).

Biochemical analyses

Plasma glucose, triacylglycerol and total cholesterol were determined by colorimetric methods after enzymatic reaction with peroxidase (Cromatest^(R) diagnostics, Joaquim Costal, Montgat, Barcelona, Spain) while, High density lipoprotein (HDL)-Cholesterol and HDL-triglyceride were also determined colorimetrically after HDL was isolated as described by Gidez *et al.* [9,10,11,12]. Low-density lipoprotein (LDL) cholesterol was estimated by the Friedewald formula, which is reliable when triacylglycerol levels are lower than 400 mg/dL [13]. The lipid content of the liver was extracted with chloroform and methanol [14]. After washing with 0.05M KCl solution, aliquots of the chloroform-methanol extract were then used for the determination of cholesterol and triglycerides concentrations. Cholesterol was determined in an aliquot of the chloroform-methanol extract of each organ as described for erythrocytes while, determination of phospholipids followed the same procedure as described for plasma. Triglyceride concentrations in aliquots of the chloroform-methanol extracts of each organ were determined following the procedure described by Kriketos *et al.* [15]. Briefly, an aliquot of the chloroform-methanol extract in Eppendorf tubes was evaporated to dryness at 60°C. After cooling, 200 µl of ethanol (97%) was added to the tube to resuspend the triglyceride. Commercially available triglyceride reagent (1.0mL) (Spin React S. A., Santa Colona, Sant Esteve De Bas, Spain) was then added and vortexed. After incubating in the dark at room temperature for 20 minutes, triglyceride content was determined spectrophotometrically. Hepatic protein concentration was determined colorimetrically at 560nm [16].

Statistical analysis

Data analyses were performed using SPSS software (SPSS 15.0 for Windows, SPSS Inc, Chicago, IL). All data are expressed as mean ± SEM. One-way analysis of variance was used to test for differences between the groups. While, the Duncan's Multiple Range Test was used for mean separation at the probability level of p<0.05.

RESULTS

The weight gain, food intake, food efficiency and fecal output of the rats are shown in Table 2. The mean body weight of untreated diabetic rats was significantly decreased as compared to normal rats. Also the group fed *V. unguiculata ssp. dekindtiana*, recorded decrease in body weight when compared with the non-diabetic animals. The

rats treated with *V. unguiculata ssp. unguiculata* had more than 100% increase in body weight over the non-diabetic rats. The legume-based diabetic rats consumed more food than the control groups with rats fed African yam bean having the highest food intake. Also *S. stenocarpa* fed rats had the highest faecal output with over 2 fold higher output than the normal rats and about 1.8 fold, 1.8 fold and 1.2 fold higher than *V. unguiculata ssp. dekindtiana*, *V. unguiculata ssp. unguiculata* and *V. subterranea* fed groups, respectively.

Figure 1 depicts the levels of fasting blood glucose in the normal and diabetic rats. The mean baseline blood glucose concentration of the rats was 68.75 mg/dL (n=42). After one week of alloxan administration, the blood glucose concentrations of the surviving rats had risen to over 200% of the baseline values. Consumption of the corresponding legume-based diets by all the diabetic rats led to a significant ($p < 0.05$) decrease in the blood glucose concentration. It was also noted that the diabetic control group also had decrease in the blood glucose concentration, however, *V. subterranea* gave the best response with about 59.9% reduction.

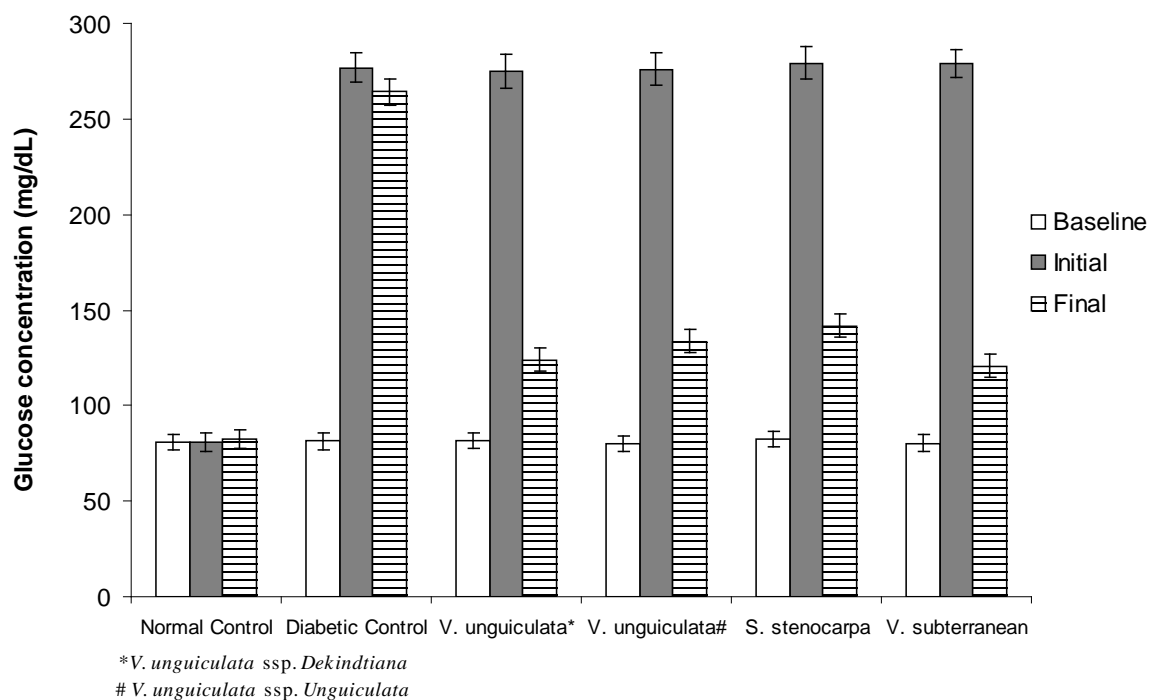


Figure 1: Effects of the legume-based diets on plasma glucose concentration normal and diabetic rats

Data on plasma lipid profile of rats are summarized in Table 3. Total cholesterol, triacylglycerols, HDL-cholesterol, HDL-triacylglycerols and LDL-cholesterol levels were significantly ($p < 0.05$) increased in alloxan induced diabetic rats. Feeding rats with the legume-based diets for five weeks resulted in marked decrease in plasma LDL-cholesterol, triacylglycerols and HDL triacylglycerol levels compared to

diabetic control rats. The rats fed on *V. unguiculata ssp. dekindtiana* had the highest cholesterol level, which was over two folds higher than that of the normal rats. However, over 75% of this was associated with HDL. Consumption of the legume-based diets by the diabetic rats resulted in a significant ($p < 0.05$) reduction in the LDL-cholesterol level. Among the legume, *V. unguiculata ssp. dekindtiana* produced the highest levels of total triglyceride and HDL triacylglycerol.

The ratios of plasma total cholesterol: HDL-cholesterol and LDL-cholesterol : HDL-cholesterol in the experimental animals are illustrated in Figures 2 and 3, respectively. The diabetic rats fed on animal protein-based diet (diabetic control group) had over 80% higher value than the normal in both ratios. However, there was no significant difference ($p > 0.05$) in the ratios between the legume-fed groups and the normal rats.

The effects of legume-based diets on hepatic lipid profile are summarized in Table 4. Diabetes caused a significant ($p < 0.05$) increase in hepatic cholesterol level and significant ($p < 0.05$) decrease in the level of triglycerides. *V. unguiculata ssp. dekindtiana* and *V. subterranea* fed diabetic rats had their hepatic cholesterol levels significantly ($p < 0.05$) reduced to the level of the normal control. Although *V. unguiculata ssp. unguiculata* and *S. stenocarpa* significantly ($p < 0.05$) reversed decrease in hepatic triglyceride, the normal control rats still had significantly ($p < 0.05$) higher levels of hepatic triglycerides. There was no significant difference ($p > 0.05$) in the hepatic protein levels among the groups.

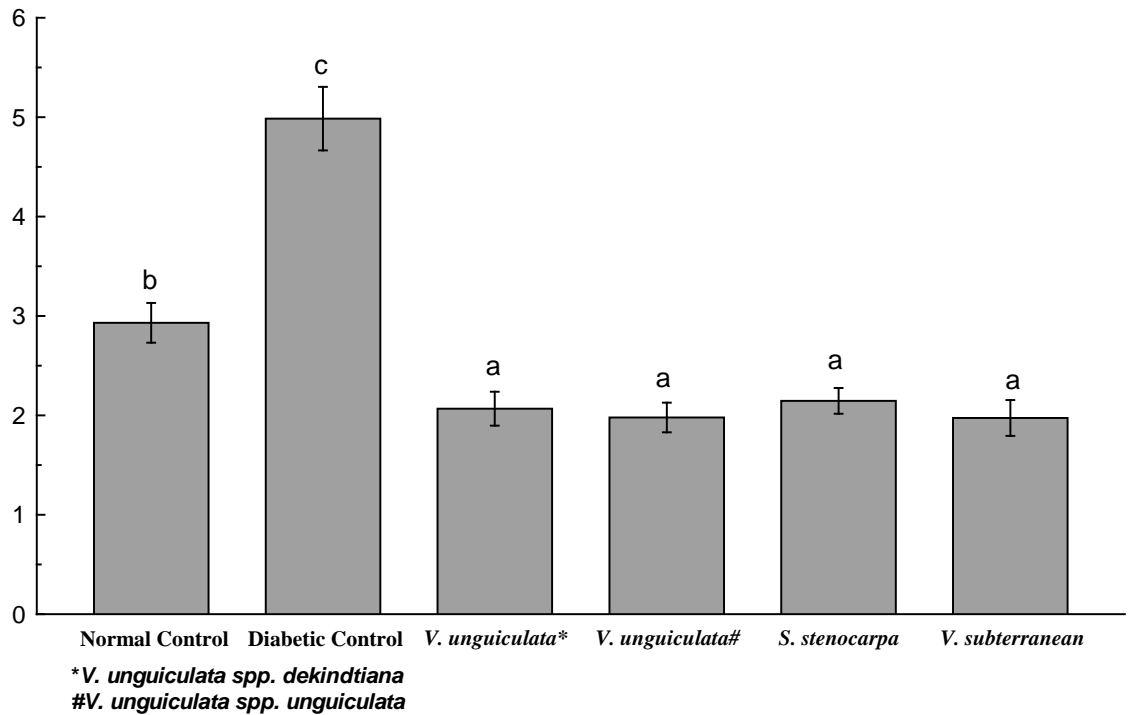


Figure 2: Effect of the legume-based diets on total Cholesterol / HDL-Cholesterol ratios normal and diabetic rats (Bars with different alphabet labels are significantly different at $p < 0.05$)

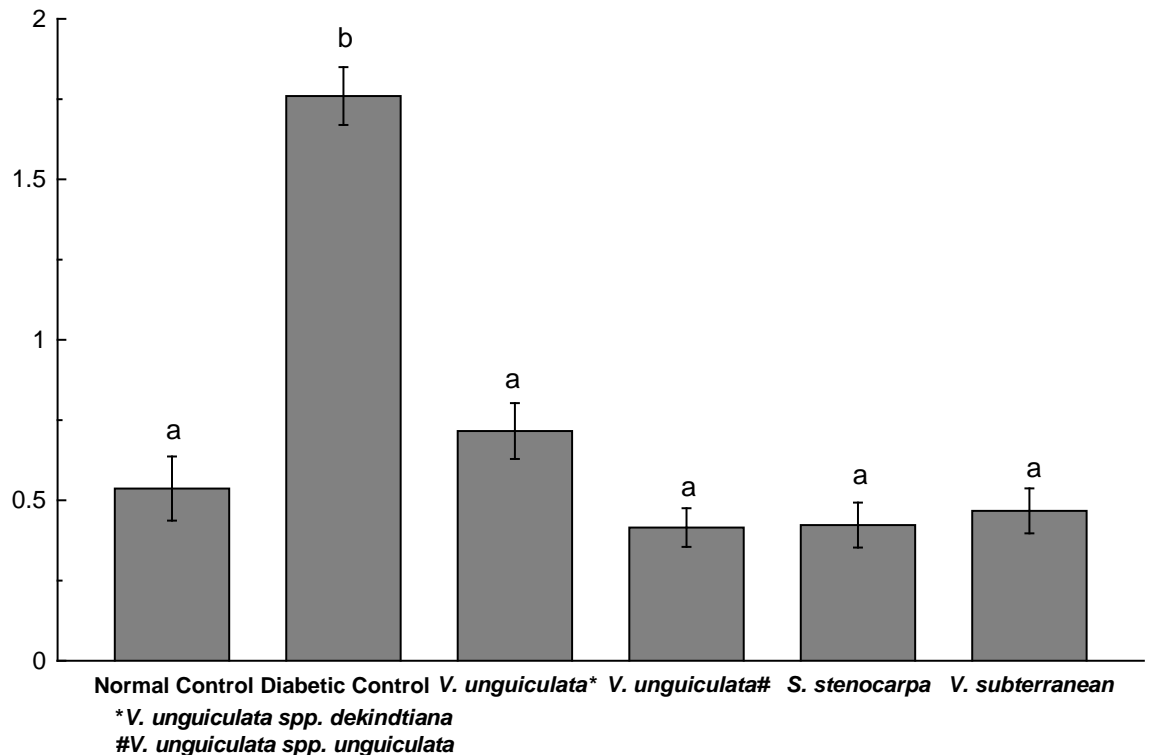


Figure 3: Effects of the legume-based diets on LDL-Cholesterol/HDL-Cholesterol ratios normal and diabetic rats (Bars with different alphabet labels are significantly different at $p < 0.05$)

DISCUSSION

In the present study, the rise in blood glucose was accompanied by reduction in body weight, an observation that was similar to previous studies [17, 18]. However, the plant extract used by Nwanjo in his study could not reverse the loss in weight as did the legumes used in this study (with the exception of *V. unguiculata* ssp. *Dekindtiana*) which further increased wasting of the diabetic rats [18]. It is recognized that gastrointestinal autonomic neuropathy associated with disordered gastrointestinal motor and sensory function occurs frequently in diabetes mellitus [19]. This may contribute to decrease in food intake and faecal output as well as eventual loss in weight. The findings from this study indicate that consumption of legume-based diets by diabetic rats resulted in increase in both food intake and faecal output. It has been established that acute changes in blood glucose concentration – both hyper- and hypoglycemia – have a marked, reversible, effect on gut motility [19]. Legumes have been reported to be rich in dietary fibre and antinutrients such as phytate, resulting in slow digestion and production of low glycemic index and insulin response [1, 17]. Hence, they have been reported to be useful in the risk reduction from vascular

complications, which is thought to have multiple causes including insulin resistance, hyperglycemia, as well as dyslipidemia [1, 20].

The consumption of the legume-based diets by the diabetic rats resulted in significant reduction ($p < 0.05$) in plasma total cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol concentrations with an increase in HDL-cholesterol level. The least reduction in LDL-cholesterol level was from rats fed the *V. unguiculata ssp. dekindtiana*-based diet as has been reported by other investigators, that legumes have varying potentials for lowering cholesterol levels [5, 20, 21, 22]. Kingman *et al.* [22] carried out an experiment with rats fed diets similar to those used in this study and reported that, induced diet-hypercholesterolemia was significantly inhibited by diets containing baked beans, peas, butter beans and lentils. They however reported that butter beans and baked beans were the most effective at reducing LDL-cholesterol [22].

The present study, however, observed that the legumes significantly ($p < 0.05$) reduced the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol in the animals. A similar marked reversal has been reported during guar gum treatment [23]. In man, at least, these ratios are considered to increase the risk of coronary heart disease, even when total cholesterol level is elevated although, the significance of the ratios in rats is still unclear because most cholesterol is transported in HDL in this species [21]. There is supporting evidence from studies in pigs that the ratios are preserved by some legume species [22].

Many dietary factors have been reported to contribute to the hypocholesterolemic effects of dietary legumes. These effects are most often associated with gelling, mucilaginous, soluble fibers in the legumes [1, 21]. Dabai *et al.* [21] and Kingman *et al.* [22] also attributed such hypocholesterolemic effects of legumes used in their studies to non-starch polysaccharides (NSP) much of which is present in legumes in the soluble form. Lin and Anderson showed that guar gum, an isolated soluble-NSP fraction prepared from the Indian cluster bean (*Cyamopsis tetragonoloba*) is hypocholesterolemic when fed to rats with raised cholesterol levels, in contrast to insoluble NSP from cereals which has no effect [24]. It has been proposed that the presence of soluble NSP in the gastrointestinal tract increases viscosity and interferes with micelles formation and lipid absorption [25]. In addition, NSP bind or adsorb bile acids and neutral sterol, enhancing their removal from enterohepatic circulation thereby triggering reverse cholesterol transport. In reverse cholesterol transport, HDL picks up and transports cholesterol in the blood back to the liver, which leads to its elimination from the body [25]. HDL cholesterol prevents LDL accumulation in the walls of the arteries [25]. Hence, HDL cholesterol is considered as a good form of cholesterol and has been found increase by feeding soluble fiber [25].

The present research findings are also supported by the earlier studies in which 22% decrease in triglycerides was observed in guinea pigs that were fed feeding soluble fiber diet containing 2.5 g/100 g of guar gum, 5 g/100 g of psyllium and 5 g/100 g of

pectin diet [26]. Dabai *et al.* [21] however, reported that even if soluble NSP was involved, other factors may also moderate the cholesterol-lowering response. For example, soy-protein feeding compared with casein has been reported to decrease postprandial serum concentrations of insulin and glucose with a significant reduction in serum total cholesterol, LDL-cholesterol, and triglyceride concentrations [7]. Phytoestrogens have been identified as having some beneficial effects by improving serum lipid due to their estrogenic activity while, minor components such as saponins, which these legumes are rich in, have been reported to have cholesterol-lowering effects [27]. It has been argued that all these legume components work synergistically to have beneficial effects on lipid metabolism [1]. In addition, a high level of arginine and a low level of lysine or methionine (met) have been suggested to be involved in the lowering of cholesterol [1]. Arginine (arg) raises the glucagon : insulin ratio; a ratio that is known to repress 3-hydroxy-3-methyl-glutaryl-CoA reductase, (HMG CoA reductase) the rate-limiting enzyme for the synthesis of cholesterol. Also, arg was found to increase plasma thyroxine, along with the lowering of cholesterol in gerbils [7, 21]. Legume proteins are high in arginine and arginine: lysine ratio but low in methionine [1,7]. Thus, arg: lys or arg: met ratio in legumes may partly contribute to reducing cholesterol in rats.

Diabetes also led to an increase in the levels of cholesterol and decrease in the level of triacylglycerols in the liver. Diabetes-induced increase in hepatic cholesterol has been reported by other investigators [28]. This is often the consequence of decreased HDL-cholesterol leading to the depression of HMG-CoA reductase. Short chain fatty acids produced by bacterial fermentation of NSP from legumes, in particular propionate, have been suggested to down-regulate the activity of HMG-CoA reductase [29]. In agreement with the finding, Evans *et al.* [30] reported that ferugreek gum, guar gum and locust bean gum lowered hepatic cholesterol concentration and they correlated their observation to inhibition of HMG-CoA reductase. The lowering of triacylglycerols in the liver in diabetic animals may be related to the inability of peripheral tissues to effectively and efficiently utilize glucose for metabolic energy. Thus, free fatty acids are mobilized from fat depots (the liver inclusive) to supply the much needed metabolic fuel to other tissues and organs, thus depleting lipid reserves. The inability of these legumes to reverse the decreased hepatic level of triacylglycerols indicates that the diabetic rats still mobilized free fatty acids from triacylglycerols for energy.

CONCLUSION

The findings, therefore, suggest that wild cowpea, white cowpea, african yam bean and bambara groundnut equally reversed diabetes-associated dyslipidemia as indicated by the indexes of cardiovascular disorder. The legumes may be beneficial in the dietary management of elevated blood glucose and dyslipidemia associated with diabetes. Caution should, however, be taken in the use of wild cowpea as its consumption resulted in wasting.

Table 1: Composition of diet

Composition	Level (g/100 g) in diet				
	Control	V. <i>unguiculata</i> <i>ssp.</i> <i>dekindtiana</i>	V. <i>unguiculata</i> <i>ssp.</i> <i>unguiculata</i>	S. <i>stenocarpa</i>	V. <i>subterranea</i>
Fish meal	25	10	10	10	10
<i>V. unguiculata ssp.</i> <i>Dekindtiana</i>	-	35	-	-	-
<i>V. unguiculata ssp.</i> <i>Unguiculata</i>	-	-	35	-	-
<i>S. stenocarpa</i>	-	-	-	35	-
<i>V. subterranean</i>	-	-	-	-	35
Sucrose	10	10	10	10	10
Corn starch	49.5	34.5	34.5	34.5	34.5
Groundnut oil	5	5	5	5	5
Salt/Mineral mix*	5.5	5.5	5.5	5.5	5.5
Cellulose	5	-	-	-	-

*Salt/Mineral mix contains the following in g/100g of the mix: Calcium phosphate 49.50, Sodium powder 11.80, Potassium sulphate 5.20, Sodium chloride 7.40, Magnesium oxide 2.40, Potassium citrate 22.40, Ferric citrate 0.60, Manganous carbonate 0.35, Cupric carbonate 0.03, Zinc carbonate 0.16, Chromium potassium sulfate 0.055, Potassium iodate 0.001, Sodium selenate 0.001, Choline chloride 0.50, Thiamine HCl 0.06, Riboflavin 0.06, Niacine 0.30, Calcium pantothenate 0.16, Biotin 0.01, Vit B₁₂ 0.10, Vit D₃ 0.025, Vit E Acetate 1.00, Pyridoxine 0.07, folic acid 0.02, Vit A Acetate 0.08

Table 2: Effects of the legume-based diets on weight gain, food intake, food efficiency and faecal output of normal and diabetic rats

Group	Weight gain (g)	Food intake (g/day)*	Food efficiency (g weight gain/g food intake)	Faecal output (g/day)*
Normal Control	7.00 ±1.75 ^b	10.57±0.29 ^{abc}	-2.56±0.87	3.50±0.34 ^{ab}
Diabetic Control	-0.43±1.94 ^a	9.48±0.54 ^a	-1.38±1.37	2.40±0.20 ^a
<i>V. unguiculata</i> ssp. <i>dekindtiana</i>	-3.50±2.47 ^a	10.03±0.57 ^{ab}	-0.49±1.44	3.33±0.49 ^{ab}
<i>V. unguiculata</i> ssp. <i>unguiculata</i>	15.33±3.80 ^c	10.33±0.59 ^{ab}	1.11±0.49	3.33±0.42 ^{ab}
<i>S. stenocarpa</i>	1.33±1.38 ^a	11.77±0.42 ^c	-0.09±0.40	6.00±0.97 ^c
<i>V. subterranea</i>	5.33±2.86 ^b	11.43±0.20 ^{bc}	2.56±0.88	4.40±0.84 ^{bc}

Each value represented the mean±SEM of six (6) readings. Values within the same column with different superscripts are significantly different at $p < 0.05$

* mean±SEM for the 5 week treatment period

Table 3: Effects of the legume-based diets on plasma lipid profile normal and diabetic rats

Group	Total Cholesterol (mg/dL)	Total Triglyceride (mg/dL)	HDL Cholesterol (mg/dL)	HDL triglyceride (mg/dL)	VLDL Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)
Normal Control	71.77±3.13 ^a	68.33±5.32 ^a	27.27±1.35 ^a	16.53±3.85 ^a	6.48±1.06 ^a	14.63±3.72 ^a
Diabetic Control	120.07±4.26 ^b	136.91±34.56 ^c	24.08±6.02 ^a	34.96±5.30 ^{ab}	35.38±6.91 ^c	42.37±8.83 ^b
<i>V. unguiculata</i> <i>ssp. dekindtiana</i>	111.45±8.68 ^c	105.33±12.53 ^b	53.93±4.22 ^c	43.73±6.76 ^b	21.07±2.51 ^b	38.59±6.37 ^b
<i>V. unguiculata</i> <i>ssp. unguiculata</i>	86.12±6.86 ^{ab}	85.14±14.48 ^{ab}	43.53±3.89 ^{bc}	41.80±7.34 ^b	17.03±2.99 ^b	18.06±4.21 ^a
<i>S. stenocarpa</i>	112.22±6.83 ^c	79.24±22.64 ^{ab}	47.84±4.84 ^{bc}	37.07±8.11 ^{ab}	15.85±4.53 ^b	20.22±5.39 ^a
<i>V. subterranean</i>	80.26±2.79 ^{ab}	71.99±13.66 ^{ab}	40.67±2.11 ^b	29.20±8.33 ^{ab}	14.40±2.73 ^b	18.99±3.65 ^a

Each value represented the mean±SEM of six (6) readings. Values within the same column with different superscripts are significantly different at $p < 0.05$

Table 4: Effects of the legume-based diets on hepatic lipids and protein levels in normal and diabetic rats

Group	Cholesterol (mg/100 g)	Triglyceride (mg/100 g)	Protein (mg/ g)
Normal Control	96.35±2.86 ^a	110.13±3.11 ^c	118.17±23.67 ^a
Diabetic Control	123.33±4.00 ^b	66.96±1.89 ^{ab}	78.08±7.14 ^a
<i>V. unguiculata</i> <i>ssp. dekindtiana</i>	87.45±2.30 ^a	60.38±4.54 ^a	107.80±12.87 ^a
<i>V. unguiculata</i> <i>ssp. unguiculata</i>	100.49±1.33 ^c	84.00±8.62 ^b	84.17±8.65 ^a
<i>S. stenocarpa</i>	109.12±1.06 ^c	82.41±3.44 ^b	117.33±12.77 ^a
<i>V. subterranean</i>	98.53±2.77 ^a	70.69±2.63 ^a	99.47±3.87 ^a

Each value represented the mean±SEM of six (6) readings. Values within the same column with different superscripts are significantly different at $p < 0.05$

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