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Serum lipid and electrolyte profiles of Wistar rats fed with *Vernonia amygdalina* supplemented *Vigna subterranea* (Bambara groundnut) pudding

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ABSTRACT

Objective: This study investigated the effects of *Vernonia amygdalina* supplementation on *Vigna subterrenea* (bambara groundnut) pudding on serum lipid profile and electrolyte levels in *Wistar rats*.

Materials and Methods: Twenty five weanling rats were randomly selected into 5 groups of 5 rats each. Group 1 received normal rat pellet. Groups 2 and 3 received 10% and 5% (w/w) non supplemented bambara pudding, respectively. Groups 4 and 5 were fed with 10% and 5% (w/w) *Vernonia* supplemented bambara pudding, respectively, for 21 days. The animals were sacrificed using anaesthesia at the end of the experiment and blood sample collected for serum lipid profile and serum electrolyte determinations using standard methods.

Results: There was a significant increase ($p<0.05$) in total cholesterol level in groups 2 (1.64 ± 0.01 mmol/L) and 4 (1.66 ± 0.01 mmol/L) compared to normal control (1.31 ± 0.02 mmol/L). Triacylglycerol level increased significantly in groups 2, 3, and 4 compared to normal control. The high density lipoprotein cholesterol (HDL-c) and Serum sodium level were significantly increased ($p < 0.05$) in group 2 and 4 respectively compared to the normal control. Potassium, chloride, bicarbonate and calcium levels showed significant increases in group 2 relative to normal control. Phosphate level decreased significantly in group 2 (1.37 ± 0.03 mmol/L), 3 (1.43 ± 0.03 mmol/L) and 5 (1.37 ± 0.03 mmol/L) when compared to normal control (1.75 ± 0.03 mmol/L) respectively.

Conclusion: Consumption of bambara groundnut pudding supplemented with *Vernonia* leaves increases HDL-c level as well as supply appreciable amounts of electrolytes in the body.

Keywords: Bambara groundnut, *Vernonia amygdalina*, Lipid profile, Electrolytes

INTRODUCTION

Blood lipids especially high concentration of low-density lipoprotein cholesterol (LDL-c) is one of the leading risk factors for the development of the cardiovascular disease. Plant-based foods are rich in phytochemicals that help in modulating levels of total cholesterol (TC) as well as LDL-c in humans. It has been reported that increased consumption of legumes contributes to about 82% reduction in the possible risks of developing coronary heart disease.[1] Bambara groundnut (*Vigna subterranea* (L.) *Verde*), a legume is widely cultivated in the West and Central Africa. It contains about 65% carbohydrate, 18% protein, and 6.5% fat, which makes it ranked as a complete food.[2] Bambara groundnut (*V. subterranean*) belongs to family Fabaceae. It is an annual herbaceous, intermediate plant with creeping stems at ground

level. It grows easily in areas of low rainfall with little or no application of fertilizer.[3] Bambara groundnut is one of the underutilized leguminous crops due to a lack of knowledge of its health-promoting benefits.

Bambara pods are often boiled and the seeds are consumed roasted or used in soups. The seeds are highly nutritious as a good source of fiber, calcium, iron, potassium, and essential amino acid.[4] The seed has the potential for providing protein in areas where animal protein is expensive, and the climatic conditions do not favor the cultivation of other legumes. Nutritionally, *Vernonia amygdalina* is used as a vegetable in soup making in the tropics and has successfully been used as a supplement in weaning foods.[5] In Nigeria as in most tropical countries of Africa where the daily diet is mostly rich in carbohydrate content, vegetables which are readily available serve as excellent sources of proteins, vitamins, minerals, essential amino acids, and micronutrients in meeting nutritional requirement for healthy living.[6] Several researchers have reported the anti-lipid lowering effect of *V. amygdalina* supplemented diet.[7] *Vernonia* supplemented diet has been reported as being capable of lowering body weight and total body fat in obesity-induced rat models.[7]

Despite the great potential of Bambara groundnut in providing food security and contributing to the reduction of malnutrition in rural communities in Nigeria, the crop has remained underutilized which may be due to lack of knowledge of its health benefits in the biological system. Therefore, this work aimed at investigating the serum lipid and electrolyte profiles of Wistar rats fed with *V. amygdalina* supplemented *V. subterranea* (Bambara groundnut) pudding.

MATERIALS AND METHODS

Sample collection

The dried cream colored *V. subterranea* (Bambara groundnuts) and *V. amygdalina* leaves were purchased from Okuku market, Yala and Watt market, Calabar, all in Cross River State, Nigeria. They were conveyed to the laboratory of the Department of Biochemistry, University of Calabar, Nigeria. The study samples (Bambara groundnut and *V. amygdalina*) were identified and authenticated by a Botanist, Mr. Frank Apojeye of the Department of Botany, University of Calabar. Bambara groundnut was assigned voucher number BCM/2014/032 while *V. amygdalina* was assigned BCM/2014/011. The voucher numbers were deposited in the herbarium of the same department.

Sample preparation

Bambara seeds were sorted to remove extraneous materials and damaged seeds. A measured weight (300 g) of the sorted seeds was soaked in tap water for 8 h at room temperature for easy removal of the outer coat. After 8 h, the seeds were washed and drained several times to dehull the coat from the cotyledon and then wet-milled into paste using a commercial milling machine. Thereafter, 200 ml of cold water was added to the flour and mixed thoroughly with recipes (70 g of sliced onions, 50 g of blended fresh pepper, 50 g of blended crayfish, 20 ml of palm oil, 4 g of magi cubes, and 25 g of chopped fresh bitter leaf). The mixture was used to prepare two kinds of diets. The first diet was prepared supplemented with fresh chopped *V. amygdalina* leaves, whereas the second diet was prepared without *Vernonia* leaves supplementation. A clean measuring cup of 20 ml size was used to dispense the mixed paste into a clean local wrapping leaf called nkong which is one of the non conventional green leafy vegetables like *Vernonia* calvoana as reported by Ejoh *et al*.[8]. The same quantity of chopped fresh bitter leaf (25 g) was added to each wrap in the *Vernonia* supplemented puddings diet. The same procedure was repeated in preparing the second type of diet but without the addition of bitter leaves, this formed the non-supplemented puddings. Both diets were cooked for 45 min, after which the wrapping leaves were removed before the pudding was oven dried at temperature of 50–60°C. The oven-dried diets were separately blended into fine particles then subsequently incorporated into the rat feed.

Animals

Twenty-five weanling Wistar rats of both sexes, of ages 4–6 weeks old, weighing between 50 g and 53 g were purchased from Animal House of the Department of Biochemistry, University of Calabar. The rat feed was purchased from a Vital Feed dealer's outlet in Calabar Municipality, Cross River State, Nigeria.[9]

Experimental protocols

The Wistar albino rats were randomly selected into their respective groups. Group 1 (control) was fed with 100 g rat pellet only, Group 2 received a mixture of 10 g non-supplemented pudding + 90 g pellet), Group 3 received a mixture of 5 g non-supplemented pudding + 95 g pellet, Group 4 received a mixture of 10 g of supplemented pudding + 90 g pellet, and Group 5 received a mixture of 5 g of supplemented pudding + 95 g pellet. The feeding experiment lasted for 21 days. All the experimental groups were allowed access to fresh tap water throughout the experimental period. The rats were weighed and the measurement recorded at 4 days intervals. Furthermore, feed intake was monitored by taking a record of the left overfeeds on a daily basis using an electronic balance. The rats' beddings were changed at 2 days intervals throughout the period of the experiment to avoid microbial growth and subsequent infection of the animals.

Collection and preparation of blood samples for analysis

The rats were fasted overnight before the time of sacrifice. They were then sacrificed under anesthesia, the rats were dissected using surgical scissors and forceps to cut open the thoracic cavity. Whole blood was collected from the left ventricle using 5 ml sterile needle and syringe and placed in non-heparinized sample tubes. The blood was allowed to clot after 3 h and subsequently centrifuged at 3000 revolutions/min for 10 min using a benchtop centrifuge (Model SM 80 – 2, England) for easy collection of the serum. The harvested sera were used to assay for lipid profile and electrolyte levels.

Serum high-density lipoprotein cholesterol (HDL-c) was estimated using Agappe assay kit following the method described by Tietz[10]

Briefly, this method involves precipitation of LDL-c, very low density lipoprotein cholesterol (VLDL-c), and chylomicron fractions with phosphotungstic acid and magnesium ions as a cofactor. The HDL-c in the supernatant after centrifugation was determined with the colorimetric enzymatic method.

Determination of TC was done using Agappe assay kit following the method of Siedel *et al*.,[11]

The principle involved enzymatic hydrolysis of cholesterol from its ester form by cholesterol esterase and further oxidation of cholesterol to produce hydrogen peroxide as a byproduct.

Triacylglycerol (TG) estimation was carried out using Agappe assay kit (GPO-PAP method) according to the procedure described by Tietz[10]

TG was estimated following hydrolysis with lipases. Quinoneimine was the indicator used which was formed from the reaction of hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol catalyzed by peroxidase.

Estimation of LDL-c and VLDL-c by calculations was done following the method described by Friedewald *et al*.,[12]

VLDL-c and LDL-c levels were estimated based on the methods of Friedewald *et al*.[12] using the concentrations of TG, HDL-c, and TC.

Sodium concentration estimation in serum was carried out using kits from Agappe in accordance with the method described by Tietz[10]

The principle involved precipitation of sodium as the triple salt, sodium magnesium, and uranyl acetate. The excess uranium reacted with ferrocyanide to produce a chromophore whose absorbance (color intensity) was inversely proportional to the concentration of sodium in the test specimen.

Potassium concentration determination in serum was done using Agappe kits according to the method described by Tietz[10]

Sodium tetraphenylboron was used as the indicator for the determination of potassium concentration. The intensity of which was proportional to potassium concentration in the range of 2–7 mEq/L.

Chloride concentration determination in serum was carried out with Agappe assay kits following the method described by Tietz[10]

Chloride reacted with mercuric thiocyanate to liberate thiocyanate ions, which on reaction with ferric ions formed a colored complex whose intensity was proportional to the chloride concentration in the sample.

Determination of macro-elements in serum was carried out using Agappe assay kits by following the method described by Tietz[10]

Determination of electrolyte profile

The concentration of electrolyte, namely, calcium (Ca^{2+}), bicarbonate (HCO_3^-), phosphate (PO_4), iron (Fe^{2+}), and zinc was assay using reported standard kit methods as described by Tietz[10] and the assay kit used was obtained from Agappe diagnostics.

Statistical analysis

All values were expressed as mean and standard error means. Data were analyzed by one-way ANOVA, and significant differences between groups were determined by the least significant difference using SPSS version 22.0. The acceptable level of significance was at $P < 0.05$.

RESULTS AND DISCUSSION

Data obtained from the effect of Bambara groundnut pudding supplemented with *Vernonia* leaves are presented in Table 1 (serum lipid indices) and Table 2 (serum electrolytes profile).

Serum lipid profile

The results of serum lipid profile obtained from different experimental groups fed with varied proportions of the non-supplemented pudding in Groups 2 and 3, supplemented pudding in Groups 4 and 5 as well as the animals fed normal rat pellet (Group 1) are presented in Table 1. TC levels in Group 1 showed a significant decrease at $P < 0.05$ when compared to Groups 2, 3, and 4, respectively. However, TC levels in Group 2 increased significantly at $P < 0.05$ when

Table 1: Serum profile of albino rats fed with *Vernonia amygdalina* supplemented Bambara pudding.

Groups	Total cholesterol/mmol/L	Triacylglycerol/mmol/L	Very low density lipoprotein cholesterol/mmol/L	High-density lipoprotein cholesterol/mmol/L	Low-density lipoprotein cholesterol/mmol/L
1	1.31±0.02	1.15±0.02	0.52±0.01	0.33±0.01	0.45±0.02
2	1.64±0.01*	1.35±0.02*	0.61±0.01*	0.35±0.00*	0.45±0.02*
3	1.41±0.01*[a]	1.35±0.02*	0.61±0.01*	0.35±0.00[a]	0.45±0.02[a]
4	1.66±0.01*	1.54±0.02*	0.70±0.01*	0.42±0.00*	0.55±0.01
5	1.27±0.01[a,b]	1.09±0.02[a]	0.50±0.01[b]	0.32±0.00[b]	0.46±0.02

All values are in mmol/L; Values are expressed as mean±standard error means, n=5; values designated *vary significantly from Group 1 at P<0.05, [a]significantly different at P<0.05 from Group 2, [b]different significantly from Group 4 at P<0.05. UFD: Non-supplemented pudding, FBD: Supplemented Bambara pudding

compared with Groups 3 and 5, respectively. Similarly, serum TC level in Group 4 significantly increased relative to Group 5 at $P < 0.05$. Duane[13] reported that total cholesterol decreases in serum following consumption of legumes via unclear mechanisms. The present study did not follow this pattern.

Furthermore, the TG levels decreased significantly at $P < 0.05$ in Group 1 when compared to Groups 2, 3, and 4, respectively. Furthermore, the VLDL-c level in Group 1 (0.52 ± 0.01 mmol/L) decreased significantly at $P < 0.05$ when compared to Group 2 (0.61 ± 0.01 mmol/L), Group 3 (0.61 ± 0.01 mmol/L), and Group 4 (0.70 ± 0.01 mmol/L), respectively. However, there was a significant increase in VLDL-c level in Group 4 when compared to Group 5 at $P < 0.05$.

Serum HDL-c increases at $P < 0.05$ in Group 2 (0.35 ± 0.00 mmol/L) and Group 4 (0.42 ± 0.00 mmol/L), respectively, when compared to Group 1 (0.33 ± 0.01 mmol/L). Similarly, HDL-c level in Group 4 (0.42 ± 0.00 mmol/L) had higher concentrations than Group 5 (0.32 ± 0.00 mmol/L) at $P < 0.05$. There was no significant difference in LDL-c level at $P < 0.05$ in Group 1 (0.45 ± 0.02 mmol/L) when compared to Group 2 (0.45 ± 0.02 mmol/L).

The result showed that dietary intake of the high amount of the Bambara diet had a positive correlation in TC levels as observed in Groups 2 and 4. TG levels in the serum were also higher in Groups 2, 3, and 4 when compared to Group 1, respectively. The high level of TC and TG in the serum may be attributed to the increased proportion of the pudding in the diet; hence, this diet should be consumed in moderate quantity.

The VLDL-c level was significantly ($P < 0.05$) higher in Groups 2, 3, and 4 when compared to Group 1, respectively. The HDL-c levels increased significantly ($P < 0.05$) in Groups 2 and 4 compared to Groups 1, 3, and 5, respectively.

Serum LDL-c levels were increased significantly only in Groups 2 and 3 when compared to Group 1. Groups 4 and 5 showed no significant difference. This may be due to the supplementation of *V. amygdalina* leaves into the diet which is known to contain antioxidants that are involved in lipid metabolism. The LDL-c transports cholesterol from the liver to the peripheral tissues. LDL-c concentration in the blood is one of the leading risk factors of cardiovascular diseases. The data obtained from the present study agreed with other works done using other legumes. Carew *et al.*[14] reported a marked reduction in cholesterol and TG after treatment with velvet beans, according to Sugano *et al.*,[15] a similar serum cholesterol and TG lowering effect in rats fed with soybeans was obtained. Njoku *et al.*[16] reported a significant increase in HDL-c and marked decrease in LDL-c levels in rats treated with aqueous seed extracts of two varieties of *Phaseolus vulgaris*.

Table 2: Serum calcium, iron, zinc, and electrolytes levels in albino rats fed with *Vernonia amygdalina* supplemented and non-supplemented Bambara puddings for 21 days.

Groups	Na/mmol/L	K/mmol/L	Cl/mmol/L	HCO_3/mmol/L	Ca/mmol/L	PO_4/mmol/L	Fe/mmol/L	Zn/mmol/L
1	132.67±0.33	5.80±0.23	94.67±0.67	16.33±0.33	2.47±0.03	1.75±0.03	133.77±1.28	88.10±5.74
2	141.33±0.33*	8.93±0.45*	99.33±0.33*	13.67±0.33*	2.47±0.03	1.37±0.03*	123.67±2.61	85.33±5.27
3	133.00±0.58[a]	5.77±0.20[a]	94.00±1.15	17.00±0.58[a]	2.70±0.06	1.43±0.03*	142.93±1.04	106.93±1.63
4	139.00±0.67*	6.87±0.15	98.33±1.15*	15.67±0.58	2.30±0.06[a]	1.50±0.03	128.40±1.75	100.73±1.65
5	132.67±0.33[b]	5.27±0.45[b]	94.00±0.33	20.00±0.33*,[a,b]	2.80±0.03*,[b]	1.37±0.03*	100.13±2.61	67.60±5.27

All values are in mmol/L. Values are expressed as mean±standard error means n=5; values designated *shows significant increase from Group 1 at P<0.05, [a]shows significant difference at P<0.05 when compared to Group 2, [b]vary significantly at P<0.05 when compared to Group 4

Serum macroelements and electrolytes

The result of some serum macroelements and electrolyte levels in different experimental groups is presented in Table 2. Sodium levels significantly increase at $P < 0.05$ in Group 2 (141.33 ± 0.33 mmol/L) and Group 4 (139.00 ± 0.67 mmol/L), respectively, when compared to Group 1 (132.67 ± 0.33 mmol/L). However, a significant decrease at $P < 0.05$ in sodium levels was seen in Group 3 (133.00 ± 0.58 mmol/L) when compared to Group 2 (141.33 ± 0.33), whereas Group 5 (132.67 ± 0.33) decreased significantly ($P < 0.05$) as compared to Group 4 (139.00 ± 0.67 mmol/L). Furthermore, there was a significant increase at $P < 0.05$ in potassium level in Group 2 (8.93 ± 0.45 mmol/L) when compared to Group 1 (5.80 ± 0.23 mmol/L). The potassium level in Group 3 (5.77 ± 0.20 mmol/L) showed significant decrease ($P < 0.05$) when compared to Group 2 (8.93 ± 0.45 mmol/L). The pattern was similar in Group 5 (5.27 ± 0.45 mmol/L) when compared to Group 4 (6.87 ± 0.15 mmol/L).

Serum chloride level in Group 2 (99.33 ± 0.33 mmol/L) and Group 4 (98.33 ± 1.15 mmol/L), respectively, was significantly higher at $P < 0.05$ when compared to Group 1 (94.67 ± 0.67 mmol/L). In Table 2, HCO_3^- levels in Group 5 (20.00 ± 0.33 mmol/L) increased significantly ($P < 0.05$) when compared to Group 1 (16.33 ± 0.33 mmol/L), whereas Group 2 (13.67 ± 0.33 mmol/L) decreased significantly when compared to Group 1. Group 3 (17.00 ± 0.58 mmol/L) value was higher significantly at $P < 0.05$ in comparison to Group 2 (13.67 ± 0.33 mmol/L). Furthermore, a significant increase ($P < 0.05$) in chloride level was observed in Group 3 (17.00 ± 0.58 mmol/L) when compared to Group 2 (13.67 ± 0.33 mmol/L). Similarly, Group 5 (20.00 ± 0.33 mmol/L) significantly increase at $P < 0.05$ in comparison to Group 4 (15.67 ± 0.58 mmol/L). The result for serum Ca^{2+} levels increased at $P < 0.05$ in Group 5 when compared to Groups 4 and 1, respectively. Serum phosphate levels showed some decreases at $P < 0.05$ in Groups 2, 3, and 5 (1.37 ± 0.03 mmol/L), respectively, when compared to Group 1 (1.75 ± 0.03 mmol/L). There was no significant difference in the concentrations of iron and zinc, respectively, in all experimental groups.

Serum sodium (Na) level showed quantity-dependent relationship in Groups 2 and 4 when compared to Groups 1, 3, and 5, respectively. It was also observed from the result in this study that Groups 4 and 5 showed decreased Na value when compared to Groups 2 and 3, respectively. This decrease may be due to the presence of anti-nutritional factors in the *V. amygdalina* leaves as reported by Eleyinmi *et al.* and Udensi *et al.*[17,18] Serum potassium level showed a significant increase in Group 2 as compared to Group 1. There was an increase in serum potassium levels, which showed a positive correlation with the quantity of puddings incorporated into the diet in Groups 2 and 4 when compared to Groups 3 and 5, respectively. It implies that increased dietary intake of Bambara diet could lead to an increased level of serum potassium. A similar result was obtained for chloride level where values in Groups 2 and 4 were higher than in Groups 3 and 5, respectively.

Serum bicarbonate level significantly increased in Group 5 when compared to Group 1, whereas, in Group 2, there was a significant decrease when compared to Group 1. Serum calcium level showed a significant increase in Group 5 when compared to Group 1. This decrease in calcium serum level could be as a result of interference of some anti-nutritional factors presence both in Bambara and *Vernonia* leaf during absorption. Serum phosphate levels significantly increased in Groups 2, 3, and 5 when compared to Group 1, respectively. There was no significant difference in serum iron and zinc levels. This may be due to the presence of phytates, oxalates, and tannins that may have inhibited their bioavailability.[9]

CONCLUSION

Consumption of Bambara groundnut diet supplemented with *V. amygdalina* leaves was observed to increase HDL-c levels in the blood of different experimental groups. However, an increased level of HDL-c in the blood has been reported to be a key indicator for the reduction of the prevalence of cardiovascular diseases. The diet contains an appreciable quantity of some macroelements, which may contribute to normal biochemical functions in the studied animal model.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Conflicts of interest

There are no conflicts of interest.

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