



Rat Serum Electrolytes and Lipid Profiles Following Administration of Aqueous Extract of *Sphenostylis stenocarpa*

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Authors' contributions

This work was carried out in collaboration between both authors. Author ONF designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript also managed the analyses of the study. Author DPI managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Aqueous extract of the seed of African yam bean (*Sphenostylis stenocarpa*) is used in Nigerian alternative medicine for the treatment of high blood pressure. Therefore the aim of this study is to investigate the effect of *Sphenostylis stenocarpa* seed extract on some electrolyte and lipid profiles of Wistar rats.

Materials and Methods: A total of 36 wistar rats were used for this research. The rats were arranged in 4 groups and these groups had 3 subgroups containing 3 rats each. Varying doses of the aqueous extract of the *Sphenostylis stenocarpa*, were administered to the rats for a period of ten days and the biochemical parameters; cholesterol, triglycerides, sodium and potassium levels were determined.

Results: There was a significant decrease $p \leq 0.05$ in the sodium (Na^+) concentration in the serum when compared to the control. Furthermore, the potassium (K^+) concentration in the serum of the

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experimental animals showed a significant $p \leq 0.05$ increase. The study also showed that the aqueous extract of *Sphenostylis stenocarpa* had a lowering effect on the lipid profiles of the experimental rats. The highest decrease of cholesterol (2.11 ± 0.01 vs control 2.20 ± 0.12 mmol/l) was obtained on the tenth day with the highest dose of 3 ml of aqueous extract of *Sphenostylis stenocarpa*. Also the highest decrease in the level of triglyceride (0.40 ± 0.01 vs control 0.46 ± 0.11 mmol/l) was obtained at the tenth day with the highest dose of 3 ml of the extract.

Conclusion: The lowering effect on the lipid profiles studied and the decrease in sodium Na^+ and increase in K^+ may be contributing to the reduction in high blood pressure in the individuals taking this aqueous extract.

Keywords: Cholesterol; electrolytes; lipids; potassium; sodium; *Sphenostylis stenocarpa*; triglycerides.

1. INTRODUCTION

African yam bean (AYB) (*Sphenostylis stenocarpa*) is an herbaceous leguminous plant cultivated throughout tropical Africa [1]. It is grown as a minor crop in association with yam and cassava. AYB serves as security crop; it has the potential to meet year round protein requirements if grown on a large scale [2]. African yam bean known locally in Igbo as uzaaku is widely cultivated in the southern parts of Nigeria. The water drained after boiling the seeds of *Sphenostylis stenocarpa* have been taken to reduce high blood pressure levels in patients practising alternative medicine. However, there is no documented evidence to support this claim. Hence this is the motive of this research. African yam bean (AYB) is highly nutritious with high protein, mineral and fibre content. Its protein content is reported to be the same as some major and commonly consumed legumes. Its amino acid profile is comparable if not better than that of cowpea, soy bean and pigeon pea [3,4,5]. It has high metabolic energy, low true protein digestibility (62.9%), moderate mineral content, the amino and fatty acids contents are comparable to those of most edible pulses [6,7,8]. It has a higher water absorption capacity when compared to cowpea [9]. The potential role of AYB in the management of many aging and chronic non communicable diseases has been reported [10,11]. In Ghana, the water drained after boiling may be drunk by lactating mothers to increase their milk production [12]. The economic potential of AYB has been recognized, especially in reducing malnutrition among Africans [13]. These health benefits can be spoiled by the presence of anti-nutrients. Some processing methods however, such as soaking, boiling, fermentation, roasting, among others are known to achieve reduction or elimination of the anti-nutritional factors which affect the nutrients [14,15,16]. The economic potentials of AYB are immense because in

addition to the production of two major food substances, the value of proteins in the tubers and seeds is comparatively higher than what could be obtained from most African tuberous and leguminous crops [3]. The AYB seeds have protein content ranging from 21 - 29% with about 50% carbohydrate mainly as starch [17]. The protein content of AYB seed is however lower than that of soybean seed (38%) but amino acid spectrum indicated that lysine and methionine (limiting amino acid in most vegetable seed proteins) are better than most legumes including soybean [18]. In Nigeria, particularly the South South and South East, AYB seeds have been preferred to other legumes in the past because they are filling; however, for unclear cultural reasons cowpea is now the preferred legume. Brown and black AYB seeds are preferred in the lowlands and the light-coloured seeds in the mountainous regions of Nigeria [19].

Cholesterol is a sterol or modified steroid, a lipid molecule and is biosynthesized by all animal cells because it is an essential structural component of animal cell membranes that is required to maintain both membrane integrity and fluidity. In addition to its importance within cells, cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D [20].

Triglyceride is an ester derived from glycerol and three fatty acids. As a blood lipid, it helps enable the bidirectional transfer of adipose fat and blood glucose from the liver. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so [20].

The sodium element plays an important role in salt and water balance in the body. The metabolism of sodium and potassium is closely linked with the maintenance of fluid balance and with the regulation of acid-base status [21]. Elevated levels are related to acidosis as well as too much water crossing the cell membrane. The

potassium element is found primarily inside the cells of the body.

The water drained from boiled African yam bean have been used to reduce high blood pressure levels when consumed. There are no documented evidence to support this claim. Therefore the aim of this study is to investigate the effect of *Sphenostylis stenocarpa* seed extract on some electrolyte and lipid parameters such as cholesterol, triglycerides, sodium and potassium using Wistar rat as the experimental animal.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Reagent kits were bought from Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom BT29 4QY.

The African yam bean (*Sphenostylis stenocarpa*) seed used for this study was obtained from Enugu State, Nigeria. It was identified at the Federal Ministry of Agriculture, Enugu State, Nigeria. The African yam bean extract was prepared by boiling 10 grams in 200 ml of water for an hour. The extract was then drained and stored in a clean container. The method of administration was gavaging and this was done twice daily.

2.2 Experimental Animals

A total of 36 male and female Wistar rats with average weight of 100 g were used for the purpose of this research. The rats were obtained from the animal holding unit of the University of Port Harcourt and they were acclimatized for one week. The rats were arranged in 4 groups and these groups had 3 subgroups containing 3 rats each. The groups were labelled according to the dose of extract (of the boiled AYB) administered orally to the rats daily.

Group 1: (Control), this group was further divided into 3 subgroups. The animals here had access to enough feed and water daily but were not given any of the plant material.

Group 2: (1 ml), this group was further divided into 3 subgroups and 1 ml of the plant solution was administered for 10 days.

Group 3: (2 ml), this group was further divided into 3 subgroups and 2 ml of the plant solution was administered for 10 days.

Group 4: (3 ml) this group was further divided into 3 subgroups and 3 ml of the plant solution was administered for 10 days.

2.3 Collection of Blood Samples

After 3 days, the first sets of rats were sacrificed taking 3 rats from each of the major groups (control, 1 ml, 2 ml and 3 ml). The same procedure was carried out after 7 days and 10 days. Chloroform was used to anaesthetize the rats which were placed in a desiccator (an air tight) container and blood sample collected.

2.4 Cholesterol Determination

The plasma cholesterol levels were determined by enzymatic endpoint method. The principle of this method is that cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoeimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase [22,23,24]. Reagent kit contained 4-aminoantipyrine (0.30 mmol/l), phenol (6 mmol/l), peroxidase (≥ 0.5 μ /ml) cholesterol esterase (≥ 0.15 μ /ml), cholesterol oxidase (≥ 0.1 μ /ml), pipes buffer (80 mmol/l; pH 6.8) and standard 5.17 mmol/l (200 mg/dl) cholesterol in alcohol.

One millilitre of reagent was mixed with 10 μ l of the sample. The standard tube contained 1.00 ml of reagent and 0.01 ml of standard and 10 μ l of distilled water. The mixture was incubated for 10 minutes at 37°C. The absorbance was read against the reagent blank within 60 minutes at 546 nm with spectrophotometer (Bausch and Lomb spectronic 20 manufactured in United States of America).

Calculation:

$$\text{Cholesterol in sample} = \frac{(\text{Change in absorbance of sample} \times \text{Conc. of Standard})}{\text{Change in absorbance of standard}}$$

Triglycerides levels were determined by enzymatic colorimetric test with lipid clearing factor (LCF). The principle of this method was that the triglycerides were determined after enzymatic hydrolysis with lipases. Indicator was quinoneimine formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the

catalytic action of peroxidase [25,26]. Reagent kit contained buffer (pH 7.5) (50 mmol/l), 4-chlorophenol (5 mmol/l), 4 – aminoantipyrine (0.25 mmol/l), magnesium ions (4.5 mmol/l), ATP (2 mmol/l), lipases ($\geq 1.5 \mu\text{ml}$), glycerol – 3-phosphate oxidase ($\geq 1.5 \mu\text{ml}$) and 3 ml standard (200 mmol/l).

To 10 μl of the sample was added 1000 μl of the reagent and mixed. The standard tube contained 1000 μl of reagent and 10 μl of the standard. The blank tube had 1000 μl of reagent. The mixtures were incubated for 5 minutes at 37°C. The absorbance of the samples were read against the reagent blank within 60 minutes at 546 nm with spectronic 20 spectrophotometer.

Calculation:

Triglyceride in sample = (Change in absorbance of sample X 2.28 mol/l) / Change in absorbance of Standard.

2.5 Sodium Determination

Sodium levels were determined by colorimetric test. Magnesium-uranyl acetate method. The Principle of this method is that after the precipitation of sodium magnesiumuranyl acetate, in the supernatant form with uranyl ions in solution with thioglycolic acid a yellow-brown coloured complex is formed. The optical density difference between the reagent blank (without precipitation of sodium) and the result of the analysis is proportional to the sodium concentration [22]. Reagent A kit contained uranylacetate (19 mM) and magnesium acetate (140 mM) while reagent B kit contained ammonium thioglycolate (550 mM), ammonia (550 mM) and the standard aqueous solution of sodium equivalent 150 mmol. 2.00 ml of reagent A was mixed with 0.02 ml of the sample. For the standard, 2.00 ml of reagent A and 0.02 ml of the standard were mixed. The mixtures were let to stand for 5 minutes, they were then shaken thoroughly for 30 seconds. The mixtures were allowed to stand for 30 minutes. They were centrifuged at 2,000 rpm for 5 minutes. The supernatant was then separated. 0.05 ml of the clear supernatant was mixed with 2.00 ml of reagent B. For the blank, 0.05 ml of reagent A and 2.00 ml of reagent B were mixed, while the standard tube contained 0.05 ml of supernatant and 2.00 ml of reagent B. The absorbance of the mixtures was read after 10 minutes at 405 nm with spectronic – 20 spectrophotometer.

Calculations:

$$\frac{(\text{Blank O.D} - \text{Sample O.D} \times 150)}{\text{Blank O.D} - \text{Standard O.D}} = \text{mmol/L}$$

2.6 Potassium Determination

Potassium levels were determined by colorimetric endpoint method [27]. One millilitre of reagent was mixed with 0.1 ml of sample except for the controls, which had no samples. The blank tube contained 1.0 ml of reagent while the standard tube contained 1.0 ml of reagent and 0.1 ml of standard. The mixtures were incubated at 25°C for 3 mins. The absorbance was read against reagent blank at 500 nm with Spectronic -20 spectrophotometer.

Calculations:

$$\frac{\Delta A_{\text{unknown}} \times C_{\text{standard}}}{\Delta A_{\text{standard}}} = \text{potassium concentration mEq/L}$$

2.7 Statistical Analysis

Data analysis was performed using the Statistical package for the Social Sciences software (SPSS, version 11.0). The statistical method of one way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups. Differences were considered significant whenever the p-value was $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The results (Tables 1 to 4) show the effect of various doses of *Sphenostylis stenocarpa* on the sodium, potassium, cholesterol and triglyceride levels in Wistar rats. This study revealed that the aqueous extract of *Sphenostylis stenocarpa* decreased the plasma cholesterol and triglyceride levels in a concentration and time dependent manner. The highest decrease of cholesterol (2.11 ± 0.01 vs control 2.20 ± 0.12 mmol/l) was obtained on the tenth day with the highest dose of 3 ml of aqueous extract of *Sphenostylis stenocarpa*. Also the highest decrease in the level of triglyceride (0.40 ± 0.01 vs control 0.46 ± 0.11 mmol/l) was obtained at day ten. This study revealed that the animals did not have significant changes in their weight when compared to the control. This suggests that African yam bean can be used for weight management. Furthermore, the decrease in the cholesterol and triglyceride levels could be attributed to the flavonoid content of the African yam bean. Uchegbu [28] in an earlier study

stipulated that *Sphenostylis stenocarpa* contained flavonoids in them. The results of this present study is in agreement with the work of Rangika et al. [29] which showed that flavonoids from boiled aqueous of flower from *Nycantus arbor – tristis* L. may augment the activity of lecithin acyl transferase which regulates blood lipids by incorporation of blood cholesterol into high density level cholesterol thus increasing high density cholesterol. Overweight and hypercholesterolemia have been implicated in

certain health conditions like cardiovascular diseases and diabetes. This result is in line with that of Onwuka et al. [30] who studied the effect of germination on the performance characteristics of AYB seed meal on Albino rats. In their study, Onyeike et al. [31] stipulated that heat processing improved feed utilisation, feed conversion, growth and organ weights of Wistar rats. This could be due to heat inactivation of toxic factors especially trypsin inhibitors [32,33,34].

Table 1. *In vivo* effect of the administration of the aqueous extract of AYB seed on sodium (Na⁺) of wistar rats

<i>Sphenostylis stenocarpa</i> (ml/100 g body wt.)	Sodium (mmol/l)		
	Day 3	Day 7	Day 10
Group 1 (Control)	134.00 ± 20.00	134.00 ± 21.10	135.00 ± 21.00
Group 2 (1 ml)	132.00 ± 22.20 ^a	131.00 ± 23.12 ^b	130.00 ± 20.11 ^c
Group 3 (2 ml)	129.00 ± 20.11 ^a	128.50 ± 21.11 ^b	127.00 ± 20.10 ^c
Group 4 (3 ml)	128.00 ± 22.12 ^a	126.00 ± 21.10 ^b	125.00 ± 20.01 ^c

Results are means of three determinations ± standard deviation
^{abc} Different letters in a given row denote significant difference, p ≤ 0.05

Table 2. *In vivo* effect of the administration of the aqueous extract of AYB seed on potassium (K⁺) of wistar rats

<i>Sphenostylis stenocarpa</i> (ml/100 g body wt.)	Potassium (mEq/l)		
	Day 3	Day 7	Day 10
Group 1 (Control)	0.45 ± 0.00	0.45 ± 0.01	0.44 ± 0.00
Group 2 (1 ml)	0.47 ± 0.02 ^a	0.48 ± 0.01 ^b	0.48 ± 0.11 ^c
Group 3 (2 ml)	0.49 ± 0.02 ^a	0.50 ± 0.01 ^b	0.51 ± 0.11 ^c
Group 4 (3 ml)	0.52 ± 0.02 ^a	0.53 ± 0.10 ^b	0.55 ± 0.10 ^c

Results are means of three determinations ± standard deviation
^{abc} Different letters in a given row denote significant difference, p ≤ 0.05

Table 3. *In vivo* effect of the administration of the aqueous extract of AYB seed on cholesterol of wistar rats

<i>Sphenostylis stenocarpa</i> (ml/100 g body wt.)	Cholesterol (mmol/l)		
	Day 3	Day 7	Day 10
Group 1 (Control)	2.21 ± 0.12	2.20 ± 0.11	2.20 ± 0.10
Group 2 (1 ml)	2.19 ± 0.12 ^a	2.16 ± 0.11 ^b	2.15 ± 0.11 ^c
Group 3 (2 ml)	2.18 ± 0.11 ^a	2.15 ± 0.11 ^b	2.13 ± 0.10 ^c
Group 4 (3 ml)	2.16 ± 0.12 ^a	2.14 ± 0.10 ^b	2.11 ± 0.01 ^c

Results are means of three determinations ± standard deviation.
^{abc} Different letters in a given row denote significant difference, p ≤ 0.05

Table 4. *In vivo* effect of the administration of the aqueous extract of AYB seed on triglycerides of wistar rats

<i>Sphenostylis stenocarpa</i> (ml/100 g body wt.)	Triglycerides (mmol/l)		
	Day 3	Day 7	Day 10
Group 1 (Control)	0.47 ± 0.11	0.46 ± 0.12	0.46 ± 0.11
Group 2 (1ml)	0.46 ± 0.17 ^a	0.45 ± 0.10 ^b	0.43 ± 0.12 ^c
Group 3 (2ml)	0.44 ± 0.12 ^a	0.42 ± 0.10 ^b	0.41 ± 0.10 ^c
Group 4 (3ml)	0.42 ± 0.12 ^a	0.41 ± 0.10 ^b	0.40 ± 0.01 ^c

Results are means of three determinations ± standard deviation.
^{abc} Different letters in a given row denote significant difference, p ≤ 0.05

This study also showed that aqueous extract of *Sphenostylis stenocarpa* did not increase the sodium levels in rat, they had a decreasing effect. On the other hand, the extract increased the potassium levels. This is good for the sodium component. Increased levels of sodium in the bloodstream affect the body balance. Thus reducing the ability of the kidneys to remove water. The result is high blood pressure due to extra fluid and extra strain on the delicate blood vessels leading to the kidneys. Excessive NaCl ingestion or NaCl retention by the kidneys and consequent tendency towards plasma volume expansion lead to hypertension [35]. Potassium lower blood pressure by balancing the negative effects of sodium. The potassium element is found primarily inside the cells of the body. Low levels in the blood may indicate severe diarrhoea, alcoholism, or excessive use of water pills. Low potassium levels can cause muscle weakness and heart problems. Potassium is the principle cation of the intracellular fluid. It is also an important constituent of the extracellular fluid due to its influence on muscle activity. Its intracellular function parallels that of its extracellular function, namely influencing acid-base balance and osmotic pressure, including water retention [36]. Elevated potassium levels (hyperkalemia) are often associated with renal failure, dehydration, shock or adrenalin insufficiency. Decreased potassium levels (hypokalemia) are associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex [37].

4. CONCLUSION

The lowering effect on the lipids studied and the decrease in Na⁺ and the subsequent increase in K⁺ may be contributing to the reduction in high blood pressure in the individuals taking this aqueous extract of *Sphenostylis stenocarpa*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This research work was carried out with the approval of the University of Port Harcourt research ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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