

RAT SERUM ELECTROLYTE AND LIPID PROFILE FOLLOWING ADMINISTRATION OF AQUEOUS EXTRACT OF *SPHENOSTYLIS STENOCARPA*

ABSTRACT

Aim: Aqueous extract of the seed of African yam bean (*Sphenostylis stenocarpa*) is used in Nigerian traditional 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 parameters 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 *sphenostylisstenocarpa*, 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 decrease (p=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 experimental animals showed a significant increase. The study also showed that the aqueous extract of *sphenostylisstenocarpa* 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 100 mg of aqueous extract of *Sphenostylisstenocarpa*. 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.

Conclusion: The lowering effect on the lipids 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.

Key words: Cholesterol, Electrolytes, Lipids, Potassium, Sodium, *Sphenostylisstenocarpa*, Triglycerides.

1. INTRODUCTION

African yam bean (AYB) (*Sphenostylisstenocarpa*) is an herbaceous leguminous plant occurring 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 *Sphenostylisstenocarpa* 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 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 like that of some major and commonly consumed legumes. Its amino acid profile is comparable if not better than those 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 noncommunicable 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 marred 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].

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

67 Triglyceride is an ester derived from glycerol and three fatty acids. As a blood lipid, it helps
68 enable the bidirectional transfer of adipose fat and blood glucose from the liver. There are
69 many triglycerides: depending on the oil source, some are highly unsaturated, some less so
70 [20].

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

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

81 **2. MATERIALS AND METHOD**

82 **2.1 Collection and identification of plant material**

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

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

90

91 2.2 Experimental animals

92 A total of 36 wistar rats with average weight of 100g were used for the purpose of this
 93 research. The rats were arranged in 4 groups and these groups had 3 subgroups containing 3
 94 rats each. The groups were labelled according to the dose of extract (of the boiled AYB)
 95 administered orally to the rats daily.

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

98 **GROUP 2** (1ml), this group was further divided into 3 subgroups and 1ml of the plant
 99 solution was administered at days 3, 7 and 10 days respectively.

100 **GROUP 3** (2ml), this group was further divided into 3 subgroups and 2ml of the plant
 101 solution was administered at days 3, 7 and 10 days respectively.

102 **GROUP 4** (3ml) this group was further divided into 3 subgroups and 3ml of the plant
 103 solution was administered at days 3, 7 and 10 days respectively.

104

105 2.3 Collection of blood samples

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

110

111 2.4 Cholesterol determination

112 The plasma cholesterol levels were determined by enzymatic endpoint method. The principle
113 of this method is that cholesterol is determined after enzymatic hydrolysis and oxidation.
114 The indicator quinoeimine is formed from hydrogen peroxide and 4-aminoantipyrine in the
115 presence of phenol and peroxidase [22, 23, 24]. Reagent kit contained 4-aminoantipyrine
116 (0.30mmol/l), phenol (6mmol/l), peroxidase ($\geq 0.5 \mu\text{ml}$) cholesterol esterase ($\geq 0.15 \mu\text{ml}$),
117 cholesterol oxidase ($\geq 0.1 \mu\text{ml}$), pipes buffer (80mmol/l; pH 6.8) and standard 5.17mmol/l
118 (200mg/dl).

119 One millilitre of reagent was mixed with 10 μl of the sample. The standard tube contained
120 1.00ml of reagent and 0.01ml of reagent and 10 μl of distilled water. The mixture was
121 incubated for 10 minutes at 37°C. The absorbance was read against the reagent blank within
122 60 minutes at 546nm with spectrophotometer (spectronic 20).

123 Calculation:

$$\begin{aligned} 124 \quad & \text{Cholesterol in sample} = \frac{\text{Change in absorbance of sample}}{\text{Change in absorbance of Standard}} \times \text{Conc. of Standard} \\ 125 \quad & \\ 126 \quad & \\ 127 \quad & \end{aligned}$$

128

129 Triglycerides levels were determined by enzymatic colorimetric test with lipid clearing factor
130 (LCF). The principle of this method was that the triglycerides were determined after
131 enzymatic hydrolysis with lipases. Indicator was quinoneimine formed from hydrogen
132 peroxide, 4 –aminoantipyrine and 4-chlorophenol under the catalytic action of peroxidase
133 [25, 26]. Reagent kit contained buffer (pH 7.5) (50mmol/l), 4-chlorophenol (5mmol/l), 4 –
134 aminoantipyrine (0.25mmol/l), magnesium ions (4.5 mmol/l), ATP (2mmol/l), lipases (≥ 1.5
135 μml), glycerol – 3- phosphate oxidase ($\geq 1.5 \mu\text{ml}$) and 3 ml standard (200 mmol/l).
136 To 10 μl of the sample was added 1000 μl of the reagent and mixed. The standard tube
137 contained 1000 μl of reagent and 10 μl of the standard. The blank tube had 1000 μl of reagent.

138 The mixtures were incubated for 5 minutes at 37 °C. The absorbance of the samples were
139 read against the reagent blank within 60 minutes at 546nm with spectronic 20
140 spectrophotometer.

141

142 Calculation:

$$\begin{aligned} 143 \quad \text{Triglyceride in sample} &= \frac{\text{Change in absorbance of sample}}{\text{Change in absorbance of Standard}} \times 2.28\text{mol/l} \\ 144 & \\ 145 & \\ 146 & \end{aligned}$$

147 **2.5 Sodium determination**

148 Sodium levels were determined by colorimetric test. Magnesium-uranyl acetate method. The
149 Principle of this method is that after the precipitation of sodium magnesiumuranyl acetate, in
150 the supernatant form with uranyl ions in solution with thioglycolic acid a yellow-brown
151 coloured complex is formed. The optical density difference between the reagent blank
152 (without precipitation of sodium) and the result of the analysis is proportional to the sodium
153 concentration [22]. Reagent A kit contained uranylacetate (19mM) and magnesium acetate
154 (140mM) while reagent B kit contained ammonium thioglycolate (550mM), ammonia
155 (550mM) and the standard aqueous solution of sodium equivalent 150mmol. 2.00ml of reagent
156 A was mixed with 0.02 ml of the sample. For the standard, 2.00 ml of reagent A and 0.02 ml
157 of the standard were mixed. The mixtures were let to stand for 5 minutes, they were then
158 shaken thoroughly for 30 seconds. The mixtures were allowed to stand for 30 minutes. They
159 were centrifuged at 2,000rpm for 5 minutes. The supernatant was then separated. 0.05ml of
160 the clear supernatant was mixed with 2.00ml of reagent B. For the blank, 0.05 ml of reagent
161 A and 2.00 ml of reagent B were mixed, while the standard tube contained 0.05 ml of
162 supernatant and 2.00ml of reagent B. The absorbance of the mixtures was read after 10
163 minutes at 405nm with spectronic – 20 spectrophotometer.

164 Calculations: $\frac{\text{Blank O.D.} - \text{Sample O.D.}}{\text{Blank O.D.} - \text{Standard O.D.}} \times 150 = \text{mmol/L}$

165 Blank O.D – Standard O.D

166 2.6 Potassium determination

167 Potassium levels were determined by colorimetric endpoint method. The Principle of this
 168 method is that the amount of potassium is determined by using sodium tetraphenylboron
 169 (2.1mmol/l) in a specifically prepared mixture to produce a potassium concentration in the
 170 range of 2 – 7 mEq/L [27]. One millilitre of reagent was mixed with 0.1ml of sample except
 171 for the controls, which had no samples. The blank tube contained 1.0ml of reagent while the
 172 standard tube contained 1.0ml of reagent and 0.1ml of standard. The mixtures were incubated
 173 at 25oC for 3mins. The absorbance was read against reagent blank at 500nm with Spectronic
 174 -20 spectrophotometer.

175

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

177 $\Delta A_{\text{standard}}$

178

179 2.7 STATISTICAL ANALYSIS

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

184 3. RESULTS

185

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

<i>Sphenostylis sternocarpa</i> (ml/100g 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 (1ml)	132.00 ± 22.20 ^a	131.00 ± 23.12 ^b	130.00 ± 20.11 ^c
Group 3 (2ml)	129.00 ± 20.11 ^a	128.50 ± 21.11 ^b	127.00 ± 20.10 ^c
Group 4 (3ml)	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 sternocarpa</i> (ml/100g 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 (1ml)	0.47 ± 0.02 ^a	0.48 ± 0.01 ^b	0.48 ± 0.11 ^c
Group 3 (2ml)	0.49 ± 0.02 ^a	0.50 ± 0.01 ^b	0.51 ± 0.11 ^c
Group 4 (3ml)	0.52 ± 0.02 ^a	0.53 ± 0.10 ^b	0.55 ± 0.10 ^c

Results are means of three determinations ± standard deviation.

194 ^{abc} Different letters in a given row denote significant difference, p=0.05.

195

196 **Table 3:** *In vivo* effect of the administration of the aqueous extract of AYB Seed on
197 cholesterol of wistar rats

<i>Sphenostylis sternocarpa</i> (ml/100g 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 (1ml)	2.19± 0.12 ^a	2.16± 0.11 ^b	2.15± 0.11 ^c
Group 3 (2ml)	2.18± 0.11 ^a	2.15± 0.11 ^b	2.13± 0.10 ^c
Group 4 (3ml)	2.16± 0.12 ^a	2.14± 0.10 ^b	2.11± 0.01 ^c

198 Results are means of three determinations ± standard deviation.

199 ^{abc} Different letters in a given row denote significant difference, p=0.05.

200 **Table 4:** *In vivo* effect of the administration of the aqueous extract of AYB Seed on
201 triglycerides of wistar rats

<i>Sphenostylis sternocarpa</i> (ml/100g 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

202 Results are means of three determinations $\pm$ standard deviation.

203 ^{abc} Different letters in a given row denote significant difference, $p=0.05$.

204 5. DISCUSSION

205 The results (Tables 1 to 4) show the effect of various doses of *Sphenostylisstenocarpa* on the
 206 sodium, potassium, cholesterol and triglyceride levels in wistar rats. This study revealed that
 207 the aqueous extract of *Sphenostylisstenocarpa* decreased the plasma cholesterol and
 208 triglyceride levels in a concentration and time dependent manner. The highest decrease of
 209 cholesterol (2.11 ± 0.01 vs control 2.20 ± 0.12 mmol/l) was obtained on the tenth day with
 210 the highest dose of 3ml of aqueous extract of *Sphenostylisstenocarpa*. Also the highest
 211 decrease in the level of triglyceride (0.40 ± 0.01 vs control 0.46 ± 0.11 mmol/l) was obtained
 212 at day ten. This study revealed that the animals did not have significant changes in their
 213 weight when compared to the control. Furthermore, the decrease in the cholesterol and
 214 triglyceride levels could be attributed to the anti-nutritional factors and low fat content of
 215 African yam bean. These factors interfere with nutrient intake and lower plasma cholesterol.
 216 This suggests that African yam bean can be used for weight management. Overweight and
 217 hypercholesterolemia have been implicated in certain health conditions like cardiovascular
 218 diseases and diabetes. This result is in line with that of Onwuka *et al.*, [28] who studied the
 219 effect of germination on the performance characteristics of AYB seed meal on Albino rats. In
 220 their study, Onyeike *et al.*, [29] stipulated that heat processing improved feed utilisation, feed
 221 conversion, growth and organ weights of wistar rats. This could be due to heat inactivation of
 222 toxic factors especially trypsin inhibitors [30,31,32].

223 This study also showed that aqueous extract of *Sphenostylisstenocarpa* did not increase the
 224 sodium levels in rat, they had a decreasing effect. On the other hand, the extract increased
 225 the potassium levels. This is good for the sodium component. Increased levels of sodium in
 226 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 [33]. 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 [34]. 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 [35].

6. 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 *sphenostylisstenocarpa*.

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