

RAT SERUM LIVER AND KIDNEY PROFILE FOLLOWING *SPHENOSTYLIS* *STENOCARPA* SEED AQUEOUS EXTRACT ADMINISTRATION

ABSTRACT

Aim: Aqueous extract of the seed of African Yam Bean (*Sphenostylis stenocarpa*) is used in Nigerian alternative medicine for the treatment of hypertension. The objective of this study is to investigate the effect of *sphenostylisstenocarpa* seed extract on the liver and kidney functions of wistar rats. The biochemical parameters assayed were alkaline phosphatase (ALP), lactate dehydrogenase (LDH), urea and creatinine.

Materials and methods: Varying doses of the aqueous extract of the *sphenostylisstenocarpa*, were administered to the rats for a period of ten days and the biochemical parameters; ALP, LDH, urea and creatinine levels were determined using spectrophotometric methods.

Results: There was significant decrease $p=0.05$ in the urea concentration in the serum. The highest decrease of 0.70 ± 0.08 vs control 0.97 ± 0.00 mmol/l was obtained after the 10 days duration at the highest dose (3ml/100g body weight). Additionally, the creatinine concentration in the serum of the experimental animals also showed a significant decrease. The highest decrease of 81 ± 1.63 vs control 110 ± 0.00 μ mol/l was obtained after the 10 days period at the highest dose (3ml/100g body weight). The study also showed that the aqueous extract of *sphenostylisstenocarpa* had no significant effect on the ALP and LDH activities of the experimental rats.

Conclusion: The decrease on the urea and creatinine levels may be contributing to the reduction in high blood pressure in the individuals taking this tea extract. Furthermore, the study showed that the aqueous extract had no harmful effects on the liver profile of the rats.

Key words: Alkaline phosphatase, Creatinine, Lactate dehydrogenase, *Sphenostylisstenocarpa*, Triglycerides, Urea.

1. INTRODUCTION

African yam bean (AYB) *Sphenostylisstenocarpa*, known locally in Igbo as uzaaku, is a climbing legume adapted to lowland tropical conditions. It is one of the lesser-known legumes [1,2] and widely cultivated in the southern parts of Nigeria. The water drained after boiling the seeds of *Sphenostylisstenocarpa* have been used 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. The potential role of AYB in the management of many aging and chronic noncommunicable diseases has been reported [3]. In Ghana, the water drained after boiling may be drunk by lactating mothers to increase their milk production [4]. The economic potential of AYB has been recognized, especially in reducing malnutrition among Africans [5]. These health benefits can be marred by the

38 presence of anti- nutrients. Some processing methods however, such as soaking, boiling,
39 fermentation, roasting, among others are known to achieve reduction or elimination of the
40 anti-nutritional factors which affect the nutrients [5,6,7,8,9,10,11].The legumes are a good
41 source of dietary protein [12]. They are cheaper than animal products such as meat, fish,
42 poultry and egg, therefore they are consumed worldwide as a major source of cheap protein
43 and especially in the developing or poor countries where consumption of animal protein may
44 be limited as a result of economic, social, cultural or religious factors [13]. Global food
45 security however is becoming shaky with increasing dependence on a few major staple crops.
46 This has resulted in an alarming reduction not only in crop diversity but also in the variability
47 within crops. This therefore emphasizes the need for the collection and conservation of
48 diversity within species. Usually when this done, there is the possibility for stockpiling crops
49 of converse characteristics - for example, stockpiling low yielding varieties with high
50 yielding ones.

51 AYB is cultivated both for the seeds and tubers, because of its valuable and prominent source
52 of plant protein (amino acids –lysine and methionine) in the diets of consumers [14]. The
53 tuber which is found beneath the ground has resemblance with sweet potatoes or Irish
54 potatoes. Above ground it produces good yields of edible seeds. It could be found in forests,
55 open wooded grasslands, rocky fields, and marshy grounds as weed and cultivated crops [15].
56 It grows on a wide range of soils including acid and highly leached sandy soils at altitudes
57 from sea level to 1950m [16]. In the area of cultivation, it serves as security crop prior to the
58 new season harvest of main staples such as maize, sorghum, yam and cassava in the tropics.
59 Different studies on nutritional, chemical composition, and extraction condition for optimum
60 solubilization of the pulse protein have been studied [17,18,19]. African yam beans (*S.*
61 *stenocarpa*) however has anti-nutritional factors such as phytin and lectin.

62 It is of great importance to know the nutrient, toxic substance as well as the anti-
63 physiological substance composition and organoleptic properties of locally available foods in
64 any community or country. Knowledge and use of local foods can help eliminate
65 malnutrition. One of the problems of planning therapeutic diets with local foods is limited
66 information on their nutrient composition [20]. It has been proposed that the fight against
67 malnutrition in developing countries should be on the use of mixtures of tubers, cereals and
68 legumes indigenous to them [21]. Urbanization has made people forget their traditional foods
69 and favour convenient foods which are mostly nutritionally inadequate and expensive. The
70 most dietary deficit is protein of high biological value and this was attributed to the high cost

71 of animal protein [20]. Vegetable proteins however complement each other if well-chosen
72 and will have a nutritive value as good as animal protein [2,13,21].

73 For quite some time, legumes were considered not too important; but now, their food use is
74 increasing with recent discoveries concerning their many nutritional and health properties
75 [20]. It has been documented that legumes contain 2-3 times the protein of cereal grains and
76 no other plant food is as rich in protein as legumes in their natural state [2,20,21]. Water
77 soluble nonstarch polysaccharides (NSP) that have viscous properties occur mostly in
78 legumes and its benefit in the prevention/management of diabetes and cardiovascular diseases
79 have been reported [22]. One such legume of interests is African yam bean
80 (*Sphenostylisstenocarpa*) (AYB).

81 African yam bean (AYB) is highly nutritious with high protein, mineral and fiber content. Its
82 protein content is reported to be like that of some major and commonly consumed legumes.
83 Its amino acid profile is comparable if not better than those of cowpea, soy bean and pigeon
84 pea [1, 17, 23]. It has high metabolic energy, low true protein digestibility (62.9%), moderate
85 mineral content, the amino and fatty acids contents are comparable to those of most edible
86 pulses [17,24,25]. It has a higher water absorption capacity when compared to cowpea [20].
87 The alkaline phosphatases are a group of enzymes that hydrolyze organic phosphates at high
88 pH. They are present in most tissues, but are in particularly high concentrations in the
89 osteoblasts of bones and the cells of the hepatobiliary tract, intestinal wall, renal tubules and
90 placenta. The primary importance of measuring alkaline phosphatase is to check the
91 possibility of bone disease or liver disease. Thus, the serum alkaline phosphatase is a measure
92 of the hepatobiliary system and the flow of bile into the small intestine [26]. The main
93 function of LDH is the conversion of pyruvic acid, the final product of glycolysis, to lactic
94 acid and vice versa in muscle through production of cellular energy [26]. It is an enzyme that
95 helps facilitate the process of turning sugar into energy for cells to use. However, it is
96 normally present in small amounts in most active organs. Thus, high level of this enzyme
97 may indicate unusual conditions that can result from liver disease, muscular disorder (e.g.
98 acute myocardial infarction) and even cancer disease [26]. Urea is synthesized in the body of
99 many organisms as part of the urea cycle, either from the oxidation of amino acids or from
100 ammonia. Amino acids from ingested food that are not used for the synthesis of proteins and
101 other biological substances are oxidized by the body, yielding urea and carbon dioxide, as an
102 alternative source of energy [27]. The oxidation pathway starts with the removal of the amino

group by a transaminase; the amino group is then fed into the urea cycle. Creatinine is a protein breakdown product and its level is a reflection of the bodies muscle mass. Creatinine is a metabolite of creatine. Production is endogenous, determined by muscle mass, unlike urea is almost independent of diet. It increases in renal failure [26,28,29].

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. So the aim of this study, is to investigate the effects of this aqueous extract of *sphenostylisstenocarpa* on the liver enzymes and also on the urea and creatinine levels using wistar rat as the experimental animal.

2. MATERIALS AND METHOD

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 (*Sphenostylisstenocarpa*) 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 was prepared by boiling 10 grams in 200ml of water for an hour. The method of administration was forced feeding and this was done twice daily.

2.2 Experimental animals

A total of 36 wistar rats were used for the purpose of this research. 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 (1ml), this group was further divided into 3 subgroups and 1ml of the plant solution was administered at days 3, 7 and 10 days respectively.

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

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

2.3 Collection of blood samples

After 3 days, the first set of rats were sacrificed taking 3 rats from each of the major groups (control, 1ml, 2ml and 3ml). 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 Alkaline phosphatase determination

Reagent kit contained 1: Buffer Diethanolamine buffer (1mol/l,pH 9.8), $MgCl_2$ 0.5mmol/l. 2: Substrate p-nitrophenylphosphate (10 mmol).

ALP levels were determined by colorimetric method. 0.05ml of sample was mixed with 3.00ml of reagent in a 1cm light path cuvette. The absorbance of the mixture was read against air at 37°C at 405nm using spectronic - 20 spectrophotometer. A timer was started simultaneously and the absorbance of the mixture was read again after 1, 2 and 3 minutes.

Calculation:

The ALP activity was calculated using the following formulae

$$IU/L = 3300 \times \Delta A_{405nm}/min$$

2.5 Lactate dehydrogenase analysis:

Reagent kit contained: R1a: Buffer/substrate: Phosphate buffer (50mmol/l, pH 9.8), Pyruvate (0.6mmol/l), R1b: NADH (0.18mmol/l).

LDH levels were determined by UV kinetic method. 0.05ml of sample was mixed with 3.00ml of reagent in a 1cm light path cuvette. The absorbance of the mixture was read against air at 37°C at 340nm using spectronic - 20 spectrophotometer. A timer was started simultaneously and the absorbance of the mixture was read again after 1, 2 and 3 minutes

2.6 Estimation of urea using Diacetylmonoxime method [30].

Principle of Method: Urea in the presence of strong acid and a reducing agent reacts with Diacetylmonoxime to give a pink coloured compound, which was measured at 520nm. A serum sample free of haemolysis was used.

Reagent and standards:

(A) Acid Solution; Conc. Sulphuric acid, Conc. Ortho phosphoric acid and Ferric chloride.

(B) Colour Solution; Diacetylmonoxime, Sodium chloride, and 4-aminoantipyrine.

(C) Standard; A 60mmol/l standard serially diluted to 3, 6, 15, 30 and 45mmol/l.

Procedure 1: For Blood urea.

	STD	TEST	QC	BLANK
Reagent A	2.5ml	2.5ml	2.5ml	2.5ml
Reagent B	2.5ml	2.5ml	2.5ml	2.5ml
Standards	50u/l			
Sample		50u/l		
QC			50u/l	

Absorbance was read at 520nm with Spectronic – 20 spectrophotometer.

CALCULATIONS:

$$\text{Concentration of Sample} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = \text{mg/dL urea} \times 0.1665 = \text{mmol/l}$$

Reference Range for Blood urea nitrogen: 2.5 – 6.6mmol/l.

2.7 Creatinine determination

Creatinine levels were determined by colorimetric method (with deproteinization)

The principle of this method is that creatinine in alkaline solution reacts with picrate to form a coloured complex [31,32,33].

The Reagent kit contained solution 1: Standard (177umol/l), solution 2: Picric acid (35 mmol/l), solution 3: Sodium hydroxide (1.6 mol/l), TA 651 Trichloroacetic acid (TCA)

(1.2mol/l). The Working reagent was prepared by mixing 10ml of solution 2 and 10ml of solution 3.

The sample was first deproteinized by mixing 1.0ml of Trichloroacetic acid (TCA) and 1.0ml of sample. The mixture was vigorously stirred with a glass rod to evenly disperse the precipitate. The mixture was then centrifuged at 2500 rpm for 10 minutes, the supernatant was then separated and used for the assay as listed below:

1.00ml of the working reagent was mixed with 1.00ml of the supernatant. The standard tube contained 1.00ml of the working reagent, 0.5ml of TCA and 0.5 ml of solution 1. The blank tube had 1.00ml of working reagent, 0.5ml of TCA and 0.5ml of distilled water. The mixture was let to stand for 20 minutes at 25°C and the absorbance of the sample and standard were read against the blank at 520nm with Spectronic -20 spectrophotometer.

Calculations

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 177 = \text{umol/l}$$

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

Normal values: 44 – 80 umol/l

2.8Statistical analysis

All data are expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism version 7.0. The data was analyzed using one-way analysis of variance (ANOVA) followed by post Hoc Turkey's multiple comparison tests, p = 0.05 were considered as significant.

3. RESULTS

211 **Table 1:***In vivo* effect of the Administration of the Aqueous Extract of AYB Seed on ALP of
212 wistar rats

<i>Sphenostylissternocarpa</i> conc. ml/100g body wt.	Day 3 (IU/L)	Day 7 (IU/L)	Day 10 (IU/L)
Group 1 (Control)	22.73 ± 2.66	19.20 ± 1.50	20.12 ± 2.27
Group 2 (1ml)	22.34 ± 2.01	23.59 ± 1.67	24.56 ± 2.42
Group 3 (2ml)	23.12 ± 1.79	24.77 ± 2.46	25.00 ± 1.97
Group 4 (3ml)	24.21 ± 2.49	24.67 ± 2.72	25.40 ± 1.25

213 No significant difference was observed at p= 0.05 for ALP at different concentrations.

214 Values are shown as Mean ± Standard deviation

215

216 **Table 2:***In vivo* effect of the Administration of the Aqueous Extract of AYB Seed on LDH of
217 wistar rats

<i>Sphenostylissternocarpa</i> conc. ml/100g body wt.	Day 3 (IU/L)	Day7 (IU/L)	Day 10 (IU/L)
Group 1 (Control)	18.47 ± 1.45	19.18 ± 1.18	15.46 ± 1.02
Group 2 (1ml)	19.71 ± 1.95	20.44 ± 7.25	20.87 ± 1.91
Group 3 (2ml)	19.78 ± 3.81	19.86 ± 4.04	19.67 ± 1.58
Group 4 (3ml)	19.26 ± 3.62	20.58 ± 1.94	20.45 ± 2.80

218 No significant difference was observed at p=0.05 for LDH at different concentrations.

219 Values are shown as Mean ± Standard deviation

220

221 **Table 3:***In vivo* effect of the Administration of the Aqueous Extract of AYB Seed on Urea of
222 wistar rats

<i>Sphenostylissternoca</i> <i>rpa</i> conc. ml/100g body wt.	Urea (mmol/l)		
	Day 3	Day 7	Day 10
Group 1 (Control)	0.97± 0.00	0.95± 0.15	0.94± 0.00
Group 2 (1ml)	0.87± 0.18 ^a	0.83± 0.48 ^b	0.81± 0.18 ^c
Group 3 (2ml)	0.80± 0.18 ^a	0.78± 0.10 ^b	0.72± 0.08 ^c
Group 4 (3ml)	0.75± 0.18 ^a	0.71± 0.10 ^b	0.70± 0.08 ^c

223 Results are means of three determinations ± standard deviation.

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

225

226 **Table 4:***In vivo* effect of the Administration of the Aqueous Extract of AYB Seed on
227 creatinine of wistar rats

<i>Sphenostylissternoca</i> <i>rpa</i> conc. ml/100g body wt.	Creatinine (μmol/l)		
	Day 3	Day 7	Day 10
Group 1 (Control)	102± 0.00	103± 0.01	110± 0.00
Group 2 (1ml)	98± 6.83 ^a	95± 3.12 ^b	90± 2.95 ^c
Group 3 (2ml)	88± 4.50 ^a	85± 1.58 ^b	83± 1.63 ^c
Group 4	87± 4.50 ^a	84± 1.58 ^b	81± 1.63 ^c

(3ml)			
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Results are means of three determinations $\pm$ standard deviation.

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

4. DISCUSSION

There have been a lot of research work carried out on the medicinal aspects of *Sphenostylisstenocarpa*. However, not much have been done on the area of using the aqueous extract of the seed to reduce the levels of high blood pressure in humans. Although there have been claims from individuals practising alternative medicine on the potential benefits of the aqueous extracts of this plant. There is no documented scientific evidence to support this very claim. It is hoped that the present research work will be of help in this case. There are immense nutritional potentials and possibilities in neglected and underutilized crop species. African yam bean (*Sphenostylisstenocarpa*), a tropical legume is one of such species with duo-food products [8]. Apart from the nutritional potentials of this crop, there lies a lot of other health benefits that can be derived from this plant. The result of the serum alkaline phosphatase and lactate dehydrogenase levels followed a similar pattern. There was a non-significant ($p=0.05$) increase in the levels of these parameters in the test groups compared to their levels in the Control. The discrepancies in significance levels can however be attributed to the differences in experiment duration and differences in the quantity/concentration of the extract fed to the experimental animals. The increase in serum alkaline phosphatase level could be attributed to necrosis of some organs. Necrosis is because of intrahepatic cholestasis in which bile secretion from the hepatocytes into the canaliculi is impaired due to toxins. When this happens, there is an increased synthesis of alkaline phosphatase in the affected duct and this increases the level of the enzyme in the plasma [34]. African yam bean has been shown to have some anti-nutritional factors which may be responsible for these observations. These anti-nutritional factors can be reduced by using efficient processing techniques and proper cooking [35]. Good processing ensures safe consumption of African yam bean meals by human and livestock. The result obtained by Anosike *et al.*, [36] showed a decrease in alkaline phosphatase activity of wistar rats fed with soybean supplemented diet. Alkaline phosphatase is found mainly in the bile ducts of the liver. There can be high levels of the ALP enzyme found with some types of liver and bone disease. High levels may indicate there is a problem with bile flow.

258 Lactate dehydrogenase levels also showed a non-significant increase

259 In cardiology, the measurement of lactate dehydrogenase and lactate dehydrogenase
 260 isoenzymes is a good method for the late detection (>36—48 hours after the first onset of
 261 complaints) of a myocardial infarction. The lactate dehydrogenase-1 /lactate dehydrogenase-2
 262 ratio with a diagnostic efficiency of 93%—98% gives the best information. For an early
 263 diagnosis, the determination of creatine kinase MB is superior, particularly in its specificity,
 264 to the lactate dehydrogenase and lactate dehydrogenase isoenzyme determination. To monitor
 265 non-cancer disease in the liver neither lactate dehydrogenase nor its isoenzymes make a real
 266 contribution to the diagnostic process. Ample alternatives with higher sensitivity and
 267 specificity exist. Although little has been published on the use of lactate dehydrogenase and
 268 lactate dehydrogenase isoenzymes in liver malignancies, the enzyme does not emerge as the
 269 method of choice in this field of clinical enzymology at all. In hematology lactate
 270 dehydrogenase is of little diagnostic value. It makes a substantial contribution only in
 271 megaloblastic- and hemolytic anemia. However, lactate dehydrogenase-3 isoenzyme seems to
 272 be a useful marker for detecting chronic myeloid leukemia and monitoring changes of active
 273 disease into remission. Prognostic value could be attributed to lactate dehydrogenase for
 274 Hodgkin's disease and non-Hodgkin's lymphoma survival; duration and rate decrease with a
 275 higher pre-treatment lactate dehydrogenase activity [31,32,34].

276 The study results also showed that the urea and creatinine levels when compared with the
 277 control decreased significantly in a concentration and time dependent manner throughout the
 278 experimental period. For plasma urea, the highest decrease of 0.70 ± 0.08 vs control $0.97 \pm$
 279 0.00 mmol/l was obtained after the 10 days duration at the highest dose (3ml/100g body
 280 weight). For plasma creatinine, the highest decrease of 81 ± 1.63 vs control 110 ± 0.00 umol/l
 281 was obtained after the 10 days period at the highest dose (3ml/100g body weight). Some of
 282 the biochemical indices evaluated in this study are useful parameters to indicate impairment
 283 in functional capacities of the renal organs. Blood Urea Nitrogen is a waste product derived
 284 from protein breakdown in the liver. Elevated levels of plasma urea can be caused by
 285 excessive protein intake, certain drugs and low fluid intake and kidney damage. The clinical
 286 importance of the urea level in plasma is normally determined in conjunction with the plasma
 287 creatinine level [27]. Creatinine is measured primarily to assess kidney function and has
 288 certain advantages over the measurement of urea. The plasma levels of creatinine is
 289 relatively independent of protein ingestion, water intake, rate of urine production and

exercise. Since its rate of production is constant, elevation of plasma creatinine is indicative of under excretion, suggesting kidney impairment. Very low levels of plasma creatinine are rare and not clinically significant [29].

The significant decrease in the values of plasma urea and creatinine levels in table 3 and 4, point to the fact that the entire renal functions are not compromised following the administration of the aqueous extract of *sphenostylisstenocarpa*. The decrease on the urea and creatinine levels may be contributing to the reduction in high blood pressure in the individuals taking this tea extract. Furthermore, the study showed that the aqueous extract had no harmful effects on the liver profile of the rats.

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