Int. J. Plant Bas. Pharm. (2025) 5(1), 16-24

INTERNATIONAL JOURNAL OF PLANT BASED PHARMACEUTICALS
https://ijpbp.com

RESEARCH ARTICLE

OPEN ACCESS

Biochemical and histological evaluation of kidney, liver, and hematological indices in normal Wistar rats administered dietary formulations of roasted *Sphenotylis stenocarpa* seeds (African yam bean)

Nene Hephzibah Chiaka-Onyemeze[a], Chinelo Chinenye Nkwocha[a], Affiong Asuquo Edeke[a], Emmanuel Chimeh Ezeako[a*]

[a] *University of Nigeria, Department of Biochemistry, Nsukka, 410001, Enugu, Nigeria*

ARTICLE INFO

Article History:

Received: 04 October 2024
Revised: 28 December 2024
Accepted: 30 December 2024
Available online: 03 January 2025

Edited by: B. Tepe

Keywords:

Sphenostylis stenocarpa
Nephroprotective
Hepatoprotective
Antioxidant
Cardioprotective

ABSTRACT

Sphenotylis stenocarpa seeds (African yam bean) represent one of the under-exploited nutrient-rich legumes associated with African folklore and disease-remedy claims, that have not been fully authenticated scientifically. The wellness enhancement effect of roasted pulverized *S. stenocarpa* (PROSS) diet formulations on liver, renal, and hematological indices was investigated. Rats were assigned randomly into five groups ($n = 5$). Group I received standard rat chow. Groups II-V received a diet of 10%, 20%, 30%, and 40% PROSS mixed with standard rat chow to make 100 mg/kg body weight, respectively. After a five-week regular feeding regimen, animals were humanly sacrificed, and biochemical and histological indices were determined. Groups administered various diet formulations of PROSS showed a significant ($p < 0.05$) increase in catalase, superoxide dismutase, and glutathione peroxide activity and a decrease ($p < 0.05$) in MDA level compared to normal control. Although serum creatinine, total protein, and potassium levels did not differ significantly ($p > 0.05$) across the groups, however, the chloride level increased significantly ($p < 0.05$), compared to the control. Furthermore, sodium ion level decreased at low doses (10% and 20% PROSS) ($p < 0.05$) but increased at higher doses ($p < 0.05$), while serum urea level decreased with an increase in dosage ($p < 0.05$). A significant increase in HDL level ($p < 0.05$) and a decrease in TAG, LDL, and VLDL levels ($p < 0.05$) were also recorded. Also, ALP, ALT, and AST activity in the serum decreased across the groups ($p < 0.05$), while RBC, WBC, Hb, and hematocrit (Ht) levels were elevated as the dose increased. Normal organ architectures were observed in all the groups. Our data suggest that moderate consumption of PROSS enhances hepatic and renal well-being.

Reviewed by:
Akanchha Mani Tripathi: Institute of Nuclear Medicine and Allied Sciences, Defence Research & Development Organization, Delhi, India
Hager Tarek H. Ismail: Zagazig University, Zagazig City, Sharkia Province, Egypt
Shenxu Bao: Wuhan University of Technology, Wuhan, Hubei, China

* Corresponding author(s):
E-mail address:
emmanuel.ezeako.188647@unn.edu.ng
(E. C. Ezeako)
e-ISSN: 2791-7509
doi: https://doi.org/10.62313/ijpbp.2025.250

1. Introduction

The African community is prosperous in agricultural products, which are excellent sources of essential nutrients and supplements in an era plagued with constant food insecurity (Popoola et al., 2022). Plant-based foods remain the ultimate and irreplaceable source of micro- and macronutrients for most of the world's population (Alcorta et al., 2021). Legumes are nutrient-rich food sources that enjoy several advantages over other food sources in terms of availability, affordability, acceptability, and protein content (de Jager et al., 2019; Hughes et al., 2022; Polak et al., 2015). However, some nutrient-rich plants have remained under-exploited (Akinola et al., 2020). Sadly, in Africa (particularly in Nigeria and Ghana), some of these nutrient-rich and health-improving legumes have been underutilized and neglected (Ikhajiagbe et al., 2022; Popoola et al., 2019).

Please cite this article as: Chiaka-Onyemeze, N. H., Nkwocha, C. C., Edeke, A. A., & Ezeako, E. C. (2025). Biochemical and histological evaluation of kidney, liver, and hematological indices in normal Wistar rats administered dietary formulations of roasted *Sphenotylis stenocarpa* seeds (Af-rican yam bean). *International Journal of Plant Based Pharmaceuticals*, *5*(1), 16-24, https://doi.org/10.62313/ijpbp.2025.250.

Sphenostylis stenorcarpa seed (African yam bean) is an underutilized leguminous staple food in Africa with numerous medicinal properties, which are yet to be exploited fully for their health benefits for humans and livestock (Adewale & Nnamani, 2022; George et al., 2020). The African yam bean (AYB) is mainly distributed in the tropic regions of Africa and is usually dispersed in the southeastern area of Nigeria (Klu et al., 2000; Nnamani et al., 2017). Normally, AYBs are cultivated for edible seeds and tubers, which have high nutritional value and could be commercialized for profit-making (Gbenga-Fabusiwa, 2021). The plant tuber, which resembles Irish or sweet potatoes, usually lies beneath the ground, while the edible seeds are seen above the ground in appreciable yield (Eneh et al., 2016). The plant is usually cultivated in farmlands or found ubiquitously as a weed in grasslands (Gbenga-Fabusiwa, 2021; Klu et al., 2000).

The AYB plant has found several applications as a natural disease remedy in African folklore (George et al., 2020). Reports have shown that AYB showed ameliorative potential in managing cardiovascular diseases, anemia, hypertension, and diabetes (Adewale & Nnamani, 2022; George et al., 2020). However, the disease-preventive potential of AYB claimed in folklore has not been fully documented scientifically. Based on the premise that the plant seed is commonly consumed as a snack when roasted and represents a source of lunch for poor masses in the locales of developing countries, it became expedient to assess the nutritive and health-benefit potentials of the processed flour meal and to identify any possible toxicity on vital organs. We believe that the study will provide researchers with more insight and clarity on the knowledge gap on the medicinal properties and toxicological profile of AYB yet to be explored by researchers to optimize its utility. Therefore, this study aimed to ascertain the effect of various diet formulations of roasted *S. stenocarpa* seeds on kidney and liver function and some hematological indices in Wistar rats.

2. Materials and methods

2.1. Materials

2.1.1. Plant sample collection and identification

Fresh seeds of *S. stenocarpa* were collected from a farm land at Orba village, in Udenu Local Government Area, Enugu, Nigeria. The plant seeds were identified by a botanist named Mr. Afred Ozioko at the Taxonomy Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A sample of the plant seeds was deposited in the herbarium for future retrieval (Voucher no: Inter-CEDD/16325).

2.2. Methods

2.2.1. Preparation of plant material

Fresh seeds of *S. stenorcarpa* were hand-picked and made free from extraneous materials, after which the plant seeds were roasted at about 300 °C for an hour using firewood as prepared traditionally. After roasting, the epicarp (outer peel) was removed and seeds were pulverized into fine particles using a grinding machine and stored in an airtight container until needed for further studies.

2.2.2. Determination of proximate composition

The seeds were analyzed for moisture, ash, crude fiber, protein, crude fat, and carbohydrates determined by difference according to the method described by AOAC (AOAC, 2005). Determination of moisture content was gravimetrically performed allowing the samples to dry at 100 °C in an oven to a constant mass and recorded. After drying, other chemical determination was performed with the resultant dried materials. Determination of crude protein content was performed using the Kjeldahl method (AOAC Method No: 978.04). Determination of crude fat was performed following the Soxhlet extraction with petroleum ether as solvent at 60 – 80 °C (AOAC Method No: 930.09). Determination of the ash content of the sample was carried out by burning the sample to ash at 550 °C in a muffle furnace (AOAC Method No: 930.05).

2.2.3. Determination of minerals

Selected minerals, including sodium, potassium, and chloride were extracted from dry ashed samples and determined by atomic absorption spectrophotometer (AOAC Method No: 975.03) (AOAC, 2005).

2.2.4. Experimental animals

Twenty-five Wistar rats and sixteen albino mice of both sexes with a weight range of 107 - 148 g and 23 - 32 g, respectively, were employed in the study. Animals were obtained from the Department of Zoology and Environmental Biology Animal Housing Unit and allowed to acclimatize for fourteen days under optimal hygiene, temperature, pressure, and humidity conditions. Standard cages, kept in a room maintained at a congenital temperature (27 ± 1 °C) and 12-hours light/dark cycles, were employed in the study. Animals were administered solely standard rat chows (Grand Cereals Ltd, Jos, Nigeria) in pellet form and water ad libitum during the acclimatization period. The handling of experimental animals was done with utmost meticulousness and adhered to conventional ethical considerations. Ethical clearance documents were obtained from the Research ethics unit (Approval No: UNN/FBS/EC/1028).

2.2.5. Feed formulation

Diet formulations assigned to various groups were formulated by mixing pulverized roasted *S. stenorcarpa* seeds (PROSS) with standard rat chow (Grand Cereals Ltd, Jos, Nigeria) at specified ratios.

2.2.6. Acute toxicity (LD_{50}) study

The acute toxicity experiment was carried out with mice following the outlined protocols of Lorke (1983).

2.2.7. Animal groupings and experimental design

The presented study employed the completely randomized design (CRD) method of experimental design. Twenty-five (25) healthy Wistar rats were employed in the study. Animals were randomly assigned into five groups comprising five rats each and were given different feed formulations for five weeks. Rats in each group were administered group-specific diet formulation as well as water ad libitum daily for 5 weeks. In brief, Group I served as normal control and was administered oral doses of 100 % standard rat chow, while Groups II-V received 10 %, 20 %, 30 %, and 40 % roasted pulverized *S. stenorcarpa* seeds mixed with standard rat chow to make 100 mg/kg b.w., respectively.

2.2.7. Blood sample and organ collection

After five (5) weeks of a standard group-specific feeding regimen, animals were deprived of food overnight and humanely euthanized the following day by overdosing with halothane (an anesthetic). Blood samples of rats in each group were separately collected via cardiac puncture into group-specific and well-labeled plain tubes and EDTA tubes. After sacrifice, animals' vital organs were also harvested and labeled properly for further biochemical determinations, organs weighting and histological study.

2.2.8. Biochemical analysis

Blood samples collected with ethylenediaminetetraacetic acid (EDTA) tubes were adopted for the analysis of hematological indices [red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and hematocrit (Ht)] using a Sysmex KX-21N hematology analyzer. (Sysmex Corporation, Kobe-Japan) following the manufacturer's guidelines for reagents and instruments (Imai et al., 2008). The Roche Cobas 6000 biochemistry analyzer was used to determine liver function markers enzymes [alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST)], lipid profile indices [triacylglycerol (TAG), total cholesterol (Tchol), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL)], and kidney function parameters (serum electrolyte, urea, and creatinine) in the serum derived from whole blood samples of respective groups (Biadgo et al., 2016). Furthermore, liver antioxidant enzymes [catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD)] activity were determined following the outlined protocols of Xin et al. (1991), Hafeman et al. (1974), and Aebi (2012), respectively. In addition, hepatocyte lipid peroxidation status was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) based on the method of Wallin et al. (1993).

2.2.9. Histological studies

This study was performed according to the method described by Akintunde et al. (2013). Excision of organs (liver and kidney) was made separately immediately after animals were humanely sacrificed. The excised tissues were rinsed and fixed in 10% buffered formalin. Dehydration was accomplished using graded volumes of alcohol, after which xylene was used to clear the tissues before embedding it in paraffin wax. A microtome was used to excise sections of about 5-6 µm thick of the embedded tissues, followed by staining with hematoxylin and eosin. Photomicrographs showing the state of the micro-environment of the liver and kidney tissues were captured and recorded under the microscope (Nikon-Japan) operated at 100 x magnifications.

2.2.10. Statistical analysis

Obtained data were analyzed statistically using one-way and two-way analysis of variance (ANOVA) in an optimized version (23) of the Statistical Product and Service Solutions (SPSS) tool. The analyzed data were expressed as mean ± standard deviation of the mean (SD). The differences in mean across the various groups were analyzed using Duncan's post hoc study. The significance level of all determinations was fixed at $p = 0.05$.

3. Results and discussion

3.1. Proximate composition of PROSS

The proximate composition of PROSS (as presented in Table 1) showed that the legume is rich in carbohydrates (65.40%) and contains a considerable amount of protein (19.45%), with relatively smaller amounts of moisture (6.15%), crude fiber (3.66 ± 0.01%), fats (2.80%), and ash (2.56%) indicative of the minerals content. The rich composition of carbohydrates suggests that PROSS seed could be a good candidate for fostering food security. As a staple food with high carbohydrate content, its consumption could be a source of energy for the various types of menial jobs and strenuous involvements common among the poor masses in the locales of developing countries. The appreciable amounts of protein and minerals present in PROSS suggest its potential to serve as a functional food that could be employed in curbing the menace of protein deficiency and essential minerals and their consequences ranging from poor health development to kwashiorkor and even death. It could also represent a source for formulating infant feeds by its rich nutrient composition. The result obtained from this study was seen to be higher than that reported by Ndidi et al. (2014). This could have resulted from the difference in the location where the legume was gotten from or due to variation in soil types in which the seed was cultivated. However, Apata and Ologhobo (1990) reported a higher ash and protein content in the raw seeds of African yam bean. It may be that the roasting process could have reduced the intact protein concentration in the seeds. Similarly, due to the low fat and relatively high crude fiber of the PROSS diet, it would be suitable for making meals for diabetic patients. The low moisture content (6.15%) in PROSS implies that the food could have a longer shelf life. This agrees with the submission of Abioye et al. (2015), which reported low moisture content in PROSS, and opined it was indicative of high storability and durability because high moisture content in grains (of about 14.5%) results in mold, bacteria, and insects infestation, which deteriorate agricultural produce during storage, as opposed to low moisture content grains, which are more stable during storage.

Table 1. Proximate composition of PROSS

Nutrient	Percentage (%)
Fats	2.80 ± 0.02
Ash	2.56 ± 0.03
Moisture	6.15 ± 0.01
Protein	19.45 ± 0.04
Crude fibre	3.66 ± 0.01
Carbohydrate	65.40 ± 0.06
Total	100.00 ± 0.00

Energy value = 379.24 Kcal, *n* = 3

3.2. Acute toxicity of PROSS

Results of the acute toxicity study of the PROSS diet (Table 2) showed that the diet did not cause any lethal effect on mice at doses up to 5000 mg/kg b.w. (p.o.). Our result aligns with previous reports by Christopher et al. (2013) and Michael et al. (2018), which recorded that the plant seed extract did not cause any form of toxicity nor alterations in behaviors in experimental subjects up to a concentration of 5000 mg/kg b.w.

Table 2. Acute toxicity study of PROSS

Treatments (mg/kg b.w (*p.o.*) PROSS	No. of animals used	No. of death
10	3	0
100	3	0
1000	3	0
1600	3	0
2900	3	0
5000	3	0

n = 3; *p.o.* = per oral administration; b.w. = body weight

3.3. Effect of PROSS on organ weight

Furthermore, administration of various dietary formulations of PROSS did not have any effect on the liver and kidney organ weight ($p > 0.05$) of rats in the test groups compared to the control group (as shown in Table 3). These thus, suggest that the consumption of the seed as food is safe and healthy. It has been documented that alteration in the absolute or relative weight of vital organs after the administration of a pharmacological agent or food is indicative of the lethality of the substance (Orisakwe et al., 2004).

Table 3. Effect of PROSS diet formulations on the organ weight in Wistar rats

Groups (treatments)	Liver weight (g)	Kidney weight (g)
Group 1 (Normal control)	4.67 ± 0.31^{a}	1.03 ± 0.15^{a}
Group 2 (10% PROSS)	5.00 ± 0.57^{a}	1.08 ± 0.13^{a}
Group 3 (20% PROSS)	5.15 ± 0.59^{a}	1.23 ± 0.05^{a}
Group 4 (30% PROSS)	5.53 ± 0.48^{a}	1.13 ± 0.17^{a}
Group 5 (40% PROSS)	5.58 ± 0.10^{a}	1.05 ± 0.13^{a}

Results are presented as mean ± standard deviation (SD) (n = 4). Values in each column with different small-case alphabetic subscripts vary significantly ($p < 0.05$) and increase in alphabetic chronology (the lowest alphabet being 'a').

3.4. Effect of PROSS on electrolyte levels

Results (as presented in Table 4) showed that administration of PROSS improved serum electrolyte levels in a dose-dependent manner. Groups administered 10% and 20% PROSS showed decreased sodium levels ($p < 0.05$), while groups administered higher doses of PROSS showed increased sodium levels ($p < 0.05$) compared to control. On the other hand, the chloride level increased significantly, with an increase in doses of PROSS ($p < 0.05$), while the serum potassium level was unchanged. Sodium is an electrolyte essential for regulating water levels in the body. It helps to maintain blood volume, nerve function, and muscular activities (Minegishi et al., 2020). Homeostatic regulation of sodium concentration in body fluids and its compartmentalization is pivotal for normal brain function, neuromuscular coordination, cell-to-cell communication, and other electrical signal transduction (Noda & Matsuda, 2022). Alterations in sodium levels in body fluid (too little or too much) could result in cellular dysfunction and several fatalities (Veniamakis et al., 2022). It has been found that high sodium in the body fluid can lead to hypertension (Grillo et al., 2019). Studies have expounded on the need for a reduction in dietary sodium, as it helps to ameliorate blood pressure, decrease the incidence of hypertension, and reduce the morbidity and mortality associated with cardiovascular diseases (He et al., 2013). Hence, a diet that can decrease serum sodium levels will be of great importance for hypertensive patients. The result from the study showed that moderate consumption of the PROSS diet could help to maintain normal serum levels. Similarly, a non-significant decrease in serum potassium level was also observed across the treatment groups, compared to normal control. Potassium also plays a key role in maintaining normal blood pressure (Houston & Harper, 2008). Dietary potassium consumption correlates inversely to the incidence of hypertension and mortality associated with cardiovascular diseases (Aburto et al., 2013). However, an imbalance of potassium and sodium can be detrimental as it could further heighten the risk of high blood pressure, and even cardiac and thrombotic diseases (Levings & Gunn, 2014). Our result suggests that PROSS could help maintain potassium levels at permissible levels. The chloride level was increased significantly ($p < 0.05$) in a dose-dependent manner across the groups compared to the control. This suggests that the PROSS diet is a good source of chloride and its many health benefits. Chloride is pivotal for the maintenance of proper acidity of body fluid, passively balancing out positive ion concentrations in body tissues and organs. Similar to sodium, most chloride is obtained from the diet. Hence, a decrease in chloride intake could lead to dehydration (Popkin et al., 2010). More so, a decrease in chloride level is also seen in volume depletions and results in sodium and bicarbonate accumulation in extracellular fluid, which elevates pH level leading to hypochloremia metabolic alkalosis (Tinawi, 2021).

Table 4. Effect of PROSS diet formulations on serum electrolyte level in Wistar rats

Groups (treatments)	K^+ (mEq/l)	Na^+ (mEq/l)
Group 1 (Normal control)	3.13 ± 0.02^{a}	121.25 ± 0.91^{c}
Group 2 (10% PROSS)	3.11 ± 0.08^{a}	107.29 ± 1.56^{a}
Group 3 (20% PROSS)	3.00 ± 0.07^{a}	114.52 ± 1.33^{b}
Group 4 (30% PROSS)	3.05 ± 0.03^{a}	126.33 ± 6.26^{d}
Group 5 (40% PROSS)	3.12 ± 0.02^{a}	123.38 ± 1.68^{c,d}

Results are presented as mean ± standard deviation (SD) (n = 4). Values in each column with different small-case alphabetic subscripts vary significantly ($p < 0.05$) and increase in alphabetic chronology (the lowest alphabet being 'a').

3.5. Effect of PROSS on kidney function indices

Our data (Table 5) also showed that urea levels decreased significantly ($p < 0.05$) with an increase in the concentration of PROSS in the diet. Conversely, a non-significant increase ($p > 0.05$) in creatinine level was observed across the groups.

Table 5. Effect of PROSS diet formulations on the kidney function indices in Wistar rats

Groups (treatments)	Urea(mg/dl)	Creatinine (mg/dl)
Group 1 (Normal control)	17.26 ± 0.31^{d}	1.16 ± 0.14^{a}
Group 2 (10% PROSS)	15.54 ± 0.62^{c}	1.27 ± 0.18^{a}
Group 3 (20% PROSS)	14.89 ± 1.00^{c}	1.32 ± 0.07^{a}
Group 4 (30% PROSS)	10.44 ± 0.66^{b}	1.29 ± 0.19^{a}
Group 5 (40% PROSS)	9.98 ± 0.62^{a}	1.29 ± 0.20^{a}

Results are presented as mean ± standard deviation (SD) (n = 4). Values in each column with different small-case alphabetic subscripts vary significantly ($p < 0.05$) and increase in alphabetic chronology (the lowest alphabet being 'a').

Serum urea and creatinine levels are commonly used to assess kidney function. Our data agree with the proposition of Eneh et al. (2016), which reported that African yam bean reduced serum uric acid levels and proposed its potential to treat gout by mopping up urate crystals. Creatinine and urea are nitrogen-containing end-products of cellular metabolism. Creatinine results from the breakdown of muscle creatine, while urea being the end-product of dietary protein catabolism and tissue protein turnover is synthesized in the liver via the deamination of amino acids and the participation of the five enzymatically controlled steps of the urea cycle (Shambaugh III, 1977). Studies have shown that serum urea levels could be altered by diets as well as non-kidney disorders (Kramer, 2019). A rise in serum urea level is generally indicative of nephropathy particularly glomerular dysfunction (Rosner & Bolton, 2006). The ability of PROSS diet formulations to decrease serum urea levels suggests that the diet supplemented possesses an ameliorative effect on kidney function; hence its consumption should be encouraged. These suggest that the consumption of PROSS is safe and healthy for kidney function. In addition, serum total protein level did not differ significantly ($p > 0.05$) across the treatment groups compared to normal control. Total serum protein represents the various plasma proteins found in the blood except for fibrinogen and clotting factors (Smith et al., 2013). Plasma proteins, including albumin and globulin, play several key roles at physiological levels. For instance, albumin maintains the osmolarity of the blood and prevents leakage of fluids out of blood vessels or from the tissues into the blood. On the other hand, globulin proteins play roles in immune response (der Sarkissian, 2017). However, pathologic conditions, such as inflammation or infections, such as bone marrow disorders, HIV, and viral hepatitis B or C are accompanied by elevated levels of total protein in the serum, which help in their diagnosis (Soeters et al., 2019). A low level of total protein in serum could indicate liver disorder, malnutrition, and kidney dysfunction (Hebert et al., 2013). Low total protein can also lead to hypoproteinemia, a condition where the total protein level in the blood is critically low. It is evident from the result of the study that consumption of the PROSS diet will not be harmful to the kidneys but rather proffer both nutritional and medicinal effects on vital organ function.

3.6. Effect of PROSS on hematological indices

Furthermore, the result (as presented in Table 6) showed significant improvement in hematological indices (RBC, WBC, Hb, Ht) across the treatment groups ($p < 0.05$), suggesting that the consumption of PROSS could boost blood parameters and perhaps manage anemic patients. Assessment of hematological indices helps in screening the health status of animals. Laboratory screening of anemia is usually done by evaluating the levels of red cells, Hb, Ht, and white blood cells. Red blood cells play several physiological roles, including the transport of gases in and out of the lungs (CO_2 and O_2, respectively) to the body tissues and regulating circulatory acid/base equilibria. The ability of PROSS to ameliorate the levels of RBC and Hb in the experimental animals implies its anti-anemic potential. From the result, an increase in Ht was accompanied by a corresponding increase in RBC counts and Hb concentration. The significant elevated WBC level observed in the study ($p < 0.05$) could be due to a change in hepatic portal composition that accompanied dietary treatments as the body responds to exogenous substances. White blood cells help to defend the body against infection (Tamang et al., 2022). Our result aligns with the work of Christopher et al. (2013), which reported a significant improvement in RBC, Hb, and Ht in anemic animals treated with the PROSS diet ($p < 0.05$). In the same vein, Ajayi et al. (2009) reported that *S. stenorcarpa* seeds are rich in iron, an essential mineral for the synthesis of hemoglobin. Similarly, Nwaoguikpe (2008) highlighted that *S. stenorcarpa* exhibited anti-sickling activity on sickled hemoglobin.

Table 6. Effect of PROSS diet formulations on hematological indices in Wistar rats

Groups (treatments)	RBC(10^6/mm^3)	WBC (10^3/mm^3)	Hb (g/dl)	Ht (%)
Group 1 (Normal control)	8.11 ± 0.08^a	5.25 ± 1.02^a	9.58 ± 0.18^a	33.00 ± 1.63^a
Group 2 (10% PROSS)	$8.27 \pm 0.47^{a,b}$	$6.23 \pm 1.70^{b,c}$	10.14 ± 0.02^b	37.50 ± 1.29^b
Group 3 (20% PROSS)	8.56 ± 0.04^c	6.58 ± 1.71^b	10.25 ± 0.02^b	39.00 ± 0.81^b
Group 4 (30% PROSS)	$8.64 \pm 0.03^{b,c}$	$6.65 \pm 1.29^{b,c}$	10.21 ± 0.12^b	39.25 ± 0.95^b
Group 5 (40% PROSS)	$8.67 \pm 0.35^{b,c}$	6.89 ± 1.54^c	10.49 ± 0.07^c	41.00 ± 0.81^c

Results are presented as mean ± standard deviation (SD) ($n = 4$). Values in each column with different small-case alphabetic subscripts vary significantly ($p < 0.05$) and increase in alphabetic chronology (the lowest alphabet being 'a').

3.7. Effect of PROSS on liver marker enzymes

Cytosolic enzymes of hepatocytes could leak into the circulation when hepatic cell membranes are damaged. Conventionally, serum levels of these liver function marker enzymes (ALP, ALT, and AST) are employed to screen and measure alterations in liver function, changes in hepatocyte integrity, and the severity of liver organ injury (Andrade et al., 2019). Elevated levels of these enzymes often indicate inflammation or damage to hepatocytes (Kalas et al., 2021). Our data (as shown in Table 7) revealed that PROSS diet formulations effectively decreased the activity of liver function marker enzymes (ALP, ALT, and AST) in the serum of animals across the test groups ($p < 0.05$). These suggest the ameliorative potential of PROSS diet formulations (10 - 40% PROSS) on liver organ integrity and function. Our result agrees with the submission of Ukairo et al. (2017), which highlighted the hepatoprotective and anti-fibrotic potential of boiled *S. stenocarpa* seeds against CCl_4-induced liver damage in rats. Conversely, Ojuederie et al. (2020) reported that 50% and 100% of unprocessed African yam bean seed flour meal resulted in increased AST and ALT activity indicative of liver and kidney damage. This, therefore, highlights the intricacy of processing (roasting or boiling) the plant seed before consumption as well as the dosage, as higher doses may not be healthy.

Table 7. Effect of PROSS diet formulations on liver function marker indices in Wistar rats

Groups (treatments)	ALP (IU/l)	AST (U/l)	ALT (U/l)
Group 1 (Normal control)	53.63 ± 0.25^c	10.79 ± 0.28^c	10.06 ± 0.28^d
Group 2 (10% PROSS)	45.78 ± 0.67^b	9.54 ± 0.74^b	9.67 ± 0.74^c
Group 3 (20% PROSS)	44.44 ± 0.78^b	9.08 ± 0.32^b	8.96 ± 1.34^b
Group 4 (30% PROSS)	40.98 ± 0.34^a	9.00 ± 0.63^b	8.59 ± 0.88^a
Group 5 (40% PROSS)	40.42 ± 0.38^a	8.33 ± 0.41^a	8.06 ± 1.21^a

Results are presented as mean ± standard deviation (SD) ($n = 4$). Values in each column with different small-case alphabetic subscripts vary significantly ($p < 0.05$) and increase in alphabetic chronology (the lowest alphabet being 'a').

3.8. Effect of PROSS on liver antioxidant status

Liver antioxidant enzymes are the primary enzymatic antioxidant system that acts by neutralizing toxic oxidative radicals and hindering their oxidative attack on biomolecules (Mehta & Gowder, 2015). There is growing evidence of the nutritional and health importance associated with these first-line defense antioxidant enzymes and the inverse correlation they share with free radicals (Ighodaro & Akinloye, 2018). The result (as presented in Table 8) showed that the administration of PROSS diet formulation significantly increased the activity of liver antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) across the test groups ($p < 0.05$) compared to control. Hence, suggesting the antioxidant effect of the PROSS diet and its potency to protect against oxidative stress and disease pathologies. On the contrary, in addition, the MDA level significantly decreased in test groups ($p < 0.05$) compared to the control. These further suggest the potential of PROSS diets to fortify the redox status of the liver and prevent lipid peroxidation and oxidative stress-related diseases. The assessment of lipid peroxidation is usually performed by analyzing secondary oxidation products such as malondialdehyde (MDA). The level of MDA in a sample correlates directly with the level of lipid peroxidation and has been widely used to measure the extent of oxidative deterioration of lipids in biological and food systems (Ayala et al., 2014; Ito et al., 2019).

3.9. Effect of PROSS on serum lipid profile

Furthermore, it was observed from the result (as presented in Table 9) that animals administered various doses of PROSS diet formulation showed a significant increase in the HDL level ($p < 0.05$) and a decrease in the serum levels of TAG, LDL, and VLDL ($p < 0.05$). In addition, the TChol level was also increased across the treatment groups, but this was not significantly different compared to the control ($p > 0.05$). Clinically, the determination of serum lipid profile helps in the detection, diagnosis, and treatment of metabolic diseases. The LDL-cholesterol is often referred to as "bad" cholesterol because its elevated levels in the blood increase the risk of vascular and heart diseases due to heightened cholesterol deposition and plaque formation in the arterial walls. Several reports on the potential of legumes to decrease LDL cholesterol in animals have been documented (Amoah et al., 2023; Angeles et al., 2021). On the other hand, HDL-cholesterol is usually termed good cholesterol due to its propensity to harness the transport of arterial and tissue cholesterol to the liver for the biosynthesis of essential metabolites (Kjeldsen et al., 2021). Individuals with higher levels of HDL tend to have a low risk of cardiovascular diseases, while those with low levels have an increased risk of heart disease (Liu et al., 2022). HDL-cholesterol is protective against atherosclerosis; a high level of HDL is an indication of a healthy metabolic system (Nagao et al., 2018). A direct correlation exists between higher levels of HDL and improved cardiovascular health (Bardagjy & Steinberg, 2019). Hence, it could be inferred that PROSS seed possesses a protective effect against cardiovascular-related diseases.

Table 8. Effect of PROSS diet formulations on liver antioxidant enzymes and lipid peroxidation status in Wistar rats

Groups (treatments)	CAT (U/ml)	SOD (IU/l)	GPX (U/l)	MDA (mg/dl)
Group 1 (Normal control)	0.42 ± 0.05^a	1.12 ± 0.01^a	6.09 ± 0.36^a	4.94 ± 0.09^c
Group 2 (10% PROSS)	0.74 ± 0.03^c	1.14 ± 0.01^b	6.44 ± 0.76^b	4.25 ± 0.07^b
Group 3 (20% PROSS)	0.64 ± 0.09^b	1.17 ± 0.01^c	6.83 ± 0.94^c	$4.15 \pm 0.03^{a,b}$
Group 4 (30% PROSS)	0.62 ± 0.01^b	1.15 ± 0.01^b	7.74 ± 0.72^d	4.05 ± 0.02^a
Group 5 (40% PROSS)	0.78 ± 0.03^d	1.21 ± 0.00^d	7.38 ± 0.35^d	4.11 ± 0.35^a

Results are presented as mean ± standard deviation (SD) ($n = 4$). Values in each column with different small-case alphabetic subscripts vary significantly ($p < 0.05$) and increase in alphabetic chronology (the lowest alphabet being 'a'). CAT: Catalase, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, MDA: Malondialdehyde

Table 9. Effect of PROSS diet formulations on serum lipid profile in Wistar rats

Groups (treatments)	Tchol (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group 1 (Normal control)	2.94 ± 0.08^a	1.75 ± 0.03^c	1.10 ± 0.09^a	1.69 ± 0.09^c	0.45 ± 0.01^c
Group 2 (10% PROSS)	2.90 ± 0.08^a	1.38 ± 0.02^b	1.25 ± 0.04^b	1.41 ± 0.06^b	0.24 ± 0.00^a
Group 3 (20% PROSS)	2.80 ± 0.04^a	1.41 ± 0.07^b	1.32 ± 0.02^b	1.19 ± 0.06^a	0.28 ± 0.01^a
Group 4 (30% PROSS)	3.03 ± 0.03^a	1.43 ± 0.05^b	1.42 ± 0.01^c	1.45 ± 0.05^b	0.32 ± 0.01^b
Group 5 (40% PROSS)	3.01 ± 0.07^a	1.29 ± 0.04^a	1.45 ± 0.02^c	1.45 ± 0.07^b	0.38 ± 0.01^b

Results are presented as mean ± standard deviation (SD) ($n = 4$). Values in each column with different small-case alphabetic subscripts vary significantly ($p < 0.05$) and increase in alphabetic chronology (the lowest alphabet being 'a').

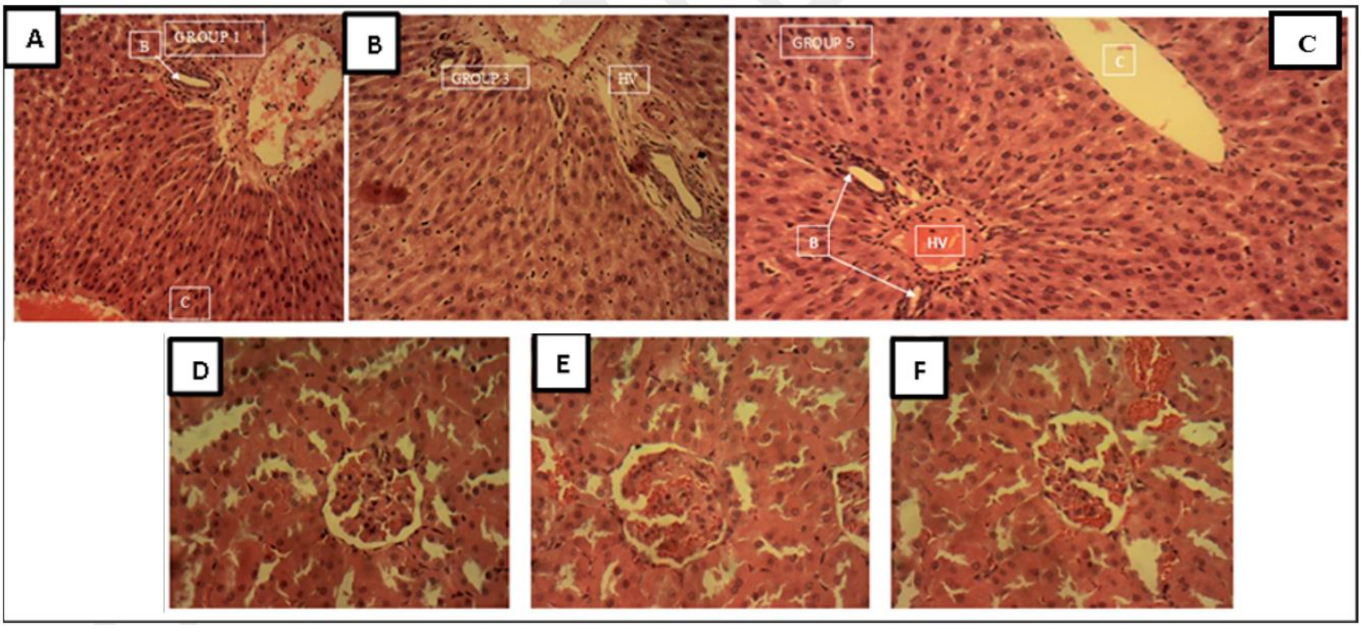

Figure 1. Microscopic representation of the liver and kidney {H&E X160}. Mag: x100.
A) Microscopic representation of the liver of the control rats. Normal liver with hepatic vein (HV), bile ducts (B), and central vein (B). B) Microscopic representation of the liver of rats fed on a 20% PROSS diet showing hepatic vein (HV), bile ducts (B), and hepatic artery (HA). C) Microscopic representation of the liver of a rat fed on a 40% PROSS diet showing hepatic vein (HV), bile ducts (B), and central vein (C). D) Microscopic representation of the kidney of a rat of the control group. E) Microscopic representation of the kidney of a rat fed on a 20% PROSS diet. F) Microscopic representation of kidney of rat fed on 40% PROSS

3.10. Effect of PROSS on liver and kidney histology

Results of histological studies of the sections of the liver and kidney randomly collected from animals in groups I, III, and V showed normal hepatic histomorphology (Figure 1A-C). Numerous normal hepatic lobules, containing normal hepatocytes arranged in radiating interconnecting cords around the central veins, with the hepatic cords being separated by normal-sized sinusoidal spaces were observed. Normal structures of the portal triads (hepatic vein, hepatic artery, and bile ducts) were also observed. This suggests that the

African yam bean seed does not pose any toxic effect on liver histomorphology. In addition, sections of the kidney collected from the animals in groups I, III, and V showed normal renal histomorphology of animals (Figure 1D-F). Normal glomeruli (G) in normal Bowman's capsules (white arrow) were observed. The glomeruli were surrounded by a sea of normal renal tubules (proximal convoluted tubules, pars recta, distal convoluted tubules, and collecting ducts) both in the cortex and the medulla. The renal interstitium was normal and consisted of thin connective tissues and blood vessels. Our data suggest that the PROSS does not pose any toxic effect on liver histomorphology at the assayed dosages. However, Ojuederie et al. (2020) reported that 50% and 100% raw seed meal of African yam bean seed floor posed mild to severe portal and cortical congestions and necrotic damage on liver and kidney tissues examined in Wistar rats. In line with their findings, it could be reasoned that the seed is not entirely innocuous when consumed unprocessed. Furthermore, while several health benefits are reported of the plant seed, it will be noteworthy to opine that the dosage should be considered carefully.

4. Conclusions

AYB is a very nutritious food with a high level of protein. The AYB meal has low caloric value and is highly recommended for people seeking weight loss. Roasted AYB could represent a healthy food that could reduce the risk of cardiovascular diseases due to its low fat content. In addition, a diet supplemented with roasted AYB diet could also reduce serum sodium levels and, hence, could be used to manage patients suffering from hypertension and diabetes. However, the low-fat content of roasted AYB limits its use for commercial oil production. Our result showed that moderate consumption of roasted African yam beans should be recommended for patients suffering from hypertension and renal dysfunction and would not affect the kidney and liver organs negatively. Our study afforded valuable insights into the short-term effects of PROSS consumption, demonstrating its potential to enhance antioxidant activity, lipid profiles, and hematological indices while supporting hepatic and renal health. However, the dose-dependent variations in electrolyte balance, particularly sodium and chloride levels, highlight the need for careful dietary recommendations, especially for individuals with hypertension or renal dysfunction. Long-term consumption of PROSS could have cumulative effects, including potential risks such as electrolyte imbalances, hepatotoxicity, or excessive hematopoietic stimulation, warranting further investigation. Future research to assess the effects of prolonged PROSS consumption on organ health in animal models with induced hypertension, diabetes, or renal impairment is warranted.

Acknowledgments

The authors express their profound gratitude to the staff of the University of Nigeria and the resource personnel from various institutions visited for their invaluable support in ensuring the success of this study.

Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

Ethical approval for the appropriate and safe execution of the study, as well as the humane and meticulous use of experimental animals, was obtained from the Ethics and Research Committee, Department of Biochemistry, University of Nigeria, Nsukka (Approval Number: UNN/BCH/2019/1014). All experimental procedures involving laboratory animals were conducted in strict adherence to international guidelines for the care and use of experimental animals.

Availability of data and materials

All data generated or analyzed during this study are included in this published article. On request, the associated author can provide more information.

Funding

None.

CRediT authorship contribution statement

Nene Hephzibah Chiaka-Onyemeze: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-reviewing & editing
Chinelo Chinenye Nkwocha: Data curation, Formal analysis, Writing-reviewing & editing, Investigation
Affiong Asuquo Edeke: Investigation, Methodology, Software, Writing-reviewing & editing
Emmanuel Chimeh Ezeako: Data curation, Formal analysis, Investigation, Methodology, Software, Writing original draft, Writing-reviewing & editing

ORCID Numbers of the Authors

N. H. Chiaka-Onyemeze: 0000-0003-1684-8973
C. C. Nkwocha: 0000-0002-7684-8441
A. A. Edeke: 0000-0002-6299-5686
E. C. Ezeako: 0000-0002-9675-8320

Supplementary File

None.

Publisher's Note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abioye, V., Olanipekun, B., & Omotosho, O. (2015). Effect of varieties on the proximate, nutritional and anti-nutritional composition of nine variants of African yam bean seeds (*Sphenostylis stenocarpa*). *Donnish Journal of Food Science and Technology, 1*(2), 17-21.

Aburto, N. J., Hanson, S., Gutierrez, H., Hooper, L., Elliott, P., & Cappuccio, F. P. (2013). Effect of increased potassium intake on cardiovascular risk factors and disease:

systematic review and meta-analyses. *BMJ, 346*, Article: f1378. https://doi.org/10.1136/bmj.f1378

Adewale, B. D., & Nnamani, C. V. (2022). Introduction to food, feed, and health wealth in African yam bean, a locked-in African indigenous tuberous legume. *Frontiers in Sustainable Food Systems, 6*, Article: 726458. https://doi.org/10.3389/fsufs.2022.726458

Aebi, H. (2012). Catalase. In H. U. Bergmeyer (Ed.), *Methods of Enzymatic Analysis (3rd ed.)* (pp. 273-286): Verlag Chemie, Weinhem. https://doi.org/10.1016/0307-4412(85)90136-0

Ajayi, F., Akande, S., Adegbite, A., & Idowu, B. (2009). Assessment of seven under-utilized grain legume foliages as feed resources for ruminants. *Livestock Research for Rural Development, 21*(9), Article: 149.

Akinola, R., Pereira, L. M., Mabhaudhi, T., De Bruin, F. M., & Rusch, L. (2020). A review of indigenous food crops in Africa and the implications for more sustainable and healthy food systems. *Sustainability, 12*(8), Article: 3493. https://doi.org/10.3390/su12083493

Akintunde, J., Ajiboye, J., Slemuri, D., Oyelowo, S., Sunday, O., Abam, E., & Irondi, A. (2013). Effects of Anti-erectile Dysfunction Drugs on some Key Tissue in Healthy Male Rats. *American Journal of Drug Discovery and Development, 3*(2), 32-46. https://doi.org/10.3923/ajdd.2013.32.46

Alcorta, A., Porta, A., Tárrega, A., Alvarez, M. D., & Vaquero, M. P. (2021). Foods for Plant-Based Diets: Challenges and Innovations. *Foods, 10*(2), Article: 293. https://doi.org/10.3390/foods10020293

Amoah, I., Ascione, A., Muthanna, F. M., Feraco, A., Camajani, E., Gorini, S., Armani, A., Caprio, M., & Lombardo, M. (2023). Sustainable strategies for increasing legume consumption: culinary and educational approaches. *Foods, 12*(11), Article: 2265. https://doi.org/10.3390/foods12112265

Andrade, R., Aithal, G., & Björnsson, E. (2019). EASL clinical practice guidelines: drug-induced liver injury. *Journal of Hepatology, 70*(6), 1222-1261. https://doi.org/10.1016/j.jhep.2019.02.014

Angeles, J. G. C., Villanueva, J. C., Uy, L. Y. C., Mercado, S. M. Q., Tsuchiya, M. C. L., Lado, J. P., Angelia, M. R. N., Bercansil-Clemencia, M. C. M., Estacio, M. A. C., & Torio, M. A. O. (2021). Legumes as functional food for cardiovascular disease. *Applied Sciences, 11*(12), Article: 5475. https://doi.org/10.3390/app11125475

AOAC. (2005). *Official Methods of Analysis (15th ed.)*: Association of Official Analytical Chemists.

Apata, D., & Ologhobo, A. (1990). Some aspects of the biochemistry and nutritive value of African Yambean seed (*Sphenostylis stenocarpa*). *Food Chemistry, 36*(4), 271-280. https://doi.org/10.1016/0308-8146(90)90066-D

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity, 2014*, Article: 360438. https://doi.org/10.1155/2014/360438

Bardagjy, A. S., & Steinberg, F. M. (2019). Relationship between HDL functional characteristics and cardiovascular health and potential impact of dietary patterns: a narrative review. *Nutrients, 11*(6), Article: 1231. https://doi.org/10.3390/nu11061231

Biadgo, B., Melku, M., Abebe, S. M., & Abebe, M. (2016). Hematological indices and their correlation with fasting blood glucose level and anthropometric measurements in type 2 diabetes mellitus patients in Gondar, Northwest Ethiopia. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 9*, 91-99. https://doi.org/10.2147/DMSO.S97563

Christopher, O. C., Obioma, N. U., & Mary, M. A. (2013). Anti-anaemic effect of methanol seed extract of *Sphenostylis stenocarpa* (African yam bean) in Wistar albino rats. *African Journal of Pharmacy and Pharmacology, 7*(45), 2907-2913. https://doi.org/10.5897/AJPP12.1141

de Jager, I., Borgonjen-Van Den Berg, K. J., Giller, K. E., & Brouwer, I. D. (2019). Current and potential role of grain legumes on protein and micronutrient adequacy of the diet of rural Ghanaian infants and young children: using linear programming. *Nutrition Journal, 18*, Article: 12. https://doi.org/10.1186/s12937-019-0435-5

der Sarkissian, C. (2017). *Total protein test*: American Association for Clinical Chemistry, University of Rochester Medical Center.

Eneh, F. U., Orjionwe, R. N., & Adindu, C. S. (2016). Effect of african yam bean (*Sphenostylis stenocarpa*) on serum calcium, inorganic phosphate, uric acid, and alkaline phosphatase concentration of male albino rats. *Journal of Agricultural Sciences, 8*(1), 148-153. https://doi.org/10.5539/jas.v8n1p148

Gbenga-Fabusiwa, F. (2021). African yam beans (*Sphenostylis stenocarpa*): A review of a novel tropical food plant for human nutrition, health and food security. *African Journal of Food Science, 15*(2), 33-47. https://doi.org/10.5897/AJFS2020.1961

George, T. T., Obilana, A. O., & Oyeyinka, S. A. (2020). The prospects of African yam bean: past and future importance. *Heliyon, 6*(11), Article: e05458. https://doi.org/10.1016/j.heliyon.2020.e05458

Grillo, A., Salvi, L., Coruzzi, P., Salvi, P., & Parati, G. (2019). Sodium intake and hypertension. *Nutrients, 11*(9), Article: 1970. https://doi.org/10.3390/nu11091970

Hafeman, D., Sunde, R., & Hoekstra, W. (1974). Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *The Journal of Nutrition, 104*(5), 580-587. https://doi.org/10.1093/jn/104.5.580

He, F. J., Li, J., & MacGregor, G. A. (2013). Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *BMJ, 346*, Article: f1325. https://doi.org/10.1136/bmj.f1325

Hebert, L. A., Parikh, S., Prosek, J., Nadasdy, T., & Rovin, B. H. (2013). Differential diagnosis of glomerular disease: a systematic and inclusive approach. *American Journal of Nephrology, 38*(3), 253-266. https://doi.org/10.1159/000354390

Houston, M. C., & Harper, K. J. (2008). Potassium, magnesium, and calcium: their role in both the cause and treatment of hypertension. *The Journal of Clinical Hypertension, 10*(7), 3-11. https://doi.org/10.1111/j.1751-7176.2008.08575.x

Hughes, J., Pearson, E., & Grafenauer, S. (2022). Legumes—a comprehensive exploration of global food-based dietary guidelines and consumption. *Nutrients, 14*(15), Article: 3080. https://doi.org/10.3390/nu14153080

Ighodaro, O., & Akinloye, O. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine, 54*(4), 287-293. https://doi.org/10.1016/j.ajme.2017.09.001

Ikhajiagbe, B., Ogwu, M. C., Ogochukwu, O. F., Odozi, E. B., Adekunle, I. J., & Omage, Z. E. (2022). The place of neglected and underutilized legumes in human nutrition and protein security in Nigeria. *Critical Reviews in Food Science and Nutrition, 62*(14), 3930-3938. https://doi.org/10.1080/10408398.2020.1871319

Imai, K., Watari, S., Sakazume, T., & Mitsuyama, S. (2008). Clinical chemistry and immunoassay testing supporting the individual healthy life. *Hitachi Review, 57*, 1-7.

Ito, F., Sono, Y., & Ito, T. (2019). Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants, 8*(3), Article: 72. https://doi.org/10.3390/antiox8030072

Kalas, M. A., Chavez, L., Leon, M., Taweesedt, P. T., & Surani, S. (2021). Abnormal liver enzymes: A review for clinicians. *World Journal of Hepatology, 13*(11), 1688-1698. https://doi.org/10.4254/wjh.v13.i11.1688

Kjeldsen, E. W., Nordestgaard, L. T., & Frikke-Schmidt, R. (2021). HDL cholesterol and non-cardiovascular disease: a narrative review. *International Journal of Molecular Sciences, 22*(9), Article: 4547. https://doi.org/10.3390/ijms22094547

Klu, G., Bansa, D., Kumaga, F., Aboagye, L., Benett-Lartey, S., & Gamedoagbao, D. (2000). The African yam bean (*Sphenostylis stenocarpa*): a neglected crop in Ghana. *West African Journal of Applied Ecology, 1*(1), 53-60.

Kramer, H. (2019). Diet and chronic kidney disease. *Advances in Nutrition, 10*, S367-S379. https://doi.org/10.1093/advances/nmz011

Levings, J. L., & Gunn, J. P. (2014). The imbalance of sodium and potassium intake: Implications for dietetic practice. *Journal of the Academy of Nutrition and Dietetics, 114*(6), 838-841. https://doi.org/10.1016/j.jand.2014.02.015

Liu, C., Dhindsa, D., Almuwaqqat, Z., Ko, Y. A., Mehta, A., Alkhoder, A. A., Alras, Z., Desai, S. R., Patel, K. J., & Hooda, A. (2022). Association between high-density lipoprotein cholesterol levels and adverse cardiovascular outcomes in high-risk populations. *JAMA Cardiology, 7*(7), 672-680. https://doi.org/10.1001/jamacardio.2022.0912

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology, 54*, 275-287. https://doi.org/10.1007/BF01234480

Mehta, S. K., & Gowder, S. J. T. (2015). Members of antioxidant machinery and their functions. In S. J. T. Gowder (Ed.), *Basic Principles and Clinical Significance of Oxidative Stress* (pp. 59-85): IntechOpen. https://doi.org/10.5772/61884

Michael, O., Etim, E., & Innocent, O. (2018). Investigation on the anti-diabetic activity of *Sphenostylis stenocarpa* seed milk extract in alloxan-induced diabetes rats. *International Journal of Scientific and Research Publications, 8*(8), 8-24.

Minegishi, S., Luft, F. C., Titze, J., & Kitada, K. (2020). Sodium handling and interaction in numerous organs. *American Journal of Hypertension, 33*(8), 687-694.

Nagao, M., Nakajima, H., Toh, R., Hirata, K., & Ishida, T. (2018). Cardioprotective effects of high-density lipoprotein beyond its anti-atherogenic action. *Journal of Atherosclerosis and Thrombosis, 25*(10), 985-993. https://doi.org/10.5551/jat.RV17025

Ndidi, U. S., Ndidi, C. U., Olagunju, A., Muhammad, A., Billy, F. G., & Okpe, O. (2014). Proximate, antinutrients and mineral composition of raw and processed (Boiled and Roasted) *Sphenostylis stenocarpa* seeds from Southern Kaduna, Northwest Nigeria. *International Scholarly Research Notices, 2014*, Article: 280837. https://doi.org/10.1155/2014/280837

Nnamani, C. V., Ajayi, S. A., Oselebe, H. O., Atkinson, C. J., Igboabuchi, A. N., & Ezigbo, E. C. (2017). *Sphenostylis stenocarpa* (ex. A. Rich.) Harms., a fading genetic resource in a changing climate: prerequisite for conservation and sustainability. *Plants, 6*(3), Article: 30. https://doi.org/10.3390/plants6030030

Noda, M., & Matsuda, T. (2022). Central regulation of body fluid homeostasis. *Trends in Neurosciences, 36*(11), 661-673. https://doi.org/10.1016/j.tins.2013.08.004

Nwaoguikpe, R. (2008). Effect of the extracts of African Yam Beans (*Sphenostylis stenocarpa*), Hyacinth beans (*Lablab purpureus*) and Bambara groundnut (*Voandzeia subterrenea*) on hemoglobin polymerization and the Fe^{2+}/Fe^{3+} ratio of sickle cell blood. *Nigerian Journal of Biochemistry and Molecular Biology, 23*(2), 46-50.

Ojuederie, O. B., Ajiboye, J. A., & Babalola, O. O. (2020). Biochemical and histopathological studies of key tissues in healthy male Wistar rats fed on African yam bean seed and tuber meals. *Journal of Food Quality, 2020*, Article: 8892618. https://doi.org/10.1155/2020/8892618

Orisakwe, O. E., Husaini, D. C., & Afonne, O. J. (2004). Testicular effects of sub-chronic administration of *Hibiscus sabdariffa* calyx aqueous extract in rats. *Reproductive Toxicology, 18*(2), 295-298. https://doi.org/10.1016/j.reprotox.2003.11.001

Polak, R., Phillips, E. M., & Campbell, A. (2015). Legumes: health benefits and culinary approaches to increase intake. *Clinical Diabetes, 33*(4), 198-205. https://doi.org/10.2337/diaclin.33.4.198

Popkin, B. M., D'Anci, K. E., & Rosenberg, I. H. (2010). Water, hydration, and health. *Nutrition Reviews, 68*(8), 439-458. https://doi.org/10.1111/j.1753-4887.2010.00304.x

Popoola, J., Ojuederie, O., Omonhinmin, C., & Adegbite, A. (2019). Neglected and underutilized legume crops: Improvement and future prospects. In F. Shah, Z. Khan, A. Iqbal, M. Turan, & M. Olgun (Eds.), *Recent Advances in Grain Crops Research* (pp. 123-139): IntechOpen.

Popoola, J. O., Aworunse, O. S., Ojuederie, O. B., Adewale, B. D., Ajani, O. C., Oyatomi, O. A., Eruemulor, D. I., Adegboyega, T. T., & Obembe, O. O. (2022). The exploitation of orphan legumes for food, income, and nutrition security in Sub-Saharan Africa. *Frontiers in Plant Science, 13*, Article: 782140. https://doi.org/10.3389/fpls.2022.782140

Rosner, M. H., & Bolton, W. K. (2006). Renal function testing. *American Journal of Kidney Diseases, 47*, 174-183.

Shambaugh III, G. (1977). Urea biosynthesis I. The urea cycle and relationships to the citric acid cycle. *The American Journal of Clinical Nutrition, 30*(12), 2083-2087. https://doi.org/10.1093/ajcn/30.12.2083

Smith, G. S., Walter, G. L., & Walker, R. M. (2013). Clinical pathology in non-clinical toxicology testing. In W. Haschek, C. Rousseaux, & M. Wallig (Eds.), *Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition)* (pp. 565-594): Academic Press. https://doi.org/10.1016/B978-0-12-415759-0.00018-2

Soeters, P. B., Wolfe, R. R., & Shenkin, A. (2019). Hypoalbuminemia: pathogenesis and clinical significance. *Journal of Parenteral and Enteral Nutrition, 43*(2), 181-193. https://doi.org/10.1002/jpen.1451

Tamang, T., Baral, S., & Paing, M. P. (2022). Classification of white blood cells: A comprehensive study using transfer learning based on convolutional neural networks. *Diagnostics, 12*(12), Article: 2903. https://doi.org/10.3390/diagnostics12122903

Tinawi, M. (2021). Pathophysiology, evaluation, and management of metabolic alkalosis. *Cureus, 13*(1), Article: e12841. https://doi.org/10.7759/cureus.12841

Ukairo, D., Ojiako, O., Nwaoguikpe, R., Ibegbulem, C., Igwe, C., & Iheme, C. (2017). Ameliorative effects of Nigerian recipes on histopathological and immunohistochemical changes of CCl_4-induced hepatic fibrosis in male Wistar rats. *Journal of Pharmacognosy and Phytochemistry, 6*(6), 1624-1631.

Veniamakis, E., Kaplanis, G., Voulgaris, P., & Nikolaidis, P. T. (2022). Effects of sodium intake on health and performance in endurance and ultra-endurance sports. *International Journal of Environmental Research and Public Health, 19*(6), Article: 3651. https://doi.org/10.3390/ijerph19063651

Wallin, B., Rosengren, B., Shertzer, H. G., & Camejo, G. (1993). Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtiter plate: its use for evaluation of antioxidants. *Analytical Biochemistry, 208*(1), 10-15. https://doi.org/10.1006/abio.1993.1002

Xin, Z., Waterman, D., Hemken, R., & Harmon, R. (1991). Effects of copper status on neutrophil function, superoxide dismutase, and copper distribution in steers. *Journal of Dairy Science, 74*(9), 3078-3085. https://doi.org/10.3168/jds.S0022-0302(91)78493-2