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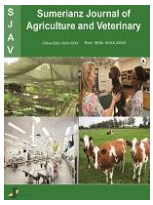

Original Article Open Access

Investigation on the Cardio-Protective Potentials of *Sphenostylis Stenocarpa* Seed Milk on Salt Loading-Induced Hypertension in Albino Rats

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Abstract

Cardio-protection is a sure way of averting the incidence of cardiovascular diseases (CVDs), which have been one of the most stubborn global causes of death. An estimated 17.9 million people died from CVDs in 2016, representing 31% of all global deaths. Of these deaths, 85% are due to heart attack and stroke. Over three quarters of CVDs deaths take place in low and middle-income countries. Out of the 17 million premature deaths (under the age of 70) due to non-communicable diseases in 2015, 82% are in low and middle-income countries, and 37% are caused by CVDs. The most preventive measure for cardiovascular disease is primary prevention of risk factors. Most cardiovascular diseases can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol using population-wide strategies. People with cardiovascular diseases or who are at high cardiovascular risk (due to the presence of one or more risk factors such as hypertension, diabetes, hyperlipidaemia or already established disease) need early detection and management using counselling and medicines, as appropriate. *Sphenostylis stenocarpa* (Hochst. ex. A. Rich) Harms is an orphan legume crop which its oil when administered to hypertensive albino rats, significantly ($p< 0.05$) lowered the atherogenic index of plasma (AIP), which is a strong marker for predicting the risk of cardiovascular disease. Hypertension was induced in rats using saturated NaCl solution. The rats were treated with the extracted oil at different concentrations. It was observed that *Sphenostylis stenocarpa* seed has low glycaemic index and that at varying concentrations and time significantly ($p < 0.05$) lowered the AIP.
Keywords: *Sphenostylis stenocarpa*; Cardio-protection; Concentration; Risk factors; Atherogen; Hypertension.

1. Introduction

Cardiovascular disease is one of the topmost causes of death world-wide and is gaining ground because of increasing trend in global civilization [1, 2]. In developing Countries, research showed that the incidence is higher in men than in women because of the reproductive hormones in women [3, 4]. The best preventive measure in cardiovascular disease is primary prevention of the risk factors and understanding their inter-related nature [1, 2, 5-7]. Hyperlipidaemia describes an abnormally high level of lipids (triacylglycerol (TAG) and total cholesterol (TC), their blood transporting lipoproteins; HDL-cholesterol and LDL-cholesterol and VLDL-cholesterol. Research has shown that there is a strong relationship between the incidence of cardiovascular disease and high level of LDL-cholesterol and also low level of HDL-cholesterol; therefore the atherogenic index-LDL-C/HDL-C ratio is often calculated to estimate cardiovascular risk [8-10]. On the other hand, high level of TAG has been related with an increased LDL-C particles and increased cardiovascular risk, therefore increase in atherogenic index of plasma (AIP), and hyperTAG, is connected with high cardiovascular risk [11-13]. Most legumes are high in proteins, minerals and fibres without saturated fat as found in animals. Nutrients in beans help improve the total blood cholesterol and triacylglycerols which made up the lipid profile [2, 14-16]. Sound cardiovascular health is very much dependent on lifestyle changes [2, 4]. This in elaborate terms is based on nutrition which is one of the best weapons to fight cardiovascular diseases. Food analysis showed that an ophan legume-*Sphenostylis stenocarpa* (Hochst. exe. A. Rich) Harms popularly called African yam beans contains novel proteins, minerals, fibres and abundant bioactive compounds which in this animal experimental model lowered the lipid profile of salt loading-induced hypertension in albino rats [10, 17, 18]. *Sphenostylis stenocarpa* (Hochst. exe. A. Rich) Harms is a legume which belongs to the family fabacea. It is distributed throughout tropical Africa [19]. Clinical studies have evaluated the effects of many different nuts and peanuts (legumes) on lipids, lipoproteins and vascular reactivity [20, 21]. Hence, the nutrients and the phytochemicals present in this legume may be the rationale behind its cardio-protective potentials-[22].

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2. Materials and Methods

2.1. Plant Material

2.1.1. Seed Collection, Identification and Preparation

A quantity, 3.0 kilogrammes of harvested Nsukka cultivar seeds of *Sphenostylis stenocarpa* (African yam bean) were purchased from Ogige Market Nsukka, Enugu State Nigeria. The seeds were identified and authenticated by a Taxonomist at the Bioresources Development and Conservation Programme (BDCP), Nsukka.

2.1.2. Methods

2.1.3. Calculation of Percentage Yield

The percentage yield of the Milk was calculated as follows:

Percentage yield = volume of milk/mass of crushed seeds x 100 %

2.1.4. Oral Acute Toxicity Study

The oral acute toxicity study was done according to the method of Lorke [23]. Eighteen albino rats were used. The test involved two stages. In stage one, the animals were divided in to (3) groups of three rats each and were administered 10, 100 and 1000 mg/kg body weight of the *Sphenostylis stenocarpa* seed milk respectively. In the second stage, 1600, 2900 and 5000 mg/kg b/w of the seed milk were administered orally to another set of animals.

2.1.5. Preparation of African Yam Bean Milk

A known weight, 2000 grammes of the African yam bean were hand-sorted in a tray, and 1000 grammes were washed with normal saline and oven-dried. The seeds were roasted using frying pan for 50 minutes at 50^0C. Using Corona manual blender, the seeds were de-hulled when hot, after which they were winnowed to remove the testa remnants from the cotyledons. The seed cotyledons were ground into fine powder and sieved using fine- pored silk. The flour obtained was transferred into a plastic container for mixing and homogenization. The flour obtained was mixed with de-ionized water in the ratio of 1:2 w/v. The middian cup of electric blender (Nakai model 462) was used for homogenization process at 10,000 revolutions per minute (rpm) for 15 minutes. After this, the African yam bean milk was ready for analytical processes. Aliquot of the milk was freeze-dried using lyophilizer. The remaining quantity was used immediately. This was repeated on daily basis to avoid contamination and auto-oxidation of labile substances. Some of the freeze-dried milk sample was used for blood pressure monitoring.

2.1.6. Characterization of the Milk

The physicochemical properties of the milk was determined using both the sensory method and instruments [24].

2.1.7. Physicochemical Analysis of the Milk

2.1.7.1. Sensory Evaluation of Milk

The colour and aroma of the milk were sensorily determined as described by Ihekoronye and Ngoddy [25].

The extracted milk was subjected to sensory analysis by 20 Panelists invited from the Department of Food Science and Technology, Federal University of Agriculture, Makurdi. The chemical properties was conducted by titration for acid value, and pH with pH metre (Jenway, 3505) and the presence of minerals was carried out using Atomic absorption spectrophotometer (AAS) (NARICT, Zaria) Nigeria.

Freshly homogenated milk of *Sphenostylis stenocarpa* was made from the milk powder. This was transported to the laboratory and immediately analysed for the elements Cu, Ca and Fe. Three replicate determinations were carried out on each sample.

2.1.8. Method

Microwave system was used for acid digestion of the milk sample. The sample was dried at 70^oC in forced stove until dry weight was obtained. An amount, 0.3 g of the sample was measured into a clean 250 ml dry Pyrex digestion flask. A volume, 10 ml of 65% Nitric acid was added, followed by the addition of 3.0 ml of 30% hydrogen peroxide. The digestion flask was heated gently until frothing subsided. The sample was then heated to dryness, dissolved in 30 ml de-ionized water and filtered with number 42 whatman filter paper. The solution was made up to volume in a 100 ml flask and stored in a special container ready for analysis [26].

2.1.9. Elemental Sample Analysis

Calibration curves were prepared for three metals using absorbance at 324.8 nm (Cu), 198.4nm (Ca) and 277.7 nm (Fe) from atomic absorption spectrophotometer (model 9190 Pye Unicam, UK). The actual concentration for each metal in the sample was there in obtained by extrapolation from the standard curves [26].

2.2. The Chemical Method

The titrable acidity test measures the amount of alkali which is required to change the pH of the milk from its initial value of about 6.6 - 6.8 to the pH of the colour change of phenolphthalein added to the milk to indicate the end- point(pH 8.3). Infact, the method measures the buffering capacity of milk and not the true acidity.

Procedure:

1. Fill the burette with 10 N NaOH solution
2. Mix the milk sample thoroughly by avoiding incorporation of air
3. Transfer 10 ml milk with the pipette in conical flask
4. Add equal quantity of distilled water
5. Add 3-4drops of phenolphthalein indicator solution and stir with glass rod
6. Take the initial reading of the alkali in the burette at the lowest point of meniscus
7. Rapidly titrate the content with 10 N NaOH solution, continue to add alkali drop by drop and stirring the content with glass rod till the first definite change to pink colour which remains constant for 10-5 seconds
8. Complete the titration within 20 seconds
9. Note down the final burette reading.

2.3. Calculation

Percentage (%) lactic acid = No. of ml of 0.1N NaOH solution rqd for neutralization x0.09/weight of sample

Weight of sample = volume of milk x specific gravity.

2.4. Mineral Composition Analysis of the Seed Milk

A quantity 2 grammes of the milk sample was placed in a crucible and later put in a furnace set at 500^{o}C for 6 hr after which it was allowed to cool for a short while in the furnace before it was transferred to a dessicator to cool to a room temperature 30^{o}C. One normal hydrochloric acid (1.0 N HCl) was used to dissolve the ash to a known volume and injected in to atomic absorption spectrophotometer to read the absorbance. The standard curve was constructed from which the concentration of each mineral was read. The actual mineral concentration was calculated as shown below:

Conc: (mg/g) = Result from AAS (mg/ml) x final volume/sample size (grammes)

2.5. Preparation of the Standard Curves for Minerals

Several samples of known concentrations of two or three solutions of different concentrations are measured in advance and the calibration curve of absorbance versus concentration is prepared. However, the absorbance of unknown sample is measured at a given wavelength and extrapolated on the standard curve to obtain the concentration of the target element.

2.6. Phytochemical Analysis

The phytochemical analysis was done using the method of Harbone [27]; Trease and Evans [28].

2.7. Determination of Alkaloids

Wagner's Test: A quantity 1 gramme of the milk sample was mixed with 10 ml 2% hydrochloric acid, boiled for five minutes and then filtered. To 1 ml of the filtrate was added 1 ml of Wagner's reagent. Creamy white precipitate indicated the presence of alkaloids. In the determination of alkaloids using Meyer's test, the oil sample, 1 gramme was mixed with 10 ml 2 % hydrochloric acid, boiled for five minutes and then filtered. To 1 ml of the filtrate was added 1 ml of Meyer's reagent and a creamy white precipitate indicated the presence of alkaloid.

2.7.1. Determination of Saponins

An amount (1 gramme) of the milk sample was added 10 ml distilled water and warmed for a minute and then filtered. The filtrate was poured into a test tube and distilled water (4 ml) was added, shaken thoroughly for 5 minutes and was allowed to stand for one minute. Persistence of foam indicated the presence of saponin.

2.7.2. Determination of Flavonoids

A quantity (1 gramme) of the milk sample was taken and macerated with 20 ml of ethyl acetate for 10 minutes and centrifuged for 5 minutes. Five millilitre (5 ml) of the supernatant were each transferred into three test tubes and 5 ml of 1 M ammonium hydroxide added and tubes were shaken vigorously for 2 to 5 minutes. The upper layers were discarded, and the absorbance of the lower layer taken at 490 nm. The blank was 1 N ammonia solution. Calculation of flavonoid quantity present was done using standard curve. Different concentrations of quercetin were prepared. The absorbance of the standard was plotted against concentration of the standard flavonoid. The slope was taken and used to calculate the quantifying of flavonoid in the test sample using the following:

(Absorbance of Sample/Slope (Standard) x Dilution factor)

2.7.3. Determination of Tannins

A quantity 0.2 gramme of the sample were measured and macerated with aqueous methanol and 1 ml was mixed 0.5 ml Folin Ciocalteau's reagent.This was followed by the addition of 1 ml of saturated sodium carbonate solution and 8 ml of water. The reaction mixtures were allowed to stand for 30 minutes at room temperature. The supernatant were obtained by centrifugation and the absorbance of the standard was plotted against the concentrations. The slope was taken and used to calculate in the quantifying of total tannins in the test sample using the following:

(Absorbance of Sample/Slope (Standard) x Dilution factor)

2.7.4. The Proximate Composition of *Sphenostylis stenocarpa* Seed Milk

2.7.4.1. Determination of Carbohydrate

The total carbohydrate content was determined by difference. The procedure described by Association of Officiating Analytical Chemists AOAC [24] was used in determining the carbohydrate content. The sum total of the moisture, fat, protein and ash content were subtracted from 100 %. Carbohydrate = 100 -(% protein+ moisture + % fat + % ash).

2.7.5. Determination of Crude Fibre [24]

A quantity, 2 grammes of finely ground sample was put in to a round bottom flask, 100 mls of 0.25 M H_2SO_4 was added and the mixture boiled for 30 minutes. The hot solution was quickly filtered. The insoluble residue was washed with hot water until it is base-free. It was dried to constant weight in an oven at 100 ^{0}C cooled in a dessicator and weighed as(C_2,) the weighed sample was incinerated in a furnace at 55^{0}C for 2 hours, cooled and re-weighed as (C_3). The crude fibre was calculated as the loss in weight on ashing.

% Crude fibre= C_2 - C_3 x 100/C_1

Where, C_1= weight of the original sample (2 grammes)

2.7.6. Determination of Crude Protein

The Micro kjeldahl method described by Association of Officiating Analytical Chemists AOAC [24] was used to determine crude protein. A quantity of sample, 2 grammes was placed into a 100 ml Kjeldahl flask and a few anti-bump granules were added. An amount, 1 gramme K_2SO_4 and 1 gramme of $CuSO_4$ catalyst were added to speed up the reaction. The flask was placed on a Kjeldahl rack and heated until a clear solution was obtained. At the end of digestion, the flask was cooled and the sample transferred to a 100 ml volumetric flask and made up to the mark with distilled water. After cooling, 20 ml of the digest was pipette- transfered in to Markham semi-micro nitrogen distiller and 10 ml of 40 % NaOH solution was added. The sample was steam-distilled liberating ammonia into a 100 ml conical flask containing 10 ml of 40% Boric acid and 2 drops of methyl red indicator. Distillation process continued until the pink colour of the indicator turns greenish. The control was titrated with 4% boric acid with end-point indicated by a change from greenish to pink colour. The percentage total nitrogen per sample was calculated as:

Percentage (%) Nitrogen = titre value of sample-blank x 0.0014 x dil NH_3 /weight of sample x 5 ml aliquot.

The Crude protein was calculated as % age crude protein (P) = 6.25 x N.

2.7.7. Determination of Moisture Content

The method described by Association of Officiating Analytical Chemists AOAC [24] was used in the determination and was based on the difference between the net weight and the weight after drying to a constant weight at (100^{0}C) for 24 hours. Crucibles were washed and dried to a constant weight in an oven at 100^{0}C .They were later removed and cooled in a dessicator and weighed (W_1), ground sample of 2 grammes was placed in the weighed moisture dish (W_2). The crucible containing the sample was kept in an oven at 100^{0}C for 5 hours and weighed. It was kept back in an oven and re-weighed after about 3 hours to ensure a constant weight (W_3). Moisture content was calculated as;

% Moisture = (W_2—W_3/W_2—W_1)

2.7.8. Determination of Fat content

The procedure outlined in Association of Officiating Analytical Chemists AOAC [24] was used to determine the fat content of the samples. A quantity, 10 grammes of the sample (milk from African yam bean) was weighed and poured into a clean thimble of known weight, and placed in the extractor or extraction flask and 50 ml of solvent (n-hexane) was introduced into the flask. Heating was done at 70^{0}C for 4 hours. The solvent was recovered and the flask was transferred which includes the oil and solvent mixture into a hot air oven. This was heated until the solvent evaporates. It was later transferred into dessicator to cool for 15 minutes before weighing the oil. Percentage fat content was calculated as:

% Fat = weight loss/ weight of sample x 100.

2.7.9. Determination of Ash Content

The method described by Association of Officiating Analytical Chemists AOAC [24] was used. The weight of a clean crucible was taken, 5 grammes of sample were added at the crucible. The crucible and content was placed on the muffle furnace rack until the sample was completely ashed. The ash in the crucible were re-weighed and the percentage ash content was calculated as:

% Ash = W_3 - W_I x 100 /W_2-W.

2.8. Preparation of Drugs

A standard anti-hypertensive drug known as propranolol was purchased from Nanka pharmaceutical/dispensing shop North Bank, Makurdi and was confirmed by the Pharmacist-in -charge of the shop.

This was bought as 80 mg/ml and was diluted using de-ionized water to concentration of 1 mg/kg b.w. and administered to the rabbits.

Atropine: This was bought as 100 mg/ml and was diluted using de-ionized water to concentrations of 10 mg/ml, 10 µg/ml, 20µg/ml and 50 µg/ml that were used.

Acetylcholine: An ampoule of 100 mg/ml bought alongside other drugs was diluted to the required concentrations using de-ionized water to 10 mg/ml, 10 µg/ml , 20 µg/ml and 50 µg/ml respectively.

2.9. Milk Preparation

The African yam bean flour and distilled water were measured in the ratio of 1:5 mass by volume. The two were put inside mortar and with pestle, were homogenized and diluted to 10 mg/ml, 10 µg/ml, 20 µg/ml and 50 µg/ml for use in the test experiment.

NB: All the drugs and extracts were generally prepared and used within 30 minutes through 1 hour from their preparation. This is to prevent contamination, phase separation and/or auto-oxidation of the labile substances.

2.10. Animal Protocol

Twenty five (25) clinically-healthy male Albino rats weighing 0.68 to 0.89 kilogrammes were purchased from the animal house of the College of Animal production, Federal University of Agriculture Makurdi, Nigeria. They were housed in clean and dried cages with wire mesh floor and standard growers mash and clean water fed them *ad libitum.* Their weight gain was measured every week by 12.00 hours so as to get the accurate weight gain at the end of acclimatization period. At the end of four weeks of acclimatization, the animals were starved of feed, but were given water alone for twelve (12) hours to hypertension- induction day. This was done to make the animals receptive to drugs and milk.

2.11. Preparation of saturated NaCl solution

An analytical grade of Sodium chloride (common salt) was purchased and an amount 8 grammes was weighed using digital top weighing balance and dissolved in approximately 100 ml of distilled water until there was the presence of undissolved solutes in the solution.

2.12. Animal Protocol

The animal models of this study are as depicted in the design below:

2.13. Experimental Design

The rats were weighed and allocated randomly into five groups of five rats each and propranolol was administered I ml while the milk was administered

Group 1: Feed + water (Normal control)
Group 2: Induced + 0.1 ml of 80 mg/kg b, w propranolol (hypertensive control)
Group 3: Induced + 100 mg/kg b. w of milk
Group 4: Induced + 200 mg/kg b. w of milk
Group 5: Induced + 300 mg/kg b.w of milk

2.14. Induction of Hypertension

Hypertension was induced in the rats after their initial baseline physical, nutritional, cardiovascular and renal parameters were assessed. This was done by intraperitoneal administration of 0.45 ml of 2.9 mg/kg b.w of saturated sodium chloride solution [29].

3. Determination of Lipid Profile Parameters

3.1. Determination of Total Cholesterol Concentration

Plasma total cholesterol concentration was assayed enzymatically with commercial test kits (Quimica Clinica Aplicada, and Biosystems, Spain) [30] The results of lipid profile assay in 3.1 are shown in Table 11.

3.2. Principle

Cholesterol is released by enzymatic hydrolysis, and the free cholesterol is oxidized with concomitant release of hydrogen peroxide, whose breakdown leads to conversion of 4-aminoantipyrine to quinoneimine (the indicator) whose concentration is determined spectrophotometrically at 500 nm.

$$\text{Choesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}O_2 + H_2O \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

3.3. Method

Three test tubes were set up labelled T_1 (blank), T_2 (standard) and T_3 (test sample) T_1 contained 0.01 ml of distilled water; T_2 contained 0.01 ml of standard cholesterol solution while T_3 contained 0.01 ml of plasma. To each tube was added 1.0 ml of Reagent A. the contents were thoroughly mixed, and incubated at room temperature for 10 minutes, after which their absorbances (A) were read at 500 nm, against the blank.

3.4. Determination of Triacylglycerol Concentration

Plasma triacylglycerol concentration was assayed enzymatically with commercial test kits (Quimica Clinica) Applicada, and Biosystems, Spain``.

3.5. Principle

In this colorimetric method, the triacylglycerol are determined after enzymatic hydrolysis with lipases. The indicator is quinoneimine formed from hydrogen-peroxide 4-amniophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Lipase
Triacylglycerols + H_2O $\xrightarrow{\text{Glycerol kinase}}$ Glycerol + Fatty acids

Glycerol + ATP $\xrightarrow{\text{GPO}}$ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dihydroxyacetone + Phosphate + H_2O_2

$2HO_2$ + 4-Aminophenazone + 4-chlorophenol $\xrightarrow{\text{POD}}$ Quinoneimine + HCI + $4H_2O$

Note: GPO = glycerol phosphate oxidase, POD = peroxidase

3.6. Procedure

Three test tubes were set up labelled T_1 (blank), T_2 (Randox triacylglyerol standard) and T_3 (test sample). T_1 contained 0.01 ml distilled water, T_2 contained 0.01 standard glucose solution while T_3 contained 0.01 ml plasma sample. To each tube was added 1.0 ml of Randox triacylglycerols reagent. The contents were thoroughly-mixed, placed in a water bath at 25 ^oC for 10 minutes, after which their absorbance (A) were read at 546 nm, against the blank in a semi auto analyzer.

3.7. Determination of High Density Lipoprotein (HDL) Concentration

Plasma HDL concentration was assayed enzymatically with commercial test kits (Quimica Clinica Applicada, and Biosystems, Spain).

3.8. Principle

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample is precipitated with phosphotungstate and magnesium ions, leaving high density lipoproteins (HDL) in the supernatant. The high density lipoprotein is then spectrophotometrically measured by means of the coupled reaction outlined below.

Cholesterol esters + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + fatty acid

Cholesterol ½ O_2 + H_2O $\xrightarrow{\text{Cholesterol oxidase}}$ cholesterol + H_2O_2

$_2H_2O_2$ +4-aminoantipyrine+ DCPS $\xrightarrow{\text{Peroxidase}}$ quinoneimine + $4H_2O$

Note: DCPS = Dichloroophenolsulphonate

3.9. Procedure

Three test tubes were set up labelled T_1 (blank), T_2 (standard) and T_3 (test sample) T_1 contained 0.50 ml of distilled water; T_2 contained 0.50 ml of standard cholesterol solution while T_3 contained 0.50 ml of plasma. To each tube was added 1 ml of Reagent A, the contents were thoroughly mixed and allowed to stand for 10 minutes at room temperature, before they were centrifuged at 400 rpm for 10 minutes before reading their absorbance's (A) at 500 nm, against the blank, in a semi auto analyzer.

3.10. Statistical Analysis

This was carried out using statistical product of service and solutions (SPSS), version 18 (SPSS Inc.). Significant differences between means were determined by Duncan's multiple range test and regarded significant at $p < 0.05$. Results were presented as mean ± SD.

3.11. Results

Table-1. The percentage yield of oil and milk extracts of *Sphenostylis stenocarpa*

S/N	Seed oil	Seed milk
1.	4.0 ± 0.22	20.0 ± 1.02

Results are expressed as mean ± SD (n= 3).

Table-2. Physical Properties of the *Sphenostylis stenocarpa* seed oil

Parameters	Inference
Colour	Pale yellow

Odour	Sweet-smelling
Viscosity	830 ± 1.00 centipoise
Refractive index at 20^0C	1.48 ± 0.04

Results are expressed as mean ± SD (n= 3)

Table-3. Chemical Properties of *Sphenostylis stenocarpa* seed oil

Parameters	**Inferences**
Acid value	2.9 ± 0.04 mg/KOH/g
Iodine value	132 ± 1.02 g/I_2/100g
Peroxide value	8.02 ± 0.04 mEq/kg
Saponification value	194.01 ± 0.05 ml/KOH
pH value	5.25 ± 1.03
Free Fatty Acids (FFAs)	1.46 ± 1.12 %

Results are expressed as mean ± SD (n= 3).

Table-4. Physical Properties of the *Sphenostylis stenocarpa* Seed Milk

Parameters	**Inference\Sensory Result**
Colour	Brown
Aroma	5.8 ± 1.02
Taste	6.4 ± 0.08
Mouthfeel	6.2 ± 1.00
Overall acceptability	6.8 ± 1.04

Results are expressed as mean ± SD (n=3)

Table-5. Chemical Properties of the *Sphenostylis stenocarpa* seed milk

Paramerters	**Inference**
Titratable acidity	25.24 ± 1.00
pH	4.76 ± 0.05

Results are expressed as mean ± SD (n= 3).

Table-6. Mineral composition analysis of *Sphenostylis stenocarpa* seed milk

Parameters	**Inference**
Calcium	0.12605 ± 1.03 mg/100 g
Iron	0.05605 ± 0.07 mg/100 g
Copper	0.0009 ± 1.02 mg/100 g
Lead	Not detected
Cadmium	Not detected

Results are expressed as mean ± SD (n= 3).

Table-7. Proximate composition of *Sphenostylis stenocarpa* seed milk

Parameters	**Values (%)**
Carbohydrates	60.26 ±1.02
Protein	19.21 ± 0.06
Fibre	9.24 ± 0.18
Moisture	5.05 ± 2.04
Ash	3.40 ± 0.04
Lipids	2.84 ± 0.14

Results are expressed as mean ± SD (n= 3). Results are significant at value $p < 0.05$.

Table 11: Effect of *Sphenostylis stenocarpa* seed Milk on Lipid Profile of Hypertensive rats

Values are expressed as means ± SD (n = 5). Results are significant at value $p < 0.05$

Group 1 = No induction, feed + water (Normal control)

Group 2 = Hypertensive rats treated with 0.1 ml of 80mg/kg b.w of propranolol

Group 3 = Hypertensive rats treated with 100 mg/kg b.w of milk

Group 4 = Hypertensive rats treated with 200 mg/kg b.w of milk

Group 5= Hypertensive rats treated with 300 mg/kg b.w of milk

Groups	**Total Chholesterol (mg/dl)**	**TAGs (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**	**AI (mg/dl)**
1	148.00 ± 0.04	60.03 ± 4.02	65.20 ± 4.00	36.40 ± 1.20	0.56 ± 0.01
2	136.20 ± 2.03	58.05 ± 3.00	63.20 ± 2.02	30.20 ± 1.20	0.48 ± 0.00
3	125.70 ± 0.02	54.60 ± 1.00	59.10 ± 3.02	27.20 ± 3.00	0.46 ± 0.01
4	112.90 ± 2.00	51.30 ± 2.02	52.40 ± 1.20	23.10 ± 1.22	0.44 ± 0.02
5	98.00 ± 1.00	47.40 ± 3.05	44.30 ± 2.04	19.24 ± 3.00	0.43 ± 0.03

Values are expressed as mean ± SEM, (n=5).

Figure-1. Effect of *Sphenostylis stenocarpa* seed milk on mean total cholesterol

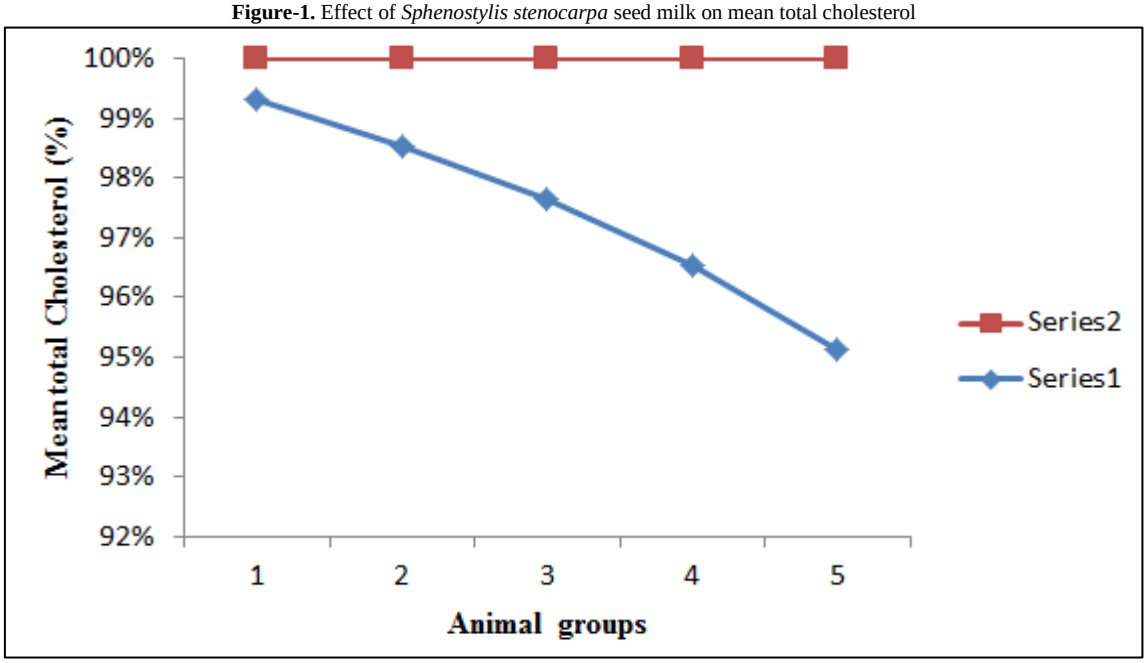


Figure-2. Effect of *Sphenostylis stenocarpa* seed milk on TAGs of Albino Rats

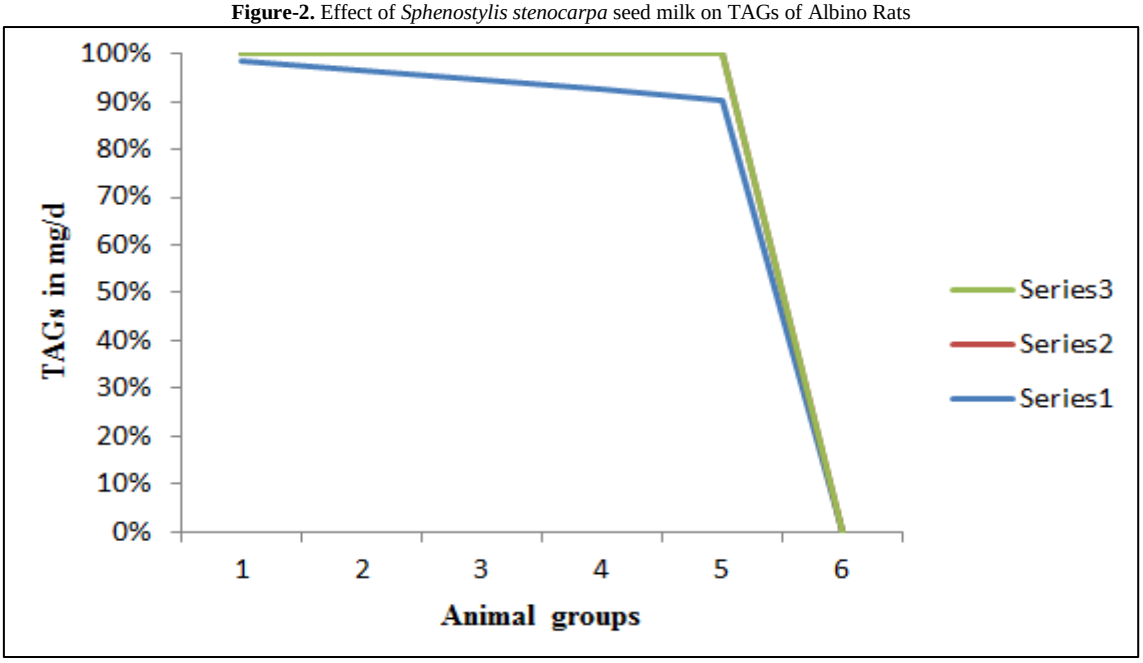


Figure-3. The effect of *Sphenostylis stenocarpa* seed milk on mean HDL of Albino Rats

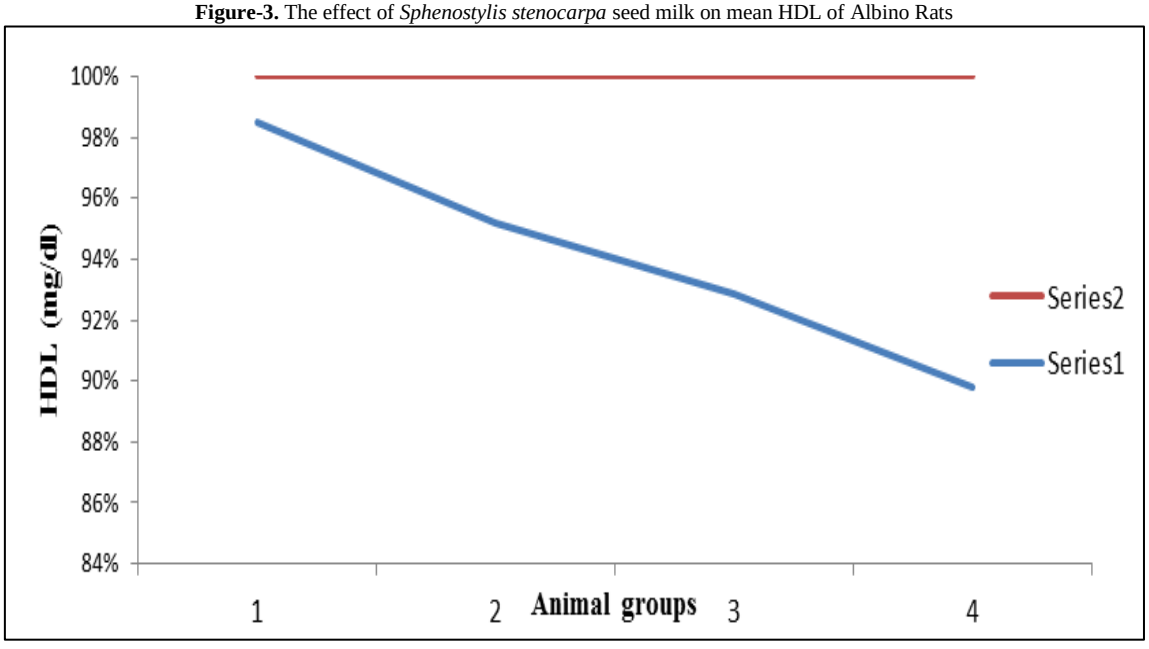


Figure-4. Effect of Sphenostylis stenocarpa on LDL

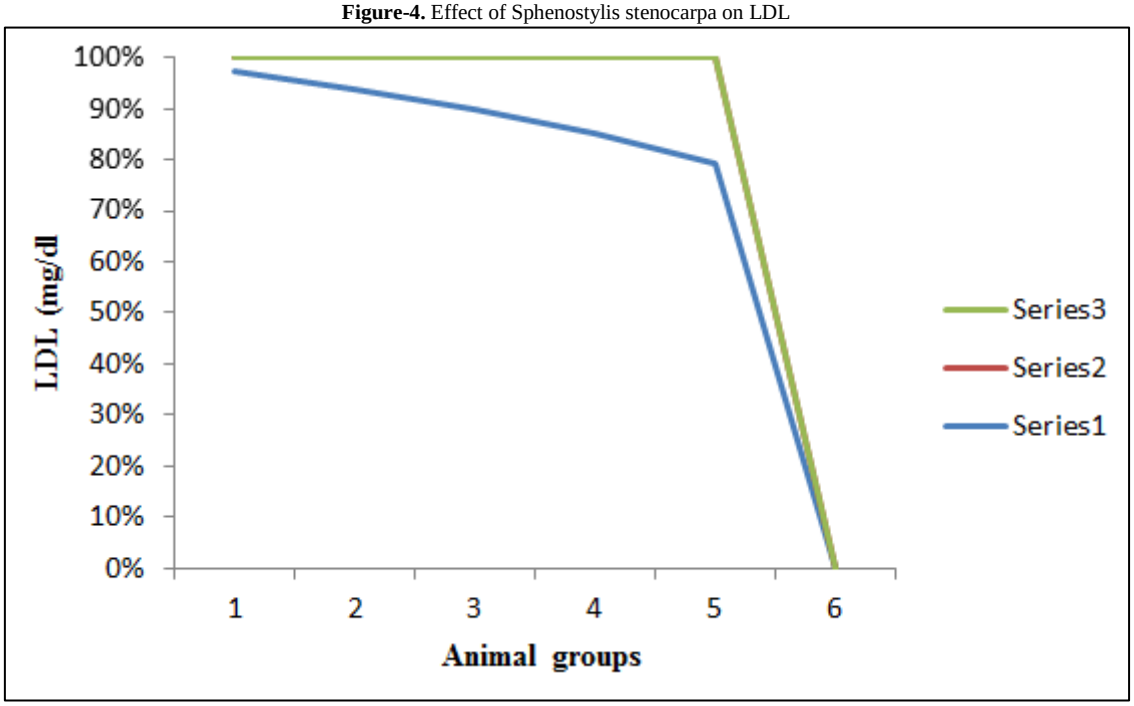
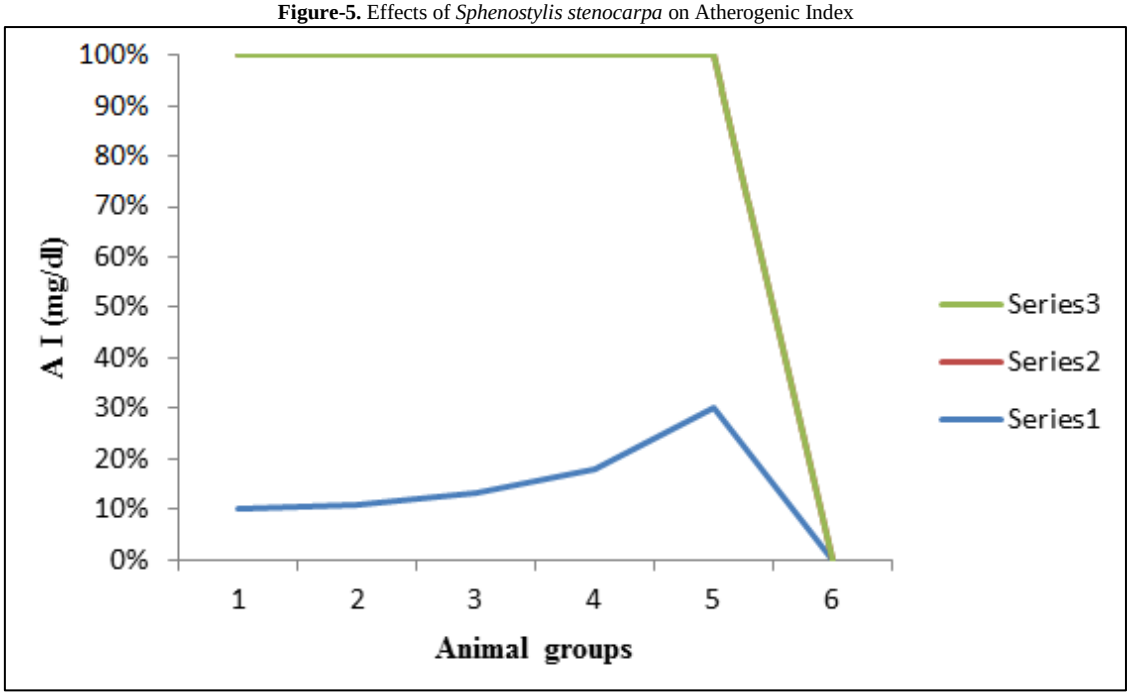


Figure-5. Effects of *Sphenostylis stenocarpa* on Atherogenic Index



4. Discussion

The results of this study showed that the seed milk of *Sphenostylis stenocarpa* had a percentage yield of 75.00 ± 1.02 from 900g of *Sphenostylis stenocarpa* seed. This agrees with the work of Nnam [31], Awonorin and Udeozor [32]. In this study, the physical properties of *Sphenostylis stenocarpa* seed milk revealed the following: colour; sand brown, the sensory evaluation of the milk as described by Ihekoronye and Ngoddy [25], gave aroma; 5.8, taste; 6.4, mouthfeel; 6.2, and overall acceptability 6.8 respectively. However, the chemical properties of the seed milk such as titratable acidity gave 25.24 and the pH was 4.76. The observed results indicate that the plant milk was slightly acidic and agrees with the work of Nnam [31], Onweluzo and Owo [33], Awonorin and Udeozor [32]. Also, the acidic nature of maintaining the buffering system remained intact. Moreover, the mineral composition of *Sphenostylis stenocarpa* seed milk revealed calcium, iron and copper in trace concentrations and the results agree with the chemical properties of Tigernut-soy milk analysis by Nnam [31], Onweluzo and Owo [33], Wong and Vanessa [34], Awonorin and Udeozor [32].

The results of the proximate composition of AYB milk showed that the mean moisture value was 5.02 ± 2.04% dry weight. This result is somehow lower when compared with the mean moisture value of legumes ranging between 7.0 % and 11.0 % as reported by Aremu, *et al.* [17], Arkroyed and Doughty [35], Chinedu and Nwinyi [36]. However, this value is comparable to the moisture content of cashew nut flour (5.7%). Aremu, *et al.* [17], Ige, *et al.* [37], had earlier reported a content of 5.0% for fruited pumpkins. The mean ash content of AYB milk in this study

was 3.40 ± 0.04. This value is comparable to the value reported by Aremu, *et al.* [17], for other nuts. Oboh and Omofoma [38], had recommended that the ash content of nuts, seeds and tubers should fall in the range of 1-2.5% in order to be suitable for animal feed [39, 40]. In this study, the ash content of AYB milk was approximately within the range and therefore may be recommended for animal feeds. The mean lipid value of 2.84 ± 0.14 reported in this study was relatively close to the values for other varieties of oil seeds ranging between 47-51.1% as reported by Ige, *et al.* [37], Fagbemi and Oshodi [41], Aremu, *et al.* [17], had earlier reported a mean lipid values of 49.2% and 47.01% respectively for pumpkin seed which is still closely related to the result obtained in this study. However, the value got in this study is comparably lower than the values of 36.7 as reported for cashew nut flour by Aremu, *et al.* [17], and 23.5% for soy bean by Pomeranz and Clifton [42]. Lipids are important in diets because they promote fat-soluble vitamins absorption [43, 44]. It is also a high energy nutrient and does not add to the bulk in the diet. The crude proteins of AYB milk was found to be 19.21 ± 0.06. This value is low compared to the protein content of soy bean, cowpeas, pigeon peas, melon, pumpkin and gourd seeds ranging between 23-33.0% [45]. The implication of the protein level was that AYB milk can supply the recommended daily intake (1,800 kcal) of protein for children [46]. Apart from the nutritional significance of proteins as source of amino acids, they also play a part in the organoleptic properties of foods [16, 47, 48]. Proteins also organize the overall metabolic processes in the body in form of enzymes, hormones and transporters [6, 18]. The crude fibre of AYB milk was found to be comparable to other legumes with mean value ranging between 5-6% as reported by Aremu, *et al.* [17]. The maintenance of internal distension for a normal peristaltic movement of the intestinal tract is the physiological role which crude fibre plays. [49] reported that a diet low in fibre is undesirable as it could cause constipation and that such diets are usually associated with diseases of the colon like piles, appendicitis and cancer [17]. The value obtained for carbohydrates by difference is 60.26 ± 1.02 %. This value is a bit higher with an acceptable mean values for legumes, 20-60% of dry weight [35]. This result justifies the *Sphenostylis stenocarpa* as a possible rich source of energy and may be capable of supplying the daily energy requirement of the body [17].

The effect of African yam bean seed milk on serum total cholesterol, triacylglycerols, high density lipoproteins, low density lipoproteins and atherogenic index of plasma showed that there was a significant ($p < 0.05$) decrease in the total cholesterol of the hypertensive rats treated with graded doses of African yam bean milk across the treatment groups (1-5) compared with the control group. This suggests that *Sphenostylis stenocarpa* seed milk may have affected cholesterol biosynthesis which resuted to reduction in the level of cholesterol in the blood. This result is in agreement with the work on other legumes [12, 38, 50]. It also agrees with the work of Ikpa [10], who reported a decrease in total serum cholesterol levels of rats fed African yam bean milk. There was also a significant ($p < 0.05$) decrease in the triacylglycerols values of the test groups after 15 days of treatment with African yam bean seed milk. This supports the report of Frota [50], who found out that cowpea whole seed decreased triacylglycerols levels of hypercholesterolemic rats. It also corroborates the work of Ikpa [10], who reported decreased triacylglycerols levels. There was a significant ($p < 0.05$) increase in high density lipoproteins (HDL) level after 15 days treatment with graded doses of the African yam bean seed milk. This was in line with the study where the incorporation of 2.5% and 25% cluster beans increased the concentration of HDL-associated cholesterol [12]. The study by also had increased HDL cholesterol after feeding African yam bean to diabetic rats. The high level of high density lipoproteins in rats treated with African yam bean has both nutritional and health implications. It is known that HDL-choesterol concentration for animals and humans has some advantages over other cholesterol [10]. The low density lipoproteins (LDL) cholesterol level of the rats treated with graded doses of African yam bean seed milk shows significant ($p < 0.05$) decrease after 15 days of treatment. The decreased value associated with increase in HDL concentration that might have caused LDL removal from the liver in to bile salts for excretion. This agrees with the work on other legumes. According to Oboh and Omofoma [38], feeding of hypercholesterolemic rats with heat-treated Lima beans for thirty days significantly ($p < 0.05$) reduced the LDL-cholesterol. Also, a study by Ikpa [10], shows reduction in LDL-cholesterol after feeding the rats with African yam bean seed milk for 15 days. However, this study showed a significant ($p < 0.05$) reduction in atherogenic index of plasma (AIP) and LDL-cholesterol, and also showed a significant increase in HDL-C in a dose and time-dependent manner. African yam bean milk had a potential cardio-protective properties.

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