



RESEARCH ARTICLE

EFFECTS OF RAW AND COOKED AQUEOUS AND METHANOL EXTRACTS OF *PHASEOLUS VULGARIS* (KIDNEY BEANS) ON RENAL FUNCTION IN ALBINO WISTAR RATS

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Abstract



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Objective: *Phaseolus vulgaris*, like other beans, is endowed with rich nutritional contents. This study evaluated the effects of raw and cooked aqueous and methanol extracts of *P. vulgaris* on renal function in albino Wistar rats.

Methods: Oral acute toxicity (LD₅₀) study of both extracts was conducted in two phases. In the main design, a total 36 wistar albino rats were used and divided into nine groups of four rats and oral administration lasted for 7 days. Group 1 served as control and 2-9 treated groups. Groups 2 and 3; 4 and 5 were administered aqueous extracts while groups 6 and 7; 8 and 9 were administered methanol extracts of 350 mg/kg and 550 mg/kg body weight raw and cooked *P. vulgaris* respectively.

Results: Results of LD₅₀ of all extracts were greater than 5000 mg/kg. Results showed a significant ($p < 0.05$) increase in concentrations of urea and chloride across test groups administered aqueous extracts, than methanol extracts; a significant ($p < 0.05$) increase in serum creatinine in test groups administered methanol extracts; a significant ($p < 0.05$) increase of serum total protein of test groups compared to control; no significant ($p < 0.05$) difference in the concentration of potassium in test groups administered compared to control group.

Conclusion: It may be concluded that *P. vulgaris* portrays potentials capable of improving renal function and its consumption may contribute to the wellness of a person due to its rich nutrients, and based on the duration of this work and standard scale of toxicity; the extracts are practically non-toxic since the LD₅₀ was greater than 5000 mg/kg.

Keywords: Creatinine, kidney function, *Phaseolus vulgaris*, potassium chloride, urea.

INTRODUCTION

The legume family is a large plant family including edible peas, peanuts, lentils, chickpeas and beans. Bean such as kidney bean, black eyed bean, pinto, pink and navy beans have been reported^{1,2}. Like other beans, *Phaseolus vulgaris* (kidney beans) is a nutrient rich food containing minerals, vitamins, and useful nutrients, which supply a reasonable amount of calories to the body. Generally, beans are reported to be composed of mineral elements such as potassium, magnesium, sodium, iron; trans fat, total fat and cholesterol may be present in small amount, and folate and fiber³. Electrolytes in food are present in the form of essential minerals such as potassium, chloride, sodium, phosphorus, magnesium and bicarbonate. Thus, foods and drinks contain electrolytes which are

essential minerals, indispensable for vigorous performance of the muscles and nerves. When levels of electrolyte in the blood become abnormally low or high, imbalances set in, which may possibly be due to dehydration, vomiting, diarrhea, kidney disease, eating disorders and severe burns⁴. Chloride is an anion that is richly found in the compartment of extracellular fluid (ECF). The concentration of chloride in the serum may abnormally become high (hyperchloremia) or low (hypochloremia). Kidney failure and acute kidney injury are reported to be associated with hyperchloremia⁵. Potassium is a cation that is abundantly found in intracellular fluid, performing an essential function in nerve and muscle cells. It is reported to be richly distributed in plant foods including kidney beans⁶. Potassium is reduced and exchanged for sodium in foods by addition of salt and

disposal of the liquid broth. In adults with hypertension, increase consumption of potassium result in blood pressure reduction, which could lower the risk of stroke and cardiovascular diseases⁷.

Kidney bean is rich in soluble fiber and its consumption is reported to be helpful in the synthesis of propionate and butyrate, short chains fatty acids capable of lowering LDL and total cholesterol, therefore reducing risk factors for hepatic disease⁸. The rich source of flavonols in kidney bean as antioxidant is linked to its function as anti-cancer food; its anti-diabetes ability is linked to its lower glycemic index as compared to other carbohydrate sources^{9,10}. The rich nutrients content of fiber and protein in kidney bean are very vital nutrients in considering diet in weight loss. The feeling of satiety is enhanced by fiber and protein is reported to excite hunger by decreasing levels the hormone, ghrelin¹¹. Kidney function is promoted in the absence of factors such as diabetes, infections, cancer, toxic chemicals, autoimmune disease and endocrine disorders¹². Kidney function can be badly affected by high blood pressure, which may result in blood vessels damage in the kidney, hampering an effective removal of waste products of excretion¹². Glomerular filtration rate (GFR) is a useful parameter that defines renal function. It can be evaluated in the blood through creatinine and urea ratio. In renal failure, GFR is decreased. In the case of kidney failure, the kidney replacement therapy often used is hemodialysis which is very significant in the removal of urea, creatinine and water as waste products from the blood. Creatinine that is produced in muscles is removed from the body as excretory non toxic waste product by the kidneys. The production and excretion of creatinine by the kidneys help to equilibrate its concentration in the blood¹². Serum creatinine concentration is said to be affected by sex, ethnicity, age, diet and life style¹³. Creatinine clearance, which is a 24hour collection of urine test, is used as an effective measurement of kidneys function. Result from creatinine clearance test reveals the quantity of creatinine that passed through the kidneys into the urine. The use of serum creatinine test alone cannot measure the effectiveness of the kidneys¹³. Urea is an organic compound excreted as waste product of dietary protein and needed in the metabolism of nitrogen containing molecules. Blood urea concentration increases in kidney failure. Though urea and creatinine are metabolic waste products but are not directly toxic as they are only used to measure kidney function¹⁴.

MATERIALS AND METHODS

Collection and Authentication of Bean Seeds

Procured bean seeds from Ogbete main market in Enugu state, Nigeria were identified and authenticated by a Taxonomist, Mr. Onyeukwu Chijioke John, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu state. A voucher number of UNH no 452 (UNH stands for University of Nigeria Herbarium), was allocated.

Preparation of raw and cooked aqueous extract of kidney bean

In the preparation of raw sample, from the dried raw seeds, 500 g was weighed and ground into powder, stored with appropriate label in a clean grease free airtight bottle. Preparation of the cooked sample involves washing and cooking stone and dirt free dried seeds of *P. vulgaris* to soft and until without broth to eliminate loss of phytochemicals; and dried with careful monitoring under the sun for 14 days. Powder (flour) of the cooked sample was prepared by weighing 500 g of the dried cooked seeds, ground and stored in a clean grease free airtight container and labeled correctly.

Preparation of dry extract from samples

Methanol and aqueous extracts of raw and cooked samples were prepared from the powdered samples, by weighing 200 g of powder into 700 ml of the appropriate and respective solvent and soaked, carefully sealed, and for proper extraction, it was left standing for two days, filtered using whatman filter paper. At temperature of 70°C, with the use of water bath, the filtrate was concentrated. According to the body weight of the rats, the formula below was used to calculate the volumes of extracts administered⁶:

$$\text{Volume to be administered (ml)} = \frac{\text{Rats wt X Dose conc.}}{\text{Conc. of extract}}$$

Collection and Preparation of Blood Sample

The rats were made unconscious and 3milliliters (3mls) of blood was collected through cardiac puncture into plain bottles and EDTA bottles. Serum and plasma collection were collected by refraction and stored in the refrigerator for biochemical analysis⁷.

Study Animal

Female Wistar albino rats weighing 68-217 kg were gotten from the University of Nigeria Nsukka. Rats were kept at an ambient temperature and relative humidity in the animals' house of Department of Biochemistry, Faculty of natural sciences, Caritas University, Amorji-Nike Enugu. The rats were given standard pelletized finisher feed and clean water before the experiment, for one week period of acclimatization. The principle of laboratory animals care and ethical guidelines for investigation of experimental pain in conscious animals were followed respectively^{15,16}.

Oral Acute Toxicity (LD₅₀) Study

Lethal Dose (LD₅₀) of the aqueous and methanol extracts of raw and cooked kidney bean was determined using the method of Lorke¹⁷ on Wistar albino rats. This was done in two phases (phase 1 and 2 respectively). At phase 1, a total of twenty four (24) rats were used. The rats were divided into twelve (12) groups of two (2) rats per cage. Administered doses are 10 mg/kg, 100 mg/kg, and 1000 mg/kg, to 2 rats each. Prior to and after administration of extract, the body weight of rats was taken. At the oral administration of single dose, observations for toxic symptoms, such as behavioral changes, loco-motion, convulsion and mortality, at day time and then overnight were made. While there was no mortality at phase 1, at phase 2, higher doses were administered to two rats each, of 12 groups, of twenty four (24) rats. The higher doses of the various extracts administered were (1600, 2900 and

5000) mg/kg body weight respectively. Observation of toxic signs made include; paw licking, salivation, rubbing of nose on floor, change in body weight and death within 24 hours. The amount or lethal dose (LD₅₀), of substances given all at once, which causes the death of 50% of a group of test animals was determined by the formula below;

$$LD_{50} = \sqrt{\text{Min. Conc. that caused death} \times \text{Max. conc. that result to no death}}$$

Experimental Design

A total of thirty six (36) Wistar albino rats were used and divided into nine (9) groups of four (4) rats per cage and were treated with aqueous and methanol extracts for one week and at the end, all rats were euthanized with chloroform, blood sample was collected for biochemical analysis. The rats were arranged and treated as follows;

Group 1: Control group no extract was administered.

Group 2 and 3: Group two and three rats were treated with aqueous extract of raw kidney bean with doses of 350 mg/kg and 550 mg/kg respectively.

Group 4 and 5: Group four and five rats were administered with aqueous extract of cooked kidney bean with doses of 350 mg/kg and 550 mg/kg respectively.

Group 6 and 7: Group six and seven rats were treated with methanol extract of raw kidney bean with doses of 350 mg/kg and 550 mg/kg respectively.

Group 8 and 9: Group eight and nine rats were treated with methanol extract of cooked kidney bean with a dose of 350 mg/kg and 550 mg/kg respectively.

Statistical Analysis

One-way ANOVA (analysis of variance) was used in the analyses of results, employing a component of Graph pad prism instat 3 software version 3.05 by graph pad Inc. P value ($p < 0.05$) was considered significant and results were expressed as mean \pm standard deviation¹⁸.

RESULTS

Oral Acute Toxicity (LD₅₀) Results

Table 1 reveals results of the oral acute toxicity of aqueous and methanol extracts of fresh and cooked kidney bean. Doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg were administered to 2 rats each in the 1st phase, of which no mortality was observed. In the absence of mortality in the 1st phase, higher doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg were then administered on 2 rats each for the 2nd phase of which no mortality observed as well with strict observance on paw licking, salivation, rubbing of nose on floor, change in body weight and death within 24 hours. Results of LD₅₀ of all extracts were found to be greater than 5000 mg/kg and based on the duration of this work and standard scale of toxicity; the extracts are practically non-toxic.

Table 1: Phase I and II acute toxicity of aqueous and methanol extracts of raw Kidney Bean and cooked Kidney Bean.

Study	Dose (mg/kg)	Number of dead rats after 24 hours			
		RKBAE	CKBAE	RKBME	CKBME
Phase I	10	0/2	0/2	0/2	0/2
		0/2	0/2	0/2	0/2
	100	0/2	0/2	0/2	0/2
		0/2	0/2	0/2	0/2
1000	0/2	0/2	0/2	0/2	
Phase II	1600	0/2	0/2	0/2	0/2
		0/2	0/2	0/2	0/2
	2900	0/2	0/2	0/2	0/2
		0/2	0/2	0/2	0/2
5000	0/2	0/2	0/2	0/2	

Key: RKBME= Raw kidney bean methanol extract, CKBME= Cooked kidney bean methanol extract, RKBAE = Raw kidney bean aqueous extract, CKBAE = Cooked kidney bean aqueous extract.

The renal function test of rats after administration of raw and cooked kidney bean aqueous extracts on serum urea, creatinine, chloride and potassium is shown in Table 2. There was a significant ($p < 0.05$) increase in urea, creatinine and chloride concentrations between test groups of raw and cooked extracts as compared to control group but there was no significant ($p < 0.05$) difference in the concentration of potassium between test groups of raw and cooked extracts as compared to control group. However, the values of urea and chloride of rats administered cooked extracts is significantly ($p < 0.05$) higher than those of raw extracts irrespective of the dose and rats administered raw extracts had a significantly ($p < 0.05$) higher value of creatinine than those administered cooked extracts, in a non dose dependent manner. Results show a significant ($p < 0.05$) increase between control and test

groups of Urea and a significant ($p < 0.05$) decrease between test and control group of Creatinine, Chloride, and Potassium. Table 3 reveals result of kidney function test of rats administered 350 mg/kg and 550 mg/kg body weight of raw and Cooked Methanol Extracts. A significant ($p < 0.05$) increase in the concentrations of urea, creatinine and chloride of test groups when compared with control group. While there was no significant ($p < 0.05$) increase in the concentration of potassium in test groups as compared to control group. Meanwhile, concentrations of urea and chloride of rats administered raw extracts were significantly ($p < 0.05$) higher than those administered cooked extracts, in a non dose dependent pattern and the concentration of creatinine of rats administered cooked extract was significantly ($p < 0.05$) higher than those administered raw extract not in a dose dependent pattern.

Table 2: Rats administered with (350 mg/kg and 550 mg/kg body weight of rat) raw and cooked aqueous extracts.

Group	Control	350 mg Aqueous Extract		550 mg Aqueous Extract		Range
	1	2 _{Raw Extract}	4 _{Cooked Extract}	3 _{Raw Extract}	5 _{Cooked Extract}	
UREA(mg/dl)	8.335±11.52 ^{abcde}	5.325±0.09192 ^b	33.28±13.10 ^c	12.13±2.199 ^d	16.25±2.044 ^e	10-40
CREA(μmol/l)	94.76±62.42 ^{abcde}	55.73±30.50 ^b	36.53±5.812 ^c	40.15±3.882 ^d	35.29±4.752 ^e	
CHL(mEq/l)	95.40±8.280 ^{abcd}	67.83±3.663 ^b	96.38±6.767 ^a	67.83±5.431 ^c	54.20±3.097 ^d	95-105
POT(mEq/l)	4.035±0.03536 ^a	3.615±0.1909 ^a	3.845±0.219 ^a	3.650±0.1273 ^a	3.695±0.1768 ^a	3.4-5.0

Results are mean ± standard deviation, Values in the same row bearing different superscripts are significantly different at $p < 0.05$. (n=4). Key: 1: Control Group, Crea=Creatinine, CHL= Chloride, POT= Potassium.

There was a significant ($p < 0.05$) increase between test groups (2,3,4,5,6,7,8 and 9) and control group as shown in Figure 1 and Figure 2 respectively.

DISCUSSION

Results of LD₅₀ (Table 1) of all extracts (raw and cooked aqueous and methanol) of *P. vulgaris* were found to be greater than 5000 mg/kg and no mortality was observed in both phases of the study. While there was no mortality recorded with the use of both extracts of *P. vulgaris* even up to 5000 mg/kg body weight, it could be associated to the rich beneficial antioxidant activities it possesses^{19,20}. Thus, based on the duration of this study and standard scale of toxicity; the extracts of *P. vulgaris* (raw and cooked aqueous and methanol) are established to be practically non-toxic on the absence of coma, convulsion, restlessness and death^{21,22}.

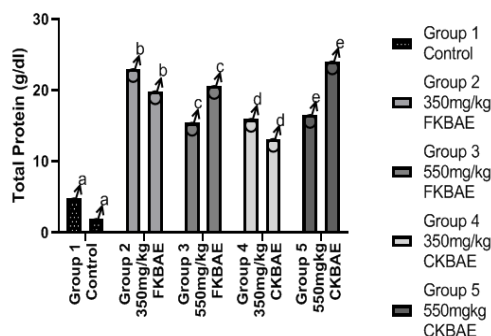


Figure 1: Concentration of total protein of rats treated with 350 mg/kg and 550 mg/kg FKBAE and CKBAE

Results are expressed as Mean±Standard Deviation (n=4). FKBAE =Fresh (Raw) kidney bean aqueous extract, CKBAE =Cooked kidney bean aqueous extract. Comparing control group 1 with test groups 2, 3, 4 and 5 respectively, alphabets; a, b, c, d, and e indicates significant difference ($p < 0.05$)

Observations made (Table 2 and Table 3) after administration of cooked and raw aqueous and methanol (350 mg/kg and 550 mg/kg body weight of rat) extracts to all groups revealed that administration of cooked extract of *P. vulgaris* resulted in a significant ($p < 0.05$) increase in concentrations of urea and chloride than those of raw extracts not minding the dose, and rats administered raw extracts had a significantly ($p < 0.05$) higher value of creatinine than those administered cooked extracts, in a non dose dependent manner. There was no significant ($p < 0.05$) difference in the concentration of potassium between test groups and control group. Increased serum urea

concentration could be due to dietary protein content of the cooked bean, since urea is synthesized from protein catabolism as a nitrogenous waste product of metabolism and because dietary protein influences the amount of blood urea^{23,24}. In a kidney disease condition, consumption of protein, vitamins, minerals and calories in the proper and healthy measure is vital to keep the kidney condition from getting worse²⁵. When urea is reabsorbed and secreted by the kidney, via glomerular filtrate, the resultant is extreme concentrated urine. This action makes urea to perform two physiologically linked functions; conservation of water and ammonia detoxification functions²³. Urea in blood is transported into glomerular filtrate in kidney undergoing glomerular filtration. Serum urea concentration is contributive of the equilibrium between its production by the liver and removal by the kidneys, via urine. Thus, serum urea concentration can be due to over production by the liver, decreased excretion by the kidneys or both²⁶.

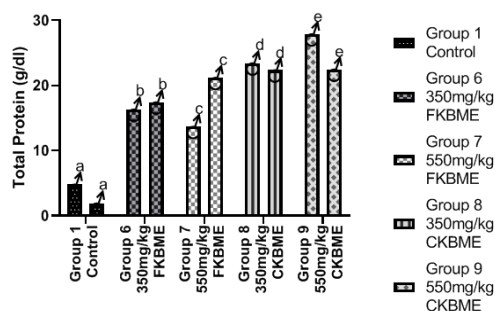


Figure 2: Concentration of total protein of rats treated with 350 mg/kg and 550 mg/kg FKBAE and CKBAE

Results are expressed as Mean ± Standard Deviation (n=4). FKBAE = Fresh (Raw) kidney bean methanol extract, CKBAE= Cooked kidney bean methanol extract. Comparing control group 1 with test groups 6, 7, 8 and 9 respectively, alphabets; a, b, c, d, and e indicates significant difference ($p < 0.05$).

Serum urea balance is also affected by loss of small amount through sweat and the gut²⁶. Clinically, kidney function is significantly measured by glomerular filtrate rate (GFR), a parameter that is rationally measured using blood creatinine and urea. In the presence of normal kidney function (normal GFR), serum urea concentration may be high, thus in testing for renal function, urea cannot be recommended for routine measurement because it is not a better choice for assessing GFR compared to creatinine since other non renal conditions, such as dietary protein can also increase the level of serum urea²⁶. Glomerular filtration rate is lowered in renal failure, with a very important relationship between aggravation of renal disease.

Table 3: Rats administered with (350 mg/kg and 550 mg/kg body weight of rat) raw and cooked methanol extracts.

Group	Control	350 mg Methanol Extract		550 mg Methanol Extract		Reference
	1	6 _{Raw Extract}	8 _{Cooked Extract}	7 _{Raw Extract}	9 _{Cooked Extract}	Range
UREA(mg/dl)	8.335±11.52 ^{abcd}	44.04±23.77 ^b	20.81±0.000 ^c	43.92±46.44 ^b	34.40±3.422 ^d	10-40
CREA(mg/dl)	94.76±62.42 ^{abcde}	29.67±21.86 ^b	25.68±11.29 ^c	20.70±7.757 ^d	40.40±10.23 ^e	
CHL(mEq/l)	95.40±8.280 ^{abcde}	109.9±6.640 ^b	80.21±11.31 ^c	64.83±1.329 ^d	101.1±36.84 ^e	95-105
POT(mEq/l)	4.035±0.04 ^a	3.695±0.11 ^a	3.490±0.09 ^a	3.600±0.01 ^a	3.525±0.22 ^a	3.4-5.0

Results are mean ± standard deviation, Values in the same row bearing different superscripts are significantly different at $p < 0.05$. (n=4). Key: 1: Control Group, Crea=Creatinine, CHL= Chloride, POT= Potassium.

The rate at which GFR decreases provides distinctions between acute renal injury and chronic renal disease. In chronic renal disease, decrease in glomerular filtration rate is a somewhat permanent or very slow in reverting, taking a period of months, years, or decades; while in acute renal injury, GFR can revert within a period of hours or days²³. In Table 2 and Table 3, the concentration of creatinine in test group is significantly ($p < 0.05$) lower when compared with control. Consistently, increase in serum creatinine is a consequence of decreased GFR and subsequent reduced kidney function or renal disease. However, since creatinine level in the blood is affected by gender, age, body size and race; assessing kidney function with how much creatinine is in the blood is not the best option either but glomerular filtration rate, which is a concurrent measurement of creatinine and estimation of urea/creatinine ratio¹². There was significant ($p < 0.05$) difference in serum level of chloride (Table 2 and Table 3) between test groups and control group. However, in comparison, the levels of chloride of rats administered (350 mg/kg and 550 mg/kg body weight) aqueous extracts (Table 2) were lower than those of methanol extracts (Table 3) of the same dose. Chloride is reported to be found in foods and drinks in appreciable content, thus, the high content of chloride in *P. vulgaris* in this study is consistent with available records, since the chloride levels in this study falls within standard reference value⁶. The transport of chloride is often coupled with sodium, the preservation of chloride equilibrium in the body. Kidney failure may result in hyperchloremia, a condition of abnormally high blood chlorine concentration. In a normal working kidney, more than 50% of the filtered chloride is absorbed shortly after the absorption of a relative portion of sodium and water. However, sodium, bicarbonates and some anions other than chloride are quickly being absorbed and eventually excreted out of the filtrate²⁷. There was no significant ($p < 0.05$) difference in serum level of potassium between test groups and control group, administered 350 mg/kg and 550 mg/kg body weight of raw and cooked aqueous and methanol extracts (Table 2 and Table 3). However, the concentrations of potassium in this study is within the standard reference range, which depict the claim that *P. vulgaris*, is a very rich dietary source of potassium²⁸. Potassium is a metal, a mineral, an essential nutrient and electrolyte that abound in foods and naturally produced in the body. It is the major intracellular cation²⁹. Diseases of the kidneys, heart and lung tend to aggravate when there is imbalance and deviation from normal range of serum potassium concentrations in the body³⁰. Since

hypokalemia affects peristalsis, results in stomach upset, intestinal paralysis, abdominal cramps and constipation, impairment of glucose tolerance and decreases secretion of insulin in response to high level of glucose; consuming *P. vulgaris* may be able to furnish potassium into affected cells, to make up for the shortage³¹. There was a significant ($p < 0.05$) difference in the concentration of total protein in test groups compared to control group administered cooked and raw, aqueous and methanol extracts irrespective of dose. A close consideration of this study (Figure 1 and Figure 2), reveals that methanol and aqueous cooked extracts gave higher protein. This is consistent with Idoko et al.,¹, who in their work, detailed that cooking of *P. vulgaris* increased the concentration of protein. Protein and other nutrients in beans serve as alternative to meat to vegan because of the content of complementary amino acids when cooked with grains³². It is reported that beans' proteins are naturally free from gluten and could serve a good combination in the diet of those who are at risk of the deficiency in vitamins B production³³. Protein function in beans is associated to weight loss, reduction in circumference of the waist, body fat mass, and reduction in blood pressure, and decrease in cholesterol³⁴. Dietary protein deficiency is associated with interrupted transport of potassium into and away from the cell³¹.

CONCLUSIONS

P. vulgaris cooked and raw aqueous and methanol extracts affected kidney function irrespective of the dose administered. It is obvious that *P. vulgaris* possesses potential ability to serve as a reliable plant source of chloride, urea, creatinine and potassium as assayed in this study. It may be concluded that *P. vulgaris* portrays potentials capable of improving kidney function and its consumption may also contribute to the general wellness of a person due to its rich nutrients (proteins) composition, the extracts are practically non-toxic since the LD₅₀ was greater than 5000 mg/kg.

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AUTHOR'S CONTRIBUTION

Idoko A: writing original draft, methodology. **Philip OC:** formal analysis, data curation, conceptualization.

Nwali ON: writing, review and editing. **Ugwudike PO:** methodology, formal analysis. **Blessing NO:** data curation, conceptualization. **Ani PN:** methodology, formal analysis. **Onyinye AN:** data interpretation. **Ayomide TA:** assisted with practical work. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

None to declare.

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