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**Research article** 

# Dietary diversity and impact of Moringa oleifera Lam. leaves supplemented – Diet on the nutritional status and CD4 cell counts of patients receiving antiretroviral therapy in Nigeria: A double - Blind randomized trial\*

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## ABSTRACT

Background: To maintain an optimal nutritional status and immunological function in PLHIV, a diet with adequate nutrients is of utmost importance. This is a major challenge among poor populations in developing worlds like Nigeria, where malnutrition and food insecurity are endemic. This study aimed to assess the type of regular diet consumed and assess the impact of supplementation of the diet with Moringa oleifera Lam. leaves on the nutritional status and CD4 cell counts of PLHIV that are on ART in Nigeria.

Method: A double-blind, randomized trial was conducted. Two hundred consented patients were randomly allocated to either the Moringa oleifera Lam. group (MOG) or the control group (COG). The FAO individual dietary diversity questionnaire was used. The regular diets of participants at baseline and six months were monitored. The measurements of weight, BMI, MUAC, and CD4 cell counts were obtained from baseline to six months of Moringa oleifera Lam. leaves supplementation.

Results: One hundred and seventy-seven patients completed the six-month follow-up (89 MOG versus 88 COG). At both baseline and sixth month, the foods most commonly consumed by the participants in both MOG and COG were cereals, spices and condiments, oils, fats and palm oil, and dark green vegetables. At baseline, significantly higher consumption of legumes, nuts & seeds (p = 0.001) was observed in the MOG and higher consumption of other vegetables (p = 0.024) in COG. Consumption of cereals, roots, and tubers was significantly higher (p = 0.024) in COG. 0.024; 0.045) in the COG in the sixth month. In both groups, participants were in the medium or low dietary diversity tercile. Throughout the study period, all the nutritional status variables observed were not significantly different between the two study groups [(p > 0.0001); weight; p = 0.5556; BMI; p = 0.5145; MUAC; p = 0.6456]. Over the study period, the treatment by time interaction shows a significant difference in CD4 counts by treatment group (p < 0.0001) and an estimate of fixed effects 10.33 folds greater in the MOG than COG. All tests were conducted at 95CI.

Conclusion: This study revealed a poor dietary diversity amongst PLHIV. Supplementation of regular diet with Moringa oleifera Lam. leaves did not affect the nutritional status but could improve the immune response of HIVpositive adults attending the antiretroviral treatment centre in the present study area.

# 1. Introduction

Nutrition is a vital component of care for people living with HIV and AIDS (PLHIV). This is particularly essential in resource-constrain settings where the prevalence of malnutrition and food insecurity is high [1]. Nutritional deficiencies in PLHIV have been shown to affect the immune status, disease progression, and mortality [2]. In chronic diseases like HIV and AIDS, adequate micro and macronutrients are vital for normal body functioning [3]. They are essential for maintaining the optimal immunological function, reduction of oxidative stress, and other

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metabolic processes [4]. Furthermore, a highly nutritious diet is essential to improve the efficacy of antiretroviral therapy (ART) taken by PLHIV and reduce its adverse side effects [5].

Dietary Diversity is defined as the number of different foods or food groups eaten by an individual or household over a reference period [6]. The dietary diversity score is an alternative indicator of the adequate intake of energy and micronutrients [6,7]. No single food has all the vital nutrients needed for optimal health [8]. Lack of a diversified diet is a major challenge amongst poor populations in developing countries like Nigeria, where diets are primarily based on starchy staples and regularly include few or no animal products and only fruits and vegetables in season [9]. Unsurprisingly, the National Bureau of Statistics (NBS) reported that 40 percent of the total population in Nigeria live below the relative poverty line of 137,430 naira (US \$381.75) per year [10]. Therefore, this high level of poverty can be a challenge in maintaining good health and consuming a nutritious diversified diet while managing an existing HIV infection [11]. Hence, an alternative solution is warranted.

Several studies reported on the importance of nutritional supplementation in PLHIV [12, 13, 14]. Evans *et al.* reported a pilot study conducted to determine the effect of a nutritional supplement called FutureLife porridge<sup>®</sup> on the immune response, body mass index (BMI), and bioelectrical impedance in HIV infected individuals commencing ART in South Africa. FutureLife porridge<sup>®</sup>, a high-protein, high-energy meal taken simultaneously with ART, was found to promote weight gain, improve immune response, and improve physical activity after six months of follow-up [13].

PLHIV uses Moringa oleifera Lam. as nutritional supplementation either alone or concurrently with antiretroviral drugs [15]. Moringa oleifera Lam. leaves powder is documented to have a high nutritional value [16, 17, 18, 19]. Moringa oleifera Lam. leaves are rich sources of both macro-and micronutrients [20]. They are a good source of natural antioxidants [21] as they are rich in minerals, vitamins, and other vital nutrients and phytochemicals. It could serve as a good supplementation in the diets of PLHIV and act as an effective and cheap solution to malnutrition [22]. Moreover, a recent study conducted by our research team reported that nutritional supplementation with Moringa oleifera leaves improves the QoL domains for PLHIV that are on ART [23]. This present study, therefore, aims to achieve two goals: first, to assess the dietary diversity of the study participants; secondly, to determine whether supplementation of their diet with Moringa oleifera Lam. leaves will have an impact on the nutritional status and immune response of PLHIV that are on ART at the S.S Wali Virology Centre, Aminu Kano Teaching Hospital, Kano State, Nigeria.

### 2. Materials and methods

# 2.1. Study setting

The study was conducted at the Sadiq Suleiman Wali HIV clinic, which is referred to as S S Wali Virology centre, situated at the Aminu Kano Teaching Hospital (AKTH), Kano State, Nigeria.

AKTH is a tertiary institution located in Kano State which is the most populous state in Nigeria with over 13 million people. It serves as a referral centre for clients within Kano State and neighbouring states. AKTH is a 700-bed tertiary health institution. It has a President's Emergency Plan for AIDS Relief (PEPFAR) - funded HIV clinic that operates 5 days a week. The hospital provides care and services to HIV- positive patients in the nation. There are 7,086 clients currently on ART in S.S Wali virology centre.

# 2.2. Research design

The study was a double-blind, randomized trial conducted from December 2017 to November 2018. This study was not a fully controlled study due to the lack of diet control of the participants. However, all research participants and team members, as well as the principal investigator, were blinded to the allocation of participants to the different study groups.

## 2.3. Ethical consideration

The study was reviewed and approved by the Aminu Kano Teaching Hospital (AKTH) Kano State, Nigeria ethics committee with number NHREC/21/08/2008/AKTH/EC/2012, and also the University of Kwazulu-Natal, Durban, South Africa Biomedical Research Ethics Committee with number BFC294/16. The trial was registered with the Pan African Clinical Trial Registry with identification number PACTR201811722056449.

Before the commencement of the study, all ethical considerations were fully adhered to. The study was performed in compliance with the principles enunciated in Helsinki's Declaration [24]. Permission to take part in the study was obtained from each participant by signing the study informed consent form or providing a thumbprint if unable to sign. Thereafter, the research team explained to each participant the aim and objectives of the study and how the study was intended to be conducted. Lastly, in the event that a participant no longer wishes to partake in the study, the participants were informed of their rights to withdraw without that action having any impact on the services being rendered to them at the HIV clinic.

# 2.4. Participants

Participants were approached to participate in this study as they presented themselves to the clinic for any HIV services offered. The participants comprised patients diagnosed with HIV infection receiving medical care at the centre. Inclusion criteria were an HIV diagnosis with CD4 counts  $\leq$ 500 cells/mm<sup>3</sup>; 18 years or older; commenced ART no less than three months; on Tenofovir + Lamivudine + Efavirenz ART drug regimen; male and female, and those who provided consent to participate and complied with the study protocol. Participants who had a previous history of allergy to *Moringa oleifera* Lam. or placebo (cornstarch powder); pregnant women; those with active opportunistic infection and participants that took supplements made from plants/Herbs within 30 days of screening were excluded. Those that lived outside the Kano State metropolis were also excluded from participation.

# 2.5. Sample size estimation

The sample size was determined by making use of G\*Power version 3.1.9.2 [25] to detect a medium effect size (Cohen's d = 0.5) [26] or 0.5 standard deviations in mean weight or CD4 cell count by Randomised Control Trial (RCT) arm with 90% power (1- $\beta$  [type 2 error probability]), and 95% confidence (or 5%  $\alpha$  error probability [type 1]) assuming a balanced 1:1 study design. The total sample size of 172 patients was obtained (86 patients in each study arm). To give room for attrition, the total sample size was rounded up to 200 study participants. The same sample size was used to assess the dietary diversity patterns and changes in participants' diets as they are secondary outcomes of the study.

Out of the 410 participants that were considered to partake in the study, 204 were excluded from participation, 6 participants declined to take part, and the remaining 200 participants were randomly assigned to either *Moringa oleifera* Lam. group (MOG) or the Control group (COG) with 100 participants in each group. Only 177 participants completed the six months study (see Figure 1).

#### 2.6. Randomization

The randomization sequence was generated using PASS 12.0 software (Wei's Urn algorithm) by an independent statistician who is not involved in the research. During study enrolment, the balance between the two study groups was achieved using block randomization. A no was assigned



Figure 1. Flow Chart of participants.

to each recruited participant that fulfilled all the inclusion criteria and provided consent. They were randomly allocated into the MOG and COG groups. As the study design was a double-blinded trial, all the people involved in the research were blinded to the allocation of participants to the respective study groups.

# 2.7. Intervention

## 2.7.1. Moringa oleifera Lam. nutritional supplement

The fresh leaves of *Moringa oleifera* Lam. used in this study were harvested from Prime Global Agricultural Industries Limited, Kano State, Nigeria. The leaves were identified and authenticated at the Herbarium of the Department of Biological Science, Bayero University Kano (BUK), Nigeria using a standard voucher.

Briefly, destalked *Moringa oleifera* Lam. leaves were thoroughly washed under running water to remove dust. The water was drained completely. Air drying of the *Moringa oleifera* Lam. leaves was done in a well-ventilated environment away from direct sunlight. The drying was done for several days with the continuous turning of the leaves after

every 24 h to avoid fungal growth and to ensure the leaves were completely dried. Grinding of the leaves to obtain powder was done using an electric grinder. To obtain a fine powder, the leaves were sieved using a 0.500 mm standard sieve (No. 35 mesh size) [27,28] and were immediately transferred to air-tight containers and stored away from humidity and direct sunlight. The *Moringa oleifera* Lam. leaves powder was packaged at Dala Foods Nigeria Limited Kano State, Nigeria.

# 2.8. Placebo

The placebo used for the study was also processed, manufactured, and packaged at Dala Foods Nigeria Limited Kano State, Nigeria by colouring corn starch powder with chlorophyll [29]. Both the *Moringa oleifera* Lam. leaf powder and the placebo were produced and packaged to look comparable in small sachets of 15 g each. A monthly prescription of the study intervention was obtained by packaging thirty (30) individual sachets inside a bigger green-colored plastic bag. Each bag was sealed, labelled with the study code, visit no, subject no, and instructions on how to take it, and was stored appropriately. At each hospital visit, a sealed bag was

dispensed to every study participant, and was instructed to consume each supplement together with meals.

Patients were assigned randomly to either MOG or COG to be either given *Moringa oleifera* Lam. or the placebo. While taking their three daily meals, the study participants were advised to divide each intervention into three (5 g) [30,31] and consume. They were further advised to abstain from taking *Moringa oleifera* Lam. from any other source except the interventions given throughout the study period while maintaining their usual diet.

To monitor compliance with the study protocol, patients were telephoned using their mobile phone numbers biweekly. They were also questioned during their monthly hospital visits to evaluate adherence.

# 2.9. Nutritional composition of Moringa oleifera Lam. leaves powder

To obtain the nutritional composition of the *Moringa oleifera* Lam. leaf powder used for the study, ASPIRATA Food and Beverage Laboratory [32], which is a South African National Accreditation System (SANAS) [33] endorsed laboratory analyzed a 100 g of *Moringa oleifera* Lam. leaf powder as shown in Table 1 below:

# 2.10. Data collection methods

## 2.10.1. Socio-demographic variables

A trained nurse administered a detailed questionnaire during a faceto-face interview with participants to obtain socio-demographic data: age, marital status, education, employment status, family size, and monthly income.

# 2.11. Dietary assessment

#### 2.11.1. FAO dietary diversity questionnaire

To assess their regular diet, a 24- hour dietary recall was conducted at baseline and every monthly hospital visit to obtain the information on participants' food intake using the FAO dietary diversity questionnaire. Two trained qualified nurses, among the research team members together with the Principal Investigator (PI) conducted it at the virology clinic. Participants were asked to give a recall of all foods consumed and beverages taken in the preceding 24 h before the interview. The dietary assessments captured at the beginning of the study (baseline) and the end of the study (6th month) are reported for this study.

# 2.11.2. Dietary diversity

To assess the dietary diversity of the study participants, a scale of thirteen food groups was used. With the data on dietary intake captured from the 24- hour dietary recall, the dietary diversity scores for participants were obtained using the FAO guidelines for measuring individual and household dietary diversity [34]. The scores are derived by awarding a point to each food group consumed over the reference period. A sum of

<b>Table 1.</b> Nutrient composition of Moringa oleifera Lam. leaf powder.									
Nutrient	100 g	15 g							
Energy (kcal)	981	147.1							
Protein (g)	28.2	4.23							
Fat (g)	3.9	0.59							
Carbohydrate (g)	22	3.3							
Soluble minerals									
Calcium (mg)	1791.82	268.7							
Potassium (mg)	4879.26	731.8							
Sodium (mg)	24	3.6							
Trace elements									
Zinc (mg)	2.88	0.43							
Iron (mg)	37.78	5.67							

all the points awarded was computed to obtain the dietary diversity score for each participant. The scores were divided into three terciles; low dietary diversity tercile for consumption of 1–4 food groups, medium tercile for consumption of 5–9 food groups, and high dietary diversity tercile for consumption of 10 above food groups [35].

#### 2.11.3. Anthropometric variables

The height and weight of participants were obtained using a portable stadiometer (Seca 217, Seca Gmbh and co. KG., Hamburg, Germany) and a calibrated standardized digital weighing scale (Tanita HD-372, Tanita Corporation, Tokyo, Japan) respectively following standard protocols. The height was measured to the nearest centimetre and weight to the nearest 0.1 kg. The BMI was calculated as the weight in kilograms divided by the square of height in meters. The BMI was categorised as underweight (BMI <18.5); normal weight (BMI 18.5–24.9); overweight (BMI 25.0-29.9) and obesity (BMI >30.0) [36]. The mid-upper arm circumference (MUAC) was measured to the nearest 0.1 cm using a non-elastic flexible meter rule wrapped around the mid-point of the elbow and tip of the shoulder to determine the circumference of the upper arm. A trained nurse conducted all anthropometric variables (nutritional status) measurements in duplicates at the virology centre at baseline and each monthly hospital visit under the supervision of the Principal Investigator (PI). Anthropometric measurements from baseline to the sixth month were used.

#### 2.11.4. Biochemical marker

Following standard protocols, CD4 cell counts were measured at baseline and each monthly hospital visit using the Partec Flow cytometry instrument (Partec, Munster, Germany) [37]. Measurements of CD4 cell counts from baseline to the sixth month were used.

# 2.12. Study outcomes

The primary outcomes assessed in this study were changes in anthropometric parameters/nutritional status [weight, BMI, MUAC], and changes in immune response [CD4 cell count]; while the assessment of dietary diversity patterns and changes in diet were the secondary outcomes addressed.

# 2.13. Statistical analysis

A descriptive, bivariate (Chi-square, Fisher's-exact, and independent sample t-test) and linear mixed-effect model analysis were utilized. Kolmogorov-Simonov and Shapiro-Wilk tests were employed to determine the normality of the study data. Box-Cox transformation was used to transform the data that were not normally distributed. The comparison between the socio-demographic characteristics of participants in each group and their food consumption and dietary diversity tercile was conducted using chi-square and fisher's exact test. An independent t-test was employed to examine the significance of the difference in mean in nutritional status variables and immune response variables between the two groups at each study period. A repeated measure linear mixed effect model analysis was further deployed using SAS version 9.4 statistical software to determine the difference in nutritional status and immune response variable outcomes between the treatment groups over the study period. An exploratory analysis was conducted to evaluate the influence of food intake (using the dietary diversity tercile) on the nutritional status variables and CD4 cell counts of the study groups over the study period. All statistical tests were performed at a 95% confidence level.

# 3. Results

# 3.1. Participants flow

Figure 1 shows the flow chart of the participants' progress in both study groups. To assess eligibility to participate in the study, 410 patients

were screened. Two hundred patients conformed to the inclusion criteria while 210 patients were excluded [204 did not meet the inclusion criteria for the study, and six refused to participate]. The two hundred recruited patients were equally randomized into two groups [MOG and COG] with 100 patients in each study group. In the MOG, 11 patients did not complete the study [8 patients did not respond to hospital appointments, and 3 patients stopped taking the intervention]. Twelve (12) patients did not complete the study in the COG [9 patients did not respond to hospital appointments, and 3 patients stopped taking the intervention]. One hundred and seventy-seven (177) patients with a Mean  $\pm$  SD age of 41.57  $\pm$  8.23 years completed the six months study, and their data were included in the analysis (89 in the MOG and 88 in COG) (Figure 1).

## 3.2. Demographic characteristics of the participants

Table 2 shows the baseline assessments of 177 participants (89 in the MOG and 88 in the COG). The participants in both groups had similar baseline measures regarding gender, age, marital status, religion, ethnicity, level of education, family size, occupation, and monthly income. The majority of the participants were between 20 to 49 years, with a mostly female population in both groups [MOG = 70 (78.7%); COG = 67(76.1%)]. Most of the participants were married [MOG = 42 (47.2%); COG= 38 (43.2%)] with Islam being the main religion [MOG = 64 (71.9%); COG= (66 (75%)]. A greater part of the participants belong to Hausa/Fulani ethnicity [MOG = 55 (61.8%0; COG = 47 (53.4%)]. A larger number of the participants in both groups had a secondary level of education [MOG = 27 (30.3%); COG = 24 (27.3%)]. The general study participants earned below the minimum monthly income of \$30,000 (\$78.23) [MOG = 67 (75.3%); COG = 66 (75%)] (Table 2).

Compliance was high as the intervention was well accepted by the study participants.

Table 3 shows the comparison of different food groups consumed and the dietary diversity terciles of the participants at baseline and 6th month of study. At baseline, the most commonly consumed food groups in MOG and COG include cereals [MOG (95.5%); COG (90.9%)], spices and condiments [MOG (89.9%); COG (85.2%)], oils, fats and palm oil [MOG (79.7%); COG (88.6%)], dark green vegetables [MOG (69.7%); COG (60.2%)] and other vegetables [MOG (52.8%); COG (69.3%)] (Table 3).

At six months, fruits [MOG (24.7%); COG (18.2%)], meat, poultry and organ meat [MOG (24.7%); COG (30.7%)], legumes, nuts and seeds [MOG (19.1%); COG (20.5%)], milk and milk products MOG [(14.6%); COG (15.9%)], fish [MOG (12.4%); COG (18.2%)] and eggs [MOG (5.6%); COG (2.3%)] are the food groups least consumed in both MOG and COG (Table 3).

At baseline, more participants consumed other vegetables in the COG than in the MOG, while the consumption of legumes, nuts & seeds was higher in the MOG than in the COG. These differences were found to be statistically significant (p < 0.05). Consumption of all other food groups between the two study groups was otherwise similar (Table 3).

In the sixth month, the consumption of cereals, roots, and tubers was observed to be significantly higher (p < 0.05) in the COG than in the MOG (Table 3).

More than half of the participants in both groups were in the medium dietary diversity tercile at baseline [MOG (61.8%); COG (53.4%)] and six months [MOG (55.1%); COG (53.4%)]. The rest of the participants were in the low dietary diversity tercile (Table 3).

Table 4 shows the mean and standard deviation of anthropometric (nutritional status) parameters and CD4 cell count of the study participants in MOG and COG at baseline and 6th month. At baseline, all the variables assessed were similar for both the MOG and COG. The means of weight (kg) for both groups were MOG = 63.8 ( $\pm$ 14.8); COG = 61.9 ( $\pm$ 12.5). The mean BMIs for MOG and COG was 24.84 ( $\pm$ 4.8) and 23.75 ( $\pm$ 3.82), respectively. The MUAC means for MOG and COG were 26.3 ( $\pm$ 2.1) and 25.9 ( $\pm$ 1.8), respectively. At baseline, the mean CD4 cell count for MOG and COG were 341.8 ( $\pm$ 106.1) and 352.34 ( $\pm$ 126.0), respectively (Table 4).

Table 2. Demographic description of study participants.

Variables	MOG (%) (1	N = 89)	COG (%) (N	1 = 88	) P-value
Gender					
Males	19 (21.3)		21 (23.9)		0.689
Female	70 (78.7)		67 (76.1)		
Age (years)					
<20	3 (3.4)		1 (1.1)		0.737
20–29	24 (27.0)		21 (23.9)		
30–39	37 (41.6)		36 (40.9)		
40–49	20 (22.5)		22 (25.0)		
50–60	5 (5.6)		8 (9.1)		
Marital Status					
Married	42 (47.2)		38 (43.2)		0.838
Single	12 (13.5)		10 (11.4)		
Divorced	19 (21.3)		20 (22.7)		
Widowed	16 (18.0)		20 (22.7)		
Religion					
Islam	64 (71.9)		66 (75.0)		0.642
Christianity	25 (28.1)		22 (25.0)		
Ethnicity					
Hausa/Fulani	55 (61.8)		47 (53.4)		0.511
Yoruba	13 (14.6)		15 (17.0)		
Igbo	9 (10.1)		15 (17.0)		
Others	12 (13.5)		11 (12.5)		
Educational Level					
Primary	14 (15.7)		12 (13.6)		0.971
Secondary	27 (30.3)		24 (27.3)		
Tertiary	20 (22.5)		21 (23.9)		
Quranic	13 (14.6)		15 (17.0)		
None	15 (16.9)		16 (18.2)		
Occupation					
Entrepreneur	15 (16.9)		10 (11.4)		0.840
Trader	23 (25.8)		25 (28.4)		
Civil Servant	15 (16.9)		17 (19.3)		
Artisan	19 (21.3)		17 (19.3)		
Unemployed	17 (19.1)		19 (21.6)		
Family Size					
2–5	38 (42.7)		32 (36.4)		0.557
6–10	26 (29.2)		25 (28.4)		
>10	25 (28.1)		31 (35.2)		
Monthly Income(#	)				
Not Indicated	11 (12.4)		6 (6.8)		0.672
<30.000	67 (75.3)		66 (75.0)		
30,001-60,000	6 (6.7)		10 (11.4)		
60,001–90.000	1 (1.1)		1 (1.1)		
90,001–120.000	3 (3.4)		2 (2.3)		
>120,000	1 (1.1)		3 (3.4)		
Statistical test	Chi course +	octi absolute	froquence	and	porcontogo -
parentheses.	= uni-square t	est, absolute	nequency	and	percentage if

At six months, the mean weight for MOG was 64.71 (±15.07), while for COG was 63.16 (±13.49). The mean BMIs for MOG and COG were 25.16 (±4.93) and 24.19 (±4.09), respectively. The MUAC means for MOG and COG were 26.50 (±2.16) and 26.08 (±1.95), respectively. All the anthropometric (nutritional status) parameters assessed at six months were not statistically (p > 0.05) different between the MOG and COG. At six months, the mean CD4 cell counts were 425.75 (±153.76) for MOG and 373.44 (±157.31) for COG. The difference in mean CD4 cell counts between the two study groups was found to be statistically significant (p < 0.05) (Table 4). Table 3. Comparison of food groups consumed and dietary diversity tercile at baseline and 6th month between MOG and COG.

Food group	Baseline			6th month								
	MOG n.	COG n. (%)	Р	Odds	OR 95%	CI	MOG n. (%)	COG n. (%)	Р	Odds	OR 95%CI	
	(%)		value	ratio (OR)	Lower	Upper			value	ratio (OR)	Lower	Upper
Cereals	85 (95.5)	80 (90.9)	0.249	0.471	0.101	1.691	73 (82.0)	82 (93.2)	0.024*	2.995	1.181	9.748
Roots & tubers	16 (16.9)	21 (23.9)	0.392	1.546	0.537	3.836	16 (17.9)	26 (29.5)	0.045*	2.069	1.007	4.248
Dark green vegetable	62 (69.7)	53 (60.2)	0.188	0.659	0.357	1.239	60 (67.4)	48 (54.5)	0.079	0.580	0.315	1.068
Other vegetables	47 (52.8)	61 (69.3)	0.024*	2.019	1.103	4.211	50 (56.2)	54 (61.4)	0.484	1.239	0.661	2.357
Fruits	7 (7.8)	7 (8.0)	0.782	1.195	0.321	4.410	22 (24.7)	16 (18.2)	0.290	0.677	0.319	1.380
Meat, poultry & organs	23 (25.8)	19 (21.6)	0.722	0.839	0.417	1.745	22 (24.7)	27 (30.7)	0.375	1.348	0.718	2.568
Eggs	6 (6.7)	5 (5.7)	0.770	0.833	0.180	3.090	5 (5.6)	2 (2.3)	0.254	0.391	0.105	1.912
Fish	10 (11.2)	12 (13.6)	0.656	1.247	0.468	3.468	11 (12.4)	16 (18.2)	0.281	1.576	0.648	4.187
Legumes, nuts & seeds	40 (44.9)	16 (18.2)	0.001*	0.272	0.122	0.552	17 (19.1)	18 (20.5)	0.821	1.089	0.512	2.346
Milk & milk products	13 (14.6)	13 (14.8)	0.975	1.013	0.383	2.318	13 (14.6)	14 (15.9)	0.810	1.106	0.463	2.568
Oil, fats & palm oil	71 (79.7)	78 (88.6)	0.069	2.065	0.935	4.504	86 (96.6)	85 (96.6)	0.719	0.781	0.152	4.019
Sugar & honey	11 (12.4)	9 (10.2)	0.813	0.808	0.284	2.368	19 (21.3)	12 (13.6)	0.177	0.582	0.248	1.342
Spices & condiments	80 (89.9)	75 (85.2)	0.372	0.649	0.220	1.656	80 (89.9)	73 (83.0)	0.178	0.548	0.166	1.438
Dietary Diversity Tercile	<b>:</b>											
Low (1–4)	33 (37.1)	40 (45.5)	0.258	1.414	0.776	2.679	39 (43.8)	41 (46.6)	0.711	1.118	0.593	2.096
Medium (5–9)	55 (61.8)	47 (53.4)	0.289	0.709	0.384	1.269	49 (55.1)	47 (53.4)	0.826	0.936	0.504	1.776
High (>10)	1 (1.1)	1 (1.1)	0.994	1.011	0.272	3.488	1 (1.1)	0 (0.0)	0.319	0.989	0.959	0.990

significant at 95CI (Chi-square test; Fishers exact test & Odd ratio).

Table 4. Comparison of participants' mean, the standard deviation of anthropometric parameters (nutritional status), and CD4 of MOG and COG at baseline and 6th month.

Month	MOG	COG	P-value
Weight			
	Mean (SD)	Mean (SD)	
0	63.8 (14.8)	61.9 (12.5)	P=0.361
6	64.71 (15.07)	63.16 (13.49)	P=0.472
BMI			
	Mean (SD)	Mean (SD)	
0	24.84 (4.8)	23.75 (3.82)	P = 0.093
6	25.16 (4.93)	24.19 (4.09)	P = 0.157
MUAC			
	Mean (SD)	Mean (SD)	
0	26.3 (2.1)	25.9 (1.8)	P = 0.145
6	26.50 (2.16)	26.08 (1.95)	P = 0.185
CD4			
	Mean (SD)	Mean (SD)	
0	341.8 (106.1)	352.34 (126.0)	P = 0.547
6	425.75 (153.76)	373.44 (157.31)	$P = 0.03^{*}$
* = significant	at 95CI (independent sam	ple t-test).	

Table 5 shows the comparison of participants' nutritional status (categorized BMI) at baseline and sixth month between MOG and COG. Most of the participants in both study groups were of normal weight (BMI = 18.5–24.9). Only a few of the participants were underweight (BMI<18.5) or obese (BMI  $\geq$ 30.0). A considerable number were overweight. These differences in BMI categories at both baseline and sixth month were not found to be statistically significant (p > 0.05) in both MOG and COG. The descriptive analysis showed that the overall mean (±SD) BMI for MOG at baseline was 24.84 (±4.78) and 25.16 (±4.93) in the 6th month while that of the COG was 23.75 (±3.82) and 24.19

Table 6 shows the linear mixed effect model results showing the differences in nutritional status and CD4 cell count between the two groups over the study period. An unstructured correlation matrix was assumed for the model analysis. The treatment by time interaction shows

 $(\pm 4.09)$  at 6th month (Table 5).

a non-significant difference in all the nutritional status variables [weight; BMI; MUAC] analysed by the treatment group over time (p > 0.0001), while a significant difference in CD4 cell count by treatment group over time was observed (p < 0.0001). An estimate of fixed effects showed that the nutritional status variables (weight; p = 0.5556; BMI; p = 0.5145 and MUAC; p = 0.6456) between the two groups were not significantly different over time, while the CD4 counts were 10.33 folds greater in the MOG than the COG throughout the study period (Table 6) [S1\_Supplementary file].

Further exploratory analysis of the influence of food intake (using the dietary diversity tercile) on the nutritional status variables and CD4 cell counts by the study groups from baseline to the 6th month was computed. The analysis shows that food intake (using the dietary diversity tercile) had a significant (p = 0.036) influence on the BMI over the study period. The changes in BMI estimates between the treatment and COG were significant (p = 0.038) after controlling for the food intake (using dietary diversity tercile). However, the food intake (using dietary diversity tercile) had no significant (p > 0.05) influence on the changes in the weights, MUAC, and CD4 counts between the two groups over the study period [S2\_Supplementary file].

## 4. Discussion

To our knowledge, this study is the first randomised interventional trial conducted to report the regular diet consumed by PLHIV that are on ART in Nigeria. The study assessed the impact of supplementing the diet with *Moringa oleifera* Lam. leaf on the anthropometric variables (nutritional status) and CD4 cell counts of the participants. The participants' socio-demographic characteristics, nutritional status, and CD4 cell counts were similar at baseline between the MOG and COG.

Throughout the study period, food groups commonly consumed by the participants in both MOG and COG included cereals; oils, fats, palm oil, spices, and condiments. This is probably because oils, fats, spices, and condiments are used culturally in their food preparation [38]. These food groups are likely to be more accessible but less nutrient-dense than others like legumes and nuts, meat, poultry, organ meat, eggs, milk, or fish [39].

At baseline, the consumption of legumes, nuts, and seeds was statistically higher in the MOG, although consumed by less than half of the study participants. Consumption of other vegetables (e.g., tomato and

fable 5. Compariso	n of participants	s' nutritional status (	(using	categorized	BMI) at	baseline an	d 6th	month	between	MOG	and	COG
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BMI Category	Baseline			6th month						
	MOG n. (%)	Mean [±SD] (Kg/m²)	COG n. (%)	Mean [±SD] (Kg/m²)	P value	MOG n. (%)	Mean [±SD] (Kg/m²)	COG n. (%)	Mean [±SD] (Kg/m <sup>2</sup> )	P value
Underweight (<18.5)	5 (5.6)	17.73 [0.55]	5 (5.7)	17.26 [0.93]	0.357	5 (5.6)	17.19 [1.00]	7 (8.0)	17.48 [0.68]	0.549
Normal Weight (18.5–24.9)	46 (51.7)	21.87 [2.00]	51 (58.0)	21.77 [1.78]	0.781	41 (46.1)	21.82 [1.66]	46 (52.3)	21.99 [1.47]	0.600
Overweight (25.0–29.9)	27 (30.3)	27.63 [1.41]	28 (31.8)	27.40 [1.56]	0.576	29 (32.6)	27.35 [1.54]	27 (30.7)	27.41 [1.31]	0.872
Obese (≥30.0)	11 (12.4)	33.67 [2.32]	4 (4.5)	31.53 [2.13]	0.130	14 (15.7)	33.27 [3.01]	8 (9.1)	31.84 [2.05]	0.248
Overall Total	89 (100.0)	24.84 [±4.78]	88 (100.0)	23.75 [±3.82]	0.093	89 (100.0)	25.16 [±4.93]	88 (100.0)	24.19 [±4.09]	0.157
Statistical test =	Independence	sample t-test; SD	= standard de	viation.						

Table 6. Linear mixed-effects model showing the differences in nutritional status and CD4 cell counts between MOG and COG over the study period.

istimates of Fixed Effects <sup>a</sup>										
t	P value	Estimate 95% CI								
		Lower Bound	Upper Bound							
42.02	0.0001	61.43	63.65							
-0.59	0.5556	-0.20	-0.11							
•	•	•								
50.98	0.0001	23.61	24.32							
-0.65	0.5145	-0.08	-0.04							
•	•	•								
69.56	0.0001	22.94	23.52							
-0.73	0.6456	-0.09	-0.02							
•			•							
27.20	0.0001	354.29	376.09							
3.89	0.0001*	5.12	15.54							
	t 42.02 -0.59 - 50.98 -0.65 - 69.56 -0.73 - 27.20 3.89	t P value 42.02 0.0001 -0.59 0.5556  50.98 0.0001 -0.65 0.5145  69.56 0.0001 -0.73 0.6456  27.20 0.0001 3.89 0.0001*	t P value Estimate 95% CI   Lower Bound Lower Bound   42.02 0.0001 61.43   -0.59 0.5556 -0.20   . . .   50.98 0.0001 23.61   -0.65 0.5145 -0.08   . . .   69.56 0.0001 22.94   -0.73 0.6456 -0.09   . . .   27.20 0.0001 354.29   3.89 0.0001* 5.12							

\* = statistically significant;<sup>a</sup> = Dependent variable (weight, BMI, MUAC, CD4);<sup>b</sup> = parameter set to zero (redundant); Statistical test = linear mixed effect model.

pepper) was significantly higher in the COG. However, other vegetables (e.g., tomato and pepper) tend to be less nutrient-dense than foods like spinach, peas, meats, or dairy products [39]. The higher consumption of these food groups could be due to the good dietary behaviour of the participants as a result of their fairly high literacy and educational levels [40], as most of our participants have up to secondary and tertiary school levels of education [8]. It could also be due to seasonal variation in food availability [9]. At six months, the consumption of all the different food groups was observed to be similar between the two groups, except for cereals, roots, and tubers, where their consumption was observed to be slightly higher in the COG.

The high consumption of cereals, as observed above, includes meals prepared with maize, rice, and wheat. This is in keeping with the cultural practices of Northern Nigeria where the study was conducted and where 'tuwo', a locally prepared meal made from cereals or grains [41], is the most commonly consumed food amongst the Hausa people. This is supported by the fact that half of the study participants in both groups are of the Hausa tribe. It is similar to what was reported by Weldegebreal *et al.* in Ethiopia, where cereal is among the most commonly consumed foods by their study participants [1]. Dark green vegetables often consumed are dried baobab leaves soup locally called 'miyar kuka', which is an accompaniment of 'tuwo' [42].

The low intake of food groups that are a good source of proteins like meat, poultry, organ meat, fish, milk, and eggs and the high consumption of cereals as a staple diet by our participants could result in a high prevalence of protein insufficiency as has been reported by Agada *et al.* [43]. This could be because most of the participants in both groups are of

poor economic status earning less than the Nigeria monthly minimum wage of ₦30,000 (US \$78) [44]. Moreover, most people in the northern part of Nigeria prefer to rear these animals as a source of income than eat them.

Half of the study participants in both MOG and COG were in the medium dietary diversity tercile at baseline and six months. However, the remaining participants were in low tercile, consuming 1–4 food groups during the reference period.

Using the mixed linear model, the study observed that all the anthropometric parameters (nutritional status) of the participants assessed were not significantly different between the MOG and COG over the study period. *Moringa oleifera* Lam. leaf supplementation was therefore not effective in improving the weight, BMI, and MUAC of the study participants on ART. Although food intake (using the dietary diversity tercile) was found to have an influence on the BMI over the study period.

However, a significant increase was observed in CD4 cell counts of the MOG participants than in the COG. This suggests that supplementing the diet of PLHIV that is taking ART with *Moringa oleifera* Lam. leaf powder effectively improved the CD4 cell counts. *Moringa oleifera* Lam. leaves which contain a wide range of essential nutrients such as vitamins, minerals, and antioxidants [45], could be associated with the improvement in CD4 cell counts as observed [46,47]. Furthermore, food intake (using the dietary diversity tercile) was found to not influence the improvement of CD4 counts over the study period.

Although the diet of our participants lacks highly nutritious food groups, it is noteworthy that, diversity is based only on the presence of food in the diet. However, to better assess the impact of the diet it would be necessary to know the amounts and daily requirements of macronutrients and micronutrients consumed by the study participants which is beyond the scope of the present study.

This finding is similar to a study conducted in Conakry, Guinea, where an increase in the CD4 count and mean BMI of PLHIV on ART was observed after six months of supplementation with Corn- Soy, and oil [48]. In Nigeria, Amlogu *et al.* also reported a similar finding where a nutritional meal called 'Amtewa' which consists of *Glycine max* 50 g (Soya bean); *Pennisetum americanum* 20 g (Millet); *Moringa oleifera* 15 g (Moringa), and *Daucus carota* spp. sativa 15 g (Carrot) increased the CD4 cell counts and Mid Upper Arm Circumference (MUAC) of PLHIV after one year of follow-up [12].

Most of our study participants had normal BMI with a significant proportion of overweight at baseline and the end of the study. This observation could be attributed to the positive effect of ART on their general well-being. Employment of participants with underweight at baseline could have resulted in a significant change in their nutritional status (anthropometric parameters). This factor is a limitation of the study.

Our study showed that participants maintained their regular diet from baseline throughout the study. This is in line with the study protocol requirement. Owing to the poor dietary diversity pattern observed in this study, supplementation with the leaves of *Moringa oleifera* Lam. could be used as a cost-effective and sustainable source of nutrients and for improvement in immune status in PLHIV. Moreover, its effectiveness in improving the CD4 counts could be responsible for an improved quality of life as reported in our recent study conducted by the same research team. With an improved physical, psychological, level of independence, and social relationship domains of quality of life, PLHIV could experience better social interaction and improved intimate partner relationships which could decrease depression and stigmatization which are documented to be the main mental health concerns among PLHIV [23].

This being a double-blind, randomized placebo trial, a gold standard of intervention studies, gives strength and credence to the findings of this study. Further limitations of the study include the distinguishable taste of *Moringa oleifera* Lam. which could be a source of bias, recall, social desirability biases, and lack of diet control of the participants.

#### 5. Conclusion

This study revealed a poor dietary diversity amongst PLHIV. Supplementation of a regular diet with *Moringa oleifera* Lam. leaves had no effect on the nutritional status but could improve the immune response of HIV-positive adults attending the antiretroviral treatment centre in the present study area.

# Declarations

#### Author contribution statement

Aisha Gambo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nceba Gqaleni: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tesleem K. Babalola: Analyzed and interpreted the data.

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#### Data availability statement

Data will be made available on request.

### Declaration of interests statement

The authors declare no conflict of interest.

#### Additional information

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