EVALUATION OF THE ADDITION OF MORINGA OLEIFERA AS A NUTRITIONAL SUPPLEMENT ON THE ANTHROPOMETRIC, VIRAL LOAD, AND CD4 COUNTS OF ADULT HIV PATIENTS ON ANTIRETROVIRAL THERAPY

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A thesis submitted to the College of Health Sciences, University of KwaZulu-Natal, Howard College, in fulfilment of the requirements for the degree of Doctor of Philosophy in Medicine (Public Health)

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PREFACE

The study described in this thesis was carried out in the Discipline of Public Health Medicine, School of Nursing and Public Health, College of Health Sciences, University of KwaZulu-Natal.

The study described in this thesis is original work done and reported by the author. The study has not been used in any form by any person or submitted to any tertiary institution to award a degree or diploma. Some of the work has been accepted for publication in accredited journals in line with the thesis guidelines of UKZN. Due acknowledgments have been accorded where other people's work has been used in the text.

DECLARATION 1: PLAGIARISM

I **AISHA GAMBO** declare that

(i) The research reported in this dissertation is my original work, except where otherwise indicated.

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DECLARATION 2: PUBLICATIONS

The publications (in print, in the press, and submitted) that constitute this thesis and my contribution to each of the manuscripts are presented here.

Publication 1:

Gambo, A., Gqaleni, N., 2021. Evaluation of the addition of Moringa oleifera as a nutritional supplement on the anthropometric; viral load, CD4 cell counts and quality of life of adult HIV patients on antiretroviral therapy in Nigeria: A study protocol. *Journal of Public Health Research (under review).*

Authors' contributions: Aisha Gambo developed the idea for the study, designed the study, and wrote the manuscript under the supervision of Prof Nceba Gqaleni. Both authors edited and critically reviewed the manuscript.

Publication 2:

Gambo, A., Gqaleni, N., Babalola, T.K., 2021. Dietary diversity and impact of Moringa oleifera Lam. leave supplemented – diet on the nutritional status and CD4 cell counts of patients receiving antiretroviral therapy in Nigeria: A double-blind randomized trial. *Heliyon*; 8; e09524:https://doi.org/10.1016/j.heliyon.2022.e09524 *(published).*

Authors' contributions: Aisha Gambo developed the idea for the study, designed the study, and wrote the manuscript under the supervision of Prof Nceba Gqaleni. Tesleem K, Babalola performed statistical analyses. All authors contributed to results interpretation, edited, and critically reviewed the manuscript.

Publication 3:

Gambo, A., Gqaleni, N., 2022. Does Moringa oleifera Lam. leaves supplementation have an impact on the weight and BMI of people living with HIV that are on antiretroviral therapy? A double blind randomized control trial. JPHIA 13 (3), 2126 DOI:https://doi.org/10.4081/jphia.2022.2126.*(published).*

Authors' contributions: Aisha Gambo developed the idea for the study, designed the study, and wrote the manuscript under the supervision of Prof Nceba Gqaleni. Both authors edited and critically reviewed the manuscript.

Publication 4:

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Authors' contributions: Aisha Gambo developed the idea for the study, designed the study, and wrote the manuscript under the supervision of Prof Indres Moodley and Prof Musa Babashani. Tesleem K, Babalola performed statistical analyses. Prof Nceba Gqaleni contributed to statistical analyses and edited the manuscript. All authors contributed to results interpretation and critically reviewed the manuscript.

Publication 5:

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Conference proceeding

Gambo, A., Moodley, I., Babashani, M., Babalola, T.K., 2021. Impact of Moringa Oleifera leaves supplementation on quality of life of people living with HIV: A double-blind, randomized controlled trial. Presented at the 2021 International Conference on AIDS and Sexually Transmitted Infections in Africa (ICASA 2021) in December in Durban, South Africa.

Authors' contributions: Aisha Gambo conceptualised and designed the study. Prof Nceba Gqaleni will supervise the preparation of the poster for the presentation. Aisha Gambo will present the study at the conference.

Signed:  Date: 10th July 2022

DEDICATION

To my late mother Malama Zainab Gambo

May this be a source of ‘‘Sadaqatul Jariya’’ for you

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Table of Contents

ABSTRACT

Background: This thesis reports on studies conducted at the S. S Wali virology centre, Aminu Kano Teaching Hospital (AKTH), Kano State, Nigeria. The studies aimed to evaluate the addition of *Moringa oleifera* Lam. leaves powder as a nutritional supplement on the anthropometric and immune status of adult HIV patients on antiretroviral therapy (ART). The studies further assessed the quality of life (QoL) and dietary diversity of PLHIV.

Method*:* The study was a six months double-blind randomized controlled trial conducted from December 2017 to November 2018. Two hundred consented patients on ART were randomly allocated to either *Moringa oleifera Lam.* group (MOG) or the control group (COG). The participants were followed for six months. The outcomes assessed were changes in anthropometric parameters (weight, body mass index [BMI], and mid-upper-arm circumference [MUAC], changes in immune status (CD4 cell count and viral load), and the impact of the intervention on quality of life (QoL) using the WHOQOLHIV-Bref questionnaire. Additionally, the dietary diversity of the patients was assessed using the FAO 24-hour dietary recall questionnaire.

Results: One hundred and seventy-seven patients completed the six-month follow-up (89 MOG versus 88 COG). At study inception, both groups had similar socio-demographic, socioeconomic, nutritional status, and immunological characteristics. At both baseline and sixth month, a poor dietary diversity pattern was observed. The food groups most commonly consumed in both MOG and COG were cereals, spices and condiments, oils, fats and palm oil, and dark green vegetables. In both groups, participants were in the medium or low dietary diversity tercile. Over the study period, *Moringa oleifera* Lam. leaf supplementation did not have an impact on any of the anthropometric parameters measured. However, *Moringa oleifera* Lam. leaf supplementation intervention and ART were effective in improving the CD4 cell counts of the study participants. No effect was observed in the viral loads in both study groups. Supplementation with *Moringa oleifera* Lam. leaf for PLHIV that are on ART improves the quality of life (QoL) domains of physical, psychological, level of independence, and social relationships.

Conclusion: The study suggests that nutritional supplementation with *Moringa oleifera* Lam. leaf has a beneficial effect among adult HIV patients on ART in a limited resource setting. In low-income settings like Nigeria, programs should consider nutritional supplementation as part of a comprehensive approach to ensure optimal treatment outcomes in people living with HIV and AIDS.

Keywords: *Moringa oleifera* Lam., Nutritional supplementation, HIV and AIDS, CD4 cell counts, anthropometric, quality of life, Antiretroviral therapy, malnutrition.

Clinical Trial Registry registration number: PACTR201811722056449

ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AKTH	Aminu Kano Teaching Hospital
ART	Antiretroviral treatment
BMI	Body Mass Index
BUK	Bayero University Kano
CD4	Cluster of differentiation 4
CI	Confidence Interval
COG	Control Group
DQQ	Diet Quality Questionnaire
FAO	Food and Agriculture Organisation
HIV	Human Immunodeficiency Virus
IDD	Individual Dietary Diversity
MOG	Moringa oleifera Group
MUAC	Mid Upper Arm Circumference
PI	Principal Investigator
PLHIV	People Living with HIV
QoL	Quality of Life
RUTF	Ready to Use Therapeutic Foods
SANAS	South African National Accreditation System
S. S. Wali	Sadiq Suleiman Wali
UKZN	University of Kwazulu-Natal
WHO	World Health Organization
WHOQOL-HIV BREF	World Health Organisation Quality of Life-HIV Brief

CHAPTER ONE

INTRODUCTION

1.1 Background and Context of the study

Globally, millions of people have been affected by the HIV and AIDS pandemic. It is of major concern as 79.4 million persons have been infected with the virus since the start of the epidemic, as reported by the 2021 UNAIDS Global statistics report [1]. Furthermore, 36.3 million deaths from AIDS-associated illnesses have been recorded [1]. In 2020, an estimate of 37.7 million people has been reported to be living with HIV worldwide, with 1.5 million that are infected newly and 680 000 deaths from illnesses that are AIDS-related [1].

Globally, the Sub- Saharan Africa region is largely affected by the HIV and AIDS epidemic. In 2018, 20.6 million people have been estimated to be living with HIV [2]. Amongst the 800,000 new HIV infections recorded, six in seven is amongst adolescent girls that are aged between 15–19 years [1].

Nigeria has the second-leading HIV pandemic globally. In 2018, available data shows an HIV prevalence of 1.5 percent amongst adults that are aged 15-49 years. Due to the size of the population of Nigeria, 1.9 million persons were reported to be living with HIV [3]. Other Sub-Saharan African countries like South Africa and Zambia, have a higher prevalence of HIV of 20.4% and 11.3%, respectively [3]. Furthermore, 130,000 new HIV infections and 53,000 AIDS-associated deaths were reported in the same year [3]. Of every new HIV infection and death from AIDS-associated illnesses recorded in the regions of Western and Central Africa, more than half is attributed to Nigeria alone. This could be ascribed to the huge population size of Nigeria in comparison to the other countries in the region [4].

In Kano State, northwest Nigeria, the 2018 Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS) reported an HIV prevalence of 0.6% (95% CI 0.2-1.0) [5].

Antiretroviral therapy (ART) has been confirmed effective in reducing morbidity and death related to HIV infection. This is through the reduction in HIV viral load and improvement in CD4 cell counts level related to decreased occurrence of opportunistic infections [6]. The high prevalence of AIDS-related deaths in Nigeria could be attributed to poor treatment coverage and adherence to ART [3].

In September 2015, the World Health Organisation (WHO) recommended the immediate initiation of ART to all persons with HIV diagnosis regardless of age and level of CD4 cell count [7]. Therefore,

despite the continuing severity of the epidemic, this brought about tremendous progress, showing the rapid pace of treatment increase in all regions [4].

Nigeria implemented a ‘test and treat’ policy in 2016, which brought steady progress on increasing access to ART [8]. As a result, Nigeria almost tripled the number of People Living with HIV (PLHIV) that have access to ART from 360,000 people in 2010 to over 1 million people in 2018 [8]. The Kano State Agency for the Control of AIDS reported that 35% of PLHIV are on ART in November 2019 [9].

Regrettably, in Nigeria, viral suppression stands at 42.3% among PLHIV aged 15–49 years. This indicates that more than half of PLHIV still do not have suppressed viral loads [3, 8].

HIV/AIDS and malnutrition are intertwined in a vicious circle [10]. Malnutrition is a common manifestation amongst individuals (adults and children) infected with HIV and AIDS [11]. It influences disease progression, increases morbidity, and reduces chances of survival [12, 13]. In addition, patients with chronic HIV infection experiences malabsorption. This could increase the loss of nutrients and appetite and increase energy expenditures, all contributing to poor nutritional status [14].

In Sub-Saharan Africa, between 2015 and 2016, the prevalence of malnutrition has risen from 20.8 to 22.7 percent. Accordingly, in 2016, malnourished people rose from 200 to 224 million [15]. These account for 25 percent of the 815 million malnourished people globally [15].

In South Africa, the 2013 National Health and Nutrition Examination Survey (SANHANES-1) shows that 26 percent of the populace had food insecurity, 28.3 percent were in danger of hunger, and 45.6 percent were food secured [16]. This high proportion of food insecurity at the household level in South Africa is also a vulnerability exaggerated by the burden of HIV and AIDS [13].

In Nigeria, the UN Food and Agriculture Organization (FAO) reports that over 9 million people face food insecurity [17]. Malnutrition is a public health challenge in Nigeria. Data demonstrated that the country has the second-highest prevalence of stunted children in the world, with a national prevalence rate of 32 percent [18]. In addition, two million children and 7 percent of women that are of childbearing age experience severe acute malnutrition [18]. The states in the northern region of Nigeria are most affected by malnutrition, with the northwest zone having the highest proportion of stunted children with a prevalence of 55% [19]. Kano state has a stunting prevalence of 46% [20].

Nutrition plays a vital role in the maintenance and improvement of the immunological status of PLHIV [21]. Adequate amounts of micro and macronutrients are essential for normal body functioning [22]. These delays disease progression and are associated with positive health outcomes and reduced incidence of mortality [23]. Furthermore, adequate nutrients are essential to improve the efficacy and minimize side effects of ART and optimize treatment outcomes [24].

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 nairas (the US $381.75) per year [25]. Due to this high poverty level in Nigeria and other developing countries, the lack of healthy, nutritious foods is a major challenge to PLHIV.

Therefore, based on the challenges mentioned above, it is evident that malnutrition in PLHIV is a global challenge. Effective nutritional supplementation strategies are essential for enhancing the effectiveness of ART to improve the quality of life of PLHIV to enable them to realise their full potential.

Thus, we offered a solution using *Moringa oleifera* Lam*.* leaves powder as a possible nutritional supplementation. *Moringa oleifera* Lam*.* is a novel, nutritious, cost-effective, culturally acceptable, effective, and regionally produced plant. In addition, several studies have provided relevant information on the use of *Moringa oleifera* Lam. as a nutritional supplementation amongst PLHIV who are taking ART [26, 27]. In Nigeria, the Nigerian Federal Government Raw Materials Research and Development Council (RMRDC) supports the farming and consumption of *Moringa oleifera* Lam. due to its nutraceutical potential [28]. *Moringa oleifera* Lam. could be used to supplement the diets of PLHIV and reduce the burden of malnutrition in Sub - Saharan Africa and Nigeria.

Firstly, we assessed the dietary diversity pattern of the participants over the study period using the FAO Individual Dietary Diversity (IDD) questionnaire. This tool has been used in studies conducted in various countries like Ethiopia [29], Rwanda [30], and Nigeria [31]. This study aimed to determine the dietary diversity consumed by the participants. This is to enable us to examine the need for nutritional supplementation to complement their regular diets. The study also assessed how *Moringa oleifera* Lam. leaves - supplemented diet affected the nutritional statuses and CD4 of PLHIV. This study confirmed consumption of poorly diversified diets indicating the need for nutritional assistance in the diets of PLHIV [32].

Secondly, we conducted a double-blind, randomised controlled trial to determine the impact of six months *Moringa oleifera* Lam. leaves supplementation on the anthropometric status of adult HIV patients on ART. We assessed changes in weight and BMI throughout the study period. After six months of follow-up, the study confirmed that *Moringa oleifera* Lam. leaf powder supplementation intervention was ineffective in improving the study participants' weight or BMI.

Thirdly, we conducted a six-month double-blind randomised trial to investigate the impact of the addition of *Moringa oleifera* Lam. leaves to the diet of adult HIV patients on their immunological status and anthropometric parameters. We assessed the changes in CD4 cell counts, viral load, weight, and BMI from baseline to six months. The study established evidence that *Moringa oleifera* Lam. leaves supplementation can improve the CD4 cell counts of PLHIV that are on ART [33].

Every intervention aims to improve the QoL. Therefore lastly, we assessed the QoL of PLHIV on ART before and after taking *Moringa oleifera* Lam. leaves supplementation. We used the World Health Organisation Quality of Life-HIV Brief (WHOQOL-HIV BREF) questionnaire to determine the impact of the intervention on the different domains of QoL. This assessment tool has been used in studies conducted in several countries, including Nigeria [34, 35]. The tool consists of six different domains: physical, psychological, level of independence, social relationships, environment, and spirituality/religion/personal beliefs [36]. The evaluation of QoL in different domains allows observing the aspects in which treatments are effective, giving the potential to assist in making decisions about the most suitable therapeutic measures with the prospect to reduce costs of health care [37]. The study revealed the potential of *Moringa oleifera* Lam. leaves supplementation to improve the physical, psychological, level of independence and social relationships QoL domains of PLHIV that are on ART [38].

From the above findings, our study confirmed the critical role of using *Moringa oleifera* Lam. as nutritional supplementation in PLHIV that are on ART in resource-limited settings like Nigeria.

1.2 Problem statement

Malnutrition is a significant challenge amongst people infected with HIV and AIDS. It brings about an imbalance between the cellular supply of nutrients and the body's requirement for these nutrients to ensure proper growth and development and to support the maintenance of specific functions [39]. Nutritional deficiencies in PLHIV are the consequence of a combination of the direct effect of HIV, reduced dietary intake, opportunistic infections, malabsorption, and an increase in energy expenditure [40]. Furthermore, under-nutrition challenges the survival, growth, and optimal development of children and women. It could cause poor cognitive development, a reduction in human capacity, premature death, and other health consequences, which could all affect the strength and capacity of nations [41]. Undernutrition significantly hinders socio-economic development and the potential to reduce poverty [41]. Throughout the world, the intensity of maternal and child under-nutrition remains unacceptably high [41].

Role of Nutritional Supplementation in People Living with HIV and AIDS

The nutritional status of people infected with HIV has been broadly studied because it influenced both humoral and cell-mediated immune functions [42]. The deficiencies of vitamins and both micro and macronutrients are likely to co-exist among HIV-infected individuals. Micronutrients are essential for maintaining optimal immunological function and reducing oxidative stress and other metabolic processes [42].

Several studies have reported the effects of different micronutrient supplementation on the immune and nutritional status of HIV- infected individuals. Zinc and vitamin A play a prominent role in maintaining cellular integrity [43]. Vitamin A has been reported to have significant immunoregulatory properties on monocyte differentiation and function and has been shown to increase the lymphocyte count, particularly the CD4+ subset, improve natural killer cell toxicity, and improve the maintenance of maintenance epithelial integrity [42]. Deficiencies in antioxidants during HIV infection may aid disease progression by contributing to immune dysregulation and viral replication. Selenium and zinc are the key trace elements that serve as antioxidants and play an essential role in disease progression among people infected with HIV-1 [44]. Selenium has been reported to play an important role in both cell-mediated and humoral immune responses. Selenium supplementation was reported to prevent, to some extent, morbidities in people infected with HIV-1 [42]. Other vitamins and micronutrients that play vital roles include vitamins E, B_{12}, C, folic acid, and iron.

A study conducted to determine the uptake of micronutrient supplements among PLHIV in Kayole, Nairobi, reported a high uptake of vitamin and mineral mix, zinc, vitamin B6, vitamin A, folate, and iron by the study participants. The study reported major reasons for micronutrient uptake were due to the untoward effects of ART, opportunistic infections, and loss of appetite. The study observed a high level of awareness of the importance of micronutrients supplements by the participants [45].

Nutritional interventions with macronutrient supplementation in PLHIV are essential in meeting additional energy needs for those with BMI < 18.5 kg/m^2 and for malnourished children as recommended by the WHO [46]. These interventions aim to improve their nutritional status and thus delay disease progression. Various approaches have been implemented, such as the use of supplemental formulas. Examples include a high-protein, high-energy meal called FutureLife porridge® [47]; a lipid-based nutrient supplements (LNS) containing whey or soya [48] and a nutritional meal called Amtewa, which consist of Glycine max 50g (Soya bean); *Pennisetum americanum* 20g (Millet); *Moringa oleifera* 15g (Moringa) and *Daucus carota* spp. sativa 15g (Carrot) [49].

A randomized controlled pilot study was conducted by Evans *et al.* to determine the effect of a nutritional supplement called FutureLife porridge® on the immune response, body mass index (BMI), and bioelectrical impedance in HIV infected individuals that are commencing ART in a public-sector hospital in Johannesburg, South Africa. Thirty-six patients were randomized to either the nutritional supplement with the ART group or the ART alone group. After six months follow up, preliminary results suggest that in patients that present with weight loss at the time of commencement of ART, the nutritional supplement, FutureLife porridge®, taken at the same time with ART, tends to promote weight gain, improve immune response and improve physical activity [47].

Similarly, Amlogu *et al.* examined the short and long-term effects of a micro and macronutrients supplementation meal called ‘Amtewa’. The study conducted in Nigeria assessed the prospect of the

intervention to delay the progression of HIV to AIDS in PLHIV. The 'Amtewa' nutritional meal consists of Glycine max 50g (Soya bean); *Pennisetum americanum* 20g (Millet); *Moringa oleifera* 15g (Moringa), and *Daucus carota* spp. sativa 15g (Carrot). After one year of follow-up, the nutritional meal was found to increase CD4 cell counts and Mid Upper Arm Circumference (MUAC) of PLHIV [49].

These studies emphasise the impact of adequate nutrients on the nutritional and immunological status of PLHIV.

The World Health Organization (WHO) supported the use of Ready to Use Therapeutic Foods (RUTF) for community-based management of severe under-nutrition to improve and maintain healthier nutritional status [50, 51]. RUTF is an energy-dense lipid paste consisting of peanut butter, milk powder, oil, sugar, mineral, vitamins, and protein mix [50]. RUTF does not need any further preparation or the addition of water before consumption and can be kept without refrigeration for long periods [52]. A study conducted on ART-treated children in Dar es salaam reported a positive association of RUTF intervention with stunting, wasting, and underweight statuses [50]. RUTF has the potential, therefore, to improve under-nutrition among HIV- positive children on ART. The popularity of RUTF in feeding program interventions and its increased success rates for the treatment of severe malnutrition has been emphasised [50].

However, the use of supplements such as RUTF might be of disadvantage in low resource settings in terms of acceptability, high price, and low regional availability [45, 53, 54]. Therefore a product that is novel, cheaper, culturally acceptable, effective, and regionally produced could be a solution in the management of malnutrition [52] such as adding the leaves or powder of *Moringa oleifera* Lam*.*, locally grown to supplement local diets [54]. Nutritional supplementation with *Moringa oleifera* Lam. could be more sustainable and cost-effective than imported supplements in developing countries like Nigeria.

***Moringa oleifera* Lam*.* Nutritional Supplement**

Moringa oleifera Lam. (syn. M. ptreygosperma Gaertn.) is a monogeneric family Moringaceae. It is one of the most recognized, naturalized, and widely cultivated species of the Moringaceae family [55]. *Moringa oleifera* Lam*.* tree is small or medium-sized and ranges from 5 to 10 m in height [55]. It has light-green tripinnately compound foliage, 25-45cm long, with pleasantly scented flowers of a creamy-white colour [56]. *Moringa oleifera* Lam*.* tree prospers best under the insular tropical climate [55]. It can survive very long dry seasons due to its tuberous roots that enable it to store water, and it is thus little affected by the drought [57].

Moringa oleifera Lam*.* is indigenous to the sub-Himalayan tracts of India, Pakistan, Bangladesh, and Afghanistan [55] and is currently cultivated in several parts of the world. *Moringa oleifera* Lam*.* is known by many names in the different parts of the globe. In English, it is called the "horseradish" or

“drumstick“ tree [58]. In Nigeria, it is found in the country's Northern, Southern, Western, and Eastern parts. It is known as “Zogale” in Hausa, “Gawara”, in Fulani, “Okwe Oyibo” in Igbo, and “Ewe Igbale” in Yoruba [59].

For centuries, humans and animals have consumed *Moringa oleifera* Lam. as every part of the tree is edible and used for various ailments [56]. *Moringa oleifera* Lam.'s medicinal uses arise because the whole plant is rich in protein, vitamins, minerals, and carbohydrate content. It is, therefore, of high nutritional value for both humans and livestock [60]. It is also valuable and used for other different purposes; this includes nutraceuticals, functional food preparations, water purification, and biodiesel production [61].

In various countries, especially in India, Pakistan, the Philippines, and several parts of Africa, the leaves, fruits, flowers, and immature pods of *Moringa oleifera* Lam. are used as a highly nutritious vegetable [55] with its nutrient-rich leaves which have high protein value [54]. The leaves are the part of the plant that is mainly used. Literature has shown the leaves are a rich source of both macro-and micronutrients and a good source of natural antioxidants [54]. They are rich in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins [55, 62]. Furthermore, *Moringa oleifera* Lam. leaves contain lots of bioactive compounds and amino acids [62, 63]. This makes it a good source of nutrients in resource-limited settings. For example, the fresh and dried leaf powder of *Moringa oleifera* Lam. can supply 1,000 mg and 4,000 mg of Ca, respectively, compared to 300 to 400 mg provided in 8 ounces of milk [64]. The protein contained in fresh leaves of *Moringa oleifera* Lam. is two times more than milk [53] and nine times that of yogurt. It has four times Ca than the amount found in milk, Fe content is 25 times that of spinach, K content is 63 times than milk, Mg is 36 times than egg, vitamin A is four times than carrot, and 13 times of spinach [63].

Various studies have been reported on the pharmacological activities, clinical trials, and multi-functional application of the *Moringa oleifera* Lam. plant in the literature. These pharmacological activities include anti-inflammatory, antidiabetic, hypotensive effects, anti- anaphylactic, anti-arthritic, anti-atherosclerotic, antibacterial, and anticancer activities. It has also been revealed to have a positive effect on hepatic and renal functions and regulation of thyroid hormone status. The leaves of *Moringa oleifera* Lam. have also been reported to offer protection against oxidative stress, hepatic fibrosis, liver damage, and liver injury [62, 65-67]. The fresh and dried leaves of *Moringa oleifera* Lam. act as a good source of vitamin A, which is essentially functional in preventing blindness, helps in reproduction and embryonic growth and development, and improves immune competence and cell differentiation [68].

Asiedu-Gyekye *et al.* reported a study in which X-ray fluorescence (XRF) analytical technique was used for the elemental and chemical analysis of powdered, solid, and liquid samples of *Moringa oleifera* Lam. dried leaves. The analyses generated a total of 35 elements (14 macro-elements and 21

micro-elements). The macro-elements in the powdered leaf samples include S, Ca, K, Mg, Na, P, Si, Cl, Al, Fe, and Mn. The minor elements produced by the analysis in the decreasing order are V, Ba, Cr, Y, Ba, Zn, Rb, Ce, La, Cu, Cs, Sn, Co, Ni, and Zr. The study concluded that the concentrations of all the elements generated were within the limits of the recommended daily allowance (RDA) [60].

In consideration of the extensive use of *Moringa oleifera* Lam. leaf powder as a food supplement and its use in the treatment of many diseases, Asiedu-Gyekye *et al.* further set out to determine if 14-day dosing of *Moringa oleifera* Lam. collected in Accra, Ghana could have any adverse effects in rats. The study reported that it is safe to consume *Moringa oleifera* Lam. taking into account the elemental composition when given to rats. Furthermore, to avoid the consumption and eventual accumulation of some of the essential elements contained in *Moringa oleifera* Lam. leaves, the study suggests that its consumption be limited to a maximum of 70 grams per day [60].

The use of *Moringa oleifera* Lam. by PLHIV either alone or concurrently with antiretroviral drugs has been reported in the literature. A cross-sectional survey was conducted by Monera and Maponga to examine the prevalence and patterns of *Moringa oleifera* Lam. used by PLHIV in Harare, Zimbabwe. The study reported that a large proportion of the study participants that are taking ART consume *Moringa oleifera* Lam. This has provided evidence of the use of *Moringa oleifera* Lam. as a nutritional supplement with friends or relatives as the most common source of recommendation to the patients [26].

Furthermore, the impact of *Moringa oleifera* Lam. leaf powder supplementation compared to nutritional counseling on the nutritional and immune status of PLHIV was examined. A single-blind randomized controlled trial was conducted in Kinshasa, the Democratic Republic of Congo. Sixty adult patients; randomly allocated to either *Moringa oleifera* Lam. group (MG) or nutritional counseling group (CG) were followed for six months. Measures were conducted monthly to observe changes in BMI, while biological parameters were measured at times of admission and the end of the study. Results showed a significant increase in BMI and albumin levels in the MG as compared to CG. A significant interaction in professional activity was also observed. The study concluded that under medical supervision, the nutritional status of PLHIV that are on ART could be locally improved with the readily accessible *Moringa oleifera* Lam. leaf powder supplementation [69].

Monera *et al.* reported the *in vitro* CYP3A4 inhibitory activity of mediated 6ß-hydroxylation of testosterone by *Moringa oleifera* Lam. leaf extracts signifying the possibility of interaction with antiretroviral drugs. The *in vitro* data alone is not enough to conclude the clinical significance of concomitant administration of *Moringa oleifera* Lam. with ART in PLHIV [70]. Besides, the interaction between *Moringa oleifera* Lam. leaf powder and tenofovir/lamivudine/efavirenz has not been reported.

No adverse clinical effects have been recorded despite its extensive and concomitant use in Sub-Saharan Africa, especially in Nigeria.

Despite widespread knowledge and reported benefits of *Moringa oleifera* Lam., few well-controlled scientific studies have been reported on the benefits of *Moringa oleifera* Lam. in patients with nutritional deficiencies. Moreover, there is a paucity of scientifically robust studies on the effects of *Moringa oleifera* Lam. on the immunological and anthropometric status of PLHIV. More so, studies on the impact of *Moringa oleifera* Lam. on their QoL are equally lacking.

1.3 Research Hypothesis

The study hypothesizes that *Moringa oleifera* Lam. leaf as a nutritional supplement could improve the anthropometric and immunological status of adult HIV-positive individuals on ART.

1.4 Research Aim

Based on the above research hypothesis, the aim of the study is:

To evaluate the effect of six months *Moringa oleifera* Lam. leaf supplementation on the anthropometric parameters; immunological development and quality of life (QoL) of HIV-positive adults on ART.

1.5 Research Objectives

The following are the objectives of the study

1. To assess individual dietary diversity (IDD) by using the FAO questionnaire
2. To determine the effect of *Moringa oleifera* Lam. leaf supplementation on anthropometric parameters [weight; BMI, MUAC].
3. To determine the effect of *Moringa oleifera* Lam. leaf supplementation on immunological parameters [CD4 count; viral load].
4. To assess the effect of *Moringa oleifera* Lam. leaf supplementation on quality of life (QoL) by using the WHOQoL-HIV BREF questionnaire.

1.6 The Study Area: Kano State, Nigeria

Nigeria is a country in West Africa. It is surrounded by the Niger Republic in the North; the Republic of Chad and Cameroun in the East; the Republic of Benin in the West; and the Atlantic Ocean in the South [71]. The nation covers a total surface area of 923, 768 sq km and 853 km of coastline and lies

within latitudes 4_10 and 13_90 North and longitudes 2_20 and 14_300 East [71]. There are 36 states in Nigeria, as well as the Federal Capital Territory (FCT) in Abuja. The states are grouped into six geopolitical zones: North - Central, North - East, North - West, South - East, South-South, and South - West. As of 2019, Nigeria is estimated to have a population of over 200.96 million, ranking 7th in the world [71, 72].

Kano State is in northwest Nigeria. It has an approximate land area of 21,276.87 km2. Kano borders four states: Jigawa to the north, northeast, and east, Bauchi to the southeast, Kaduna to the southwest, and Katsina to the west and northwest [73, 74]. The area is divided into three senatorial districts (Kano Central, Kano North, and Kano South), which are further subdivided into 44 local government areas consisting of 484 wards [74].

Kano State has a total population of more than 13 million [75] who are mostly Hausa/Fulani and predominantly Muslims. Considerable numbers are Ibo’s and Yoruba’s. Most are traders, businessmen, farmers, and civil servants [76]. It serves as centre for trade and commerce for all the Northern states and some other states from the country's different geopolitical zones, including some neighboring countries like Niger and Chad [77].

Figure 2: Map of Kano State

Source: Department of Geography, Bayero University Kano, Nigeria

1.7 General Methodology

To achieve all the objectives, a general methodology to be employed was proposed as outlined below

METHODOLOGY

1.7.1 Study Protocol

This subsection of Chapter 1 gives a detailed description of the research methodology proposed to achieve the specific research objectives raised in this study.

This section outlines the study protocol presented as a manuscript in the format of the target journal.

Submitted: Journal of Public Health Research

Evaluation of the addition of Moringa oleifera as a nutritional supplement on the anthropometric; viral load, CD4 cell counts and quality of life of adult HIV patients on antiretroviral therapy in Nigeria: A study protocol

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Abstract

Introduction: Malnutrition is a common manifestation among people infected with HIV and AIDS. It is associated with nutritional deficiencies that influence the progression of the disease, increase morbidity, and lower chances of survival. Improved nutrition with nutritional supplementation may optimize treatment outcomes and improve the quality of life (QoL) of people living with HIV (PLHIV). This study aims to evaluate the six months' impact of *Moringa oleifera* Lam. leaves on the anthropometric, immunologic status, and QoL of adult HIV patients on antiretroviral therapy (ART).

Methods: The study is expected to recruit and randomize 200 participants into two groups namely: *Moringa oleifera* Lam. group (MOG) and Control group (COG). Changes in anthropometric parameters [weight; body mass index (BMI); and mid-upper arm circumference (MUAC)] and CD4 cell counts will be assessed at baseline to six months. HIV-1 viral load and changes in QoL of the participants will be evaluated using the WHOQOL-BREF at baseline and six months. The dietary diversity of participants will be assessed using FAO individual dietary diversity questionnaire throughout the study period. Descriptive statistics and inferential statistics of repeated ANOVA; $P < 0.05$; will be used to analyse the data generated.

Discussion: The result of this study will identify and highlight the efficacy of *Moringa oleifera* Lam. leaves nutritional supplementation compared with the control in the management of nutritional deficiencies in PLHIV.

Keywords: *Moringa oleifera*; HIV and AIDS; Malnutrition; Antiretroviral therapy

Introduction

Malnutrition is a common manifestation among people (adults and children) infected with HIV and AIDS [1]. It is associated with nutritional deficiencies that influence the progression of the disease, increase morbidity, and lower chances of survival [2, 3]. Antiretroviral therapy (ART) has demonstrated efficacy in reducing morbidity and mortality associated to HIV infection by a reduction in HIV viral load and improved CD4 level, which is associated with decreased opportunistic infections episodes [4]. However, improved nutrition may enhance the effectiveness of ART [5] and directly modify disease severity indicators [6].

Several nutritional intervention strategies have been implemented in PLHIV [7, 8]. However, the use of such interventions might be of disadvantage in terms of acceptability, high production cost, and low regional availability [9, 10]. Supplementing local diets with leaves of *Moringa oleifera* Lam. locally grown could be a solution for malnutrition management in PLHIV [11]. *Moringa oleifera* Lam. (syn. M. ptreygosperma Gaertn.) leaves have been recorded to be an important source of both micro-and macronutrients [12] and high in protein quality [11]. They also act as a source of natural antioxidants due to various types of natural antioxidant compounds for instance ascorbic acid, flavonoids, phenolics, and carotenoids [13]. Studies have reported the use of *Moringa oleifera* Lam. by PLHIV [14].

Monera *et al.* reported the *in vitro* CYP3A4 inhibitory activity of mediated 6ß-hydroxylation of testosterone by *Moringa oleifera* Lam. leaf extracts signifying the possibility of interaction with antiretroviral drugs. However, no adverse effects have been recorded despite the extensive and concomitant use of *Moringa oleifera* Lam. with antiretroviral drugs in Sub-Saharan Africa [15].

Therefore, this study is proposed to evaluate the addition of Moringa oleifera Lam. as a nutritional supplement on the anthropometric, viral load, and CD4 cell counts of adult HIV patients on antiretroviral therapy in Nigeria. The dietary diversity will be assessed, and the impact of the supplement on various domains of Quality of life (QoL) will also be examined.

Aims and Objectives of Study

The study aims to evaluate the effect of six months *Moringa oleifera* Lam. supplementation on the nutritional and immunological development of HIV-positive adults on ART. The study will further assess the impact of *Moringa oleifera* Lam. supplementation on various domains of QoL.

The objectives are:

- To assess individual dietary diversity (IDD) by using the FAO questionnaire
- To determine the effect of *Moringa oleifera* Lam. leaf supplementation on anthropometric parameters [weight; BMI; MUAC].

- To assess the impact of *Moringa oleifera* Lam*.* leaf supplementation on immunological parameters [CD4 count; viral load].
- To assess the effect of *Moringa oleifera* Lam*.* leaf supplementation on QoL domains by using the WHOQoL-HIV BREF questionnaire.

Methodology

Study design

The study is a double-blind, randomized controlled trial (RCT). Double-blind, randomized controlled trials are the gold standard in intervention studies.

Study participants

The study participants will be male and female adults 18 yrs and above who present at the HIV centre with a prior or new diagnosis of HIV infection.

Inclusion criteria for the study are: patients with CD4 counts ≤ 500 cells/mm^3; commenced ART no less than three months; on Tenofovir + Lamivudine + Efavirenz ART drug regimen, and be willing to comply with the study protocol. Patients who have a known allergy to *Moringa oleifera* Lam. or placebo (starch powder), pregnant women, and those with active opportunistic infection will be excluded from the study. Furthermore, individuals taking natural health products or nutritional supplements within 30 days of screening; participation in a clinical trial of whichever investigational product a month preceding visit one or during the study will be excluded. To ease patient monitoring, those residing outside Kano State, where the study will be conducted will also be excluded. Lastly, individuals who do not give their consent to participate will also be excluded from the study.

Study setting

The setting of the study will be the HIV clinic known as the S. S Wali Virology centre situated at the Aminu Kano Teaching Hospital, Kano state (AKTH) Nigeria.

AKTH is a tertiary health institution and also a center for the referral of patients. The center runs 5 days a week HIV clinic. It also serves as a center of excellence for clinical assessment, laboratory investigations, HIV counseling and testing (HCT), treatment, and care with support by the Federal Government of Nigeria and the Institute of Human Virology, Nigeria (IHVN) in collaboration with its global partners, which includes the Centers for Disease Control and Prevention (CDC) and the Global Fund to Fight AIDS, Tuberculosis, and Malaria. The center also attends to every patient with HIV infection either diagnosed in the hospital or referred from outside the health institution.

Study team

The study team will consist of 2 clinicians, one of whom is a consultant pulmonologist, in charge of the virology center, and coordinator, Institute of Human virology AKTH, who is also a professor with over 15 years of research experience. The team will also include two pharmacists, one of whom is the Principal Investigator (PI), with almost 10 years of clinical experience with HIV and AIDS patients; two qualified nurses, one phlebotomist, and one trained research assistant. All the study team members will be healthcare professionals at the S. S Wali virology centre. The hospital dietician will be consulted.

Sample size calculation

The sample size will be determined by utilizing G*Power version 3.1.9.2 [16] to detect a medium effect size (Cohen's d^1=0.5) [17] or 0.5 standard deviations in mean weight or CD4 cell count by Randomised Controlled Trial (RCT) arm with 90% power (1-β [type 2 error probability]) and 95% confidence (or 5% α error probability [type 1]) assuming a balanced 1:1 study design. The calculated total sample size will be 172. To take care of the attrition rate, the sample size will be rounded up to 200 which will be divided into the *Moringa oleifera* Lam. group (MOG) and Control group (COG) with each group comprising of 100 participants.

Randomization and blinding

Block randomization presents a simple way to balance study arms and decrease the chances for bias and confounding. Block randomization will be employed to balance the two groups during the enrolment period. PASS 12.0 software will be employed to generate the randomisation list using Wei's Urn algorithm by a statistician who is not involved in the research. Furthermore, we will check that randomization was successful after enrolment to ensure there are no significant differences in the socio-demographic and/or other characteristics between the two study arms. A random allocation sequence will be generated to allocate and assign each participant to either the MOG or the COG as they fulfill the inclusion criteria and consent to participate. As the study design is a double-blinded trial, all the members of the study team, as well as the principal investigator (PI) and the study participants, will be blinded to allocating participants to the study groups. Fig. 1 outlines the study flow chart.

Intervention

A botanist at the Department of Biological Science, Bayero University Kano (BUK), Nigeria, will authenticate the fresh *Moringa oleifera* Lam. leaves. The placebo to be used for the study will be produced by colouring cornstarch powder with chlorophyll [18]. The *Moringa oleifera* Lam. leaves and the placebo will be processed and packaged at Dala Foods Nigeria Limited. Dala Foods Nigeria Limited is situated in Kano State. It is a Nigerian-based company that specialises in food processing.

Both the intervention and the placebo will be provided as a powder in a sealed package. The *Moringa oleifera* Lam. supplementation or placebo (5 g) will be consumed three times daily at mealtimes by adding it to foods just before eating them [19, 20].

Compliance will be monitored by questioning each patient during the monthly hospital visits to evaluate adherence. Schedule monitoring will also include weekly telephone calls to the patients in the first month and biweekly after that.

Study outcomes

The study outcomes will be obtained using the data collection tools as follows:

1. Sociodemographic information: The sociodemographic information of the study participants will be captured at the beginning of the study. These include age, gender, educational level, marital status, occupation, family size, and monthly income.
2. Dietary diversity information: The FAO/Nutrition and Consumer Protection Division recommended questionnaire for the data collection on individual Dietary Diversity will be used to determine the type of foods consumed in the last 24 hours. Dietary Diversity is the number of different foods or food groups eaten over a given reference period [21]. A dietary diversity questionnaire is an assessment tool that offers a quick, easy-to-use, and cost-effective approach to assess changes in dietary quality at the individual level as well as the household levels. The questionnaire has been used in studies conducted in Nigeria [22]. The research team members and the participants will conduct the face-to-face interview at baseline and each monthly visit to the clinic. The participants will be asked the type of foods they had consumed at definite periods to signify the different types of meal such as breakfast, lunch, dinner, as well as snacks consumed at each interview. All the foods and drinks mentioned by the participants will then be documented on a 24-hour dietary recall assessment tool in the respective periods of the day and will be sorted into the 15 food groups, i.e.; Cereals, Vitamin A-rich vegetables and tubers, white tubers, and roots, dark green leafy vegetables, other vegetables, Vitamin A-rich fruits, other fruits, organ meat (iron-rich), flesh meats, eggs, fish, legumes, nuts and seeds, milk and milk products, oils, and fats. Each food group consumed by the participants will be assigned a score of 1, and the overall individual scores will be calculated. The overall individual food score will be classified into terciles, i.e.: Low individual Dietary Diversity score (IDDS) terciles which are equal to low dietary diversity (signifying the consumption of 1 to 3 food groups); Medium IDDS terciles corresponding to 4 to 5 food groups and High IDDS terciles means consumption of six and above food groups.
3. Anthropometric variables: The weight will be taken with the participants wearing light clothes and without shoes using a digital scale, height using a stadiometer, and mid-upper arm circumference (MUAC) using a non-elastic flexible meter rule. The body mass index (BMI) will

be calculated as the weight in kilograms divided by the square of height in meters. All anthropometric measurements will be assessed at baseline and each monthly hospital visit.

4. Immunological variables: The CD4 variable will be first obtained from the patients' hospital records. Subsequently, a trained phlebotomist will conduct the CD4 counts and viral load tests at the laboratory situated at the S. S Wali Virology Center at AKTH. The CD4 count will be quantified using a Partec flow cytometer (Partec, Munster, Germany) [23], and the viral load by polymerase chain reaction (PCR) [24, 25]. CD4 test will be performed at the beginning before the start of the study and every subsequent monthly hospital visit for each study participant, whilst the viral load test will be performed twice, at the beginning before the start of the study and after the sixth month i.e. at the end of the study.
5. QoL: The World Health Organization Quality of Life Brief (WHOQoL-BREF) will be employed to examine QoL of the study participants. The WHOQOL- HIV BREF is considered a valid assessment tool to evaluate the QoL of PLHIV. The tool consists of two sections: The first section will record the sociodemographic characteristics, whilst the second section will be used to record the various domains of QoL. This section (section 2) has 31 items, with a 5-point Likert scale on every item. A scale of 1 indicates low (negative perceptions), while a scale of 5 indicates high (positive perceptions). The 31 items are distributed in six domains of QoL. They are physical health, psychological health, level of independence, social relationships, environment, and spirituality/religion/personal beliefs [26]. The WHOQOL-HIV BREF questionnaire will be administered at the beginning before the start of the study and after six months of using the study interventions i.e. at the end of the study.

All the data collection tools will be translated to the local language that is spoken. Experts will translate the tools at the Department of Languages, Bayero University Kano (BUK). The same translators will be responsible for the back translation of the filled questionnaires to the English language

Data Management

All the research data obtained from the trial will be monitored regularly to ensure its accuracy and consistency. Data will be captured electronically using specialised data management software such as Epi-Info using constraints and validation checks to reduce data entry errors. Electronic data will be stored on computers while all hard copy data will be kept under lock and key. The data will be available only to authorised research team members. Any queries arising from missing data or data anomalies will be resolved.

Data Processing and Analysis

Preliminary analysis will be performed to test the normality of the data using the Kolmogorov V-Smirnov test before the analysis. Demographic variables and all the assessment of outcomes at baseline,

monthly, and after 6-months follow-up will be transferred into Microsoft Excel spreadsheet. Subsequently, the data will be imported into SPSS version 26.0 for analysis. Descriptive statistics of frequency, percentage, mean, and standard deviation will be used to describe the participants' demographic characteristics and all assessments. Outcome measures will be calculated for each group by determining the mean differences in the various measures from baseline, monthly, and after 6-months follow-up. Significance differences in mean CD4 count or weight (i.e., primary hypothesis) at follow-up in the two arms will be assessed using the standard t-test. If the data are not normal, then the Wilcoxon rank-sum test will be used instead. A repeated measure ANOVA will be used for the analyses to determine if there is any statistically significant difference within and between groups at $p < 0.05$. Bivariate and multivariable-adjusted mixed-effects linear regression may also be used to assess the primary hypothesis.

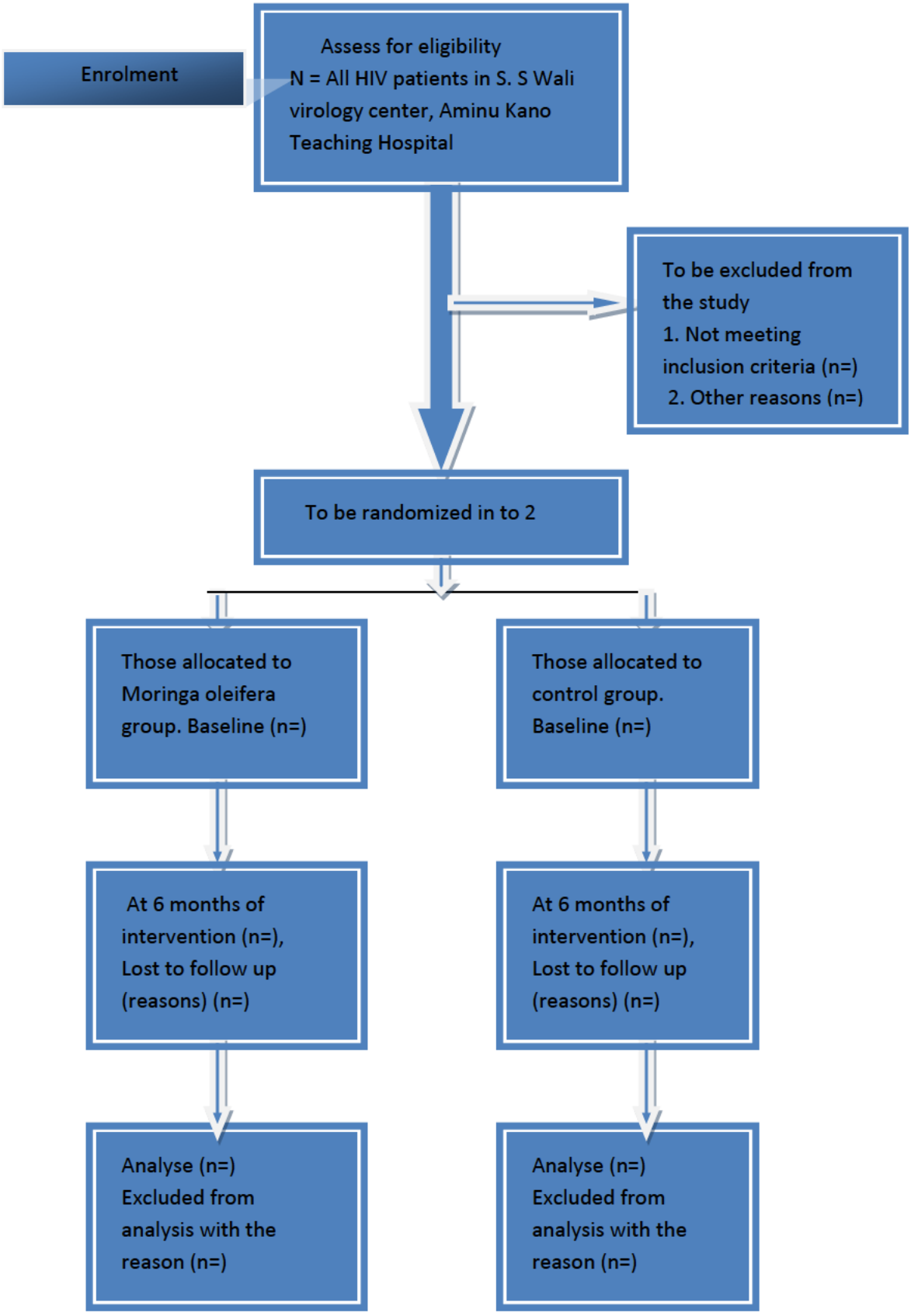

Fig. 1: Consort flowchart

Results

The outcome of this study is supposed to offer additional knowledge on the effect of consuming the leaves of *Moringa oleifera* Lam. as a nutritional supplement on the anthropometric and immune status of people living with HIV that are taking ART. The study also expects that the supplement will improve their QoL. The result of the dietary diversity is also expected to offer information on the pattern of dietary diversity consumption.

Harm

The safety of the *Moringa oleifera* Lam. plant for use for nutritional and medical purposes has been documented [27, 28]. No adverse reaction or toxicity was reported when *Moringa oleifera* Lam. leaf powder was consumed in quantity, usually taken orally. There is no reported allergy to *Moringa oleifera* Lam. in the literature. Suppose there is a known allergy to Moringa oleifera Lam. by any of the participants, in that case, these subjects will be excluded from the study as was stated as exclusionary criteria for study participation as a precautionary measure. Any adverse events will be closely monitored. However, in the event of any serious adverse reaction after taking the intervention at any point during the trial, the code will be unblinded for the safety of the study participant. All participants will be treated free of charge in the event of any adverse events relating to the consumption of the intervention.

Discussion

Globally, the Sub- Saharan Africa region is largely affected by the HIV and AIDS epidemic [29]. Nigeria has the second-leading HIV pandemic globally [30]. The prevalence of malnutrition has risen in Sub-Saharan Africa from 20.8 to 22.7 percent between 2015 and 2016. Data showed that the country has the second-highest prevalence of stunted children in the world, with a national prevalence rate of 32 percent [31]. In addition, two million children and 7 percent of women that are of childbearing age experience severe acute malnutrition [31].

PLHIV are susceptible to malnutrition as a result of intestinal damage. This causes impairment in the absorption of nutrients and a reduction in food intake as a result of vomiting and painful swallowing [32]. PLHIV uses complementary therapies because it gives them a feeling of control of their lives and as well as their illness [33]. In Nigeria, the Nigerian Federal Government Raw Materials Research and Development Council (RMRDC) supports the use of *Moringa oleifera* Lam. due to its nutraceutical potential [34].

Despite the widespread and concomitant use of *Moringa oleifera* Lam. with antiretroviral drugs in Sub-Saharan Africa, few well-controlled scientific studies have been reported on the benefits of *Moringa*

oleifera Lam. in patients with nutritional deficiencies. Therefore, it is hoped that the outcome of this study will add to the pool of knowledge in the management of nutritional deficiencies in PLHIV. Lastly, it is expected to improve treatment outcomes in PLHIV in Nigeria and other Sub- Saharan African countries.

Ethical consideration and consent to participate

The Biomedical Research Ethics Committee of the University of Kwazulu-Natal Durban, South Africa, has approved this study (reference number BFC294/16) and the Ethics Committee of Aminu Kano Teaching Hospital (AKTH) Kano, Nigeria (reference number NHREC/21/08/2008/AKTH/EC/2012). The study has been registered with the Pan African Clinical Trial Registry (www.pactr.org) with identification number PACTR201811722056449. All participants will provide a written and signed informed consent before taking part in the study. The trial will be conducted according to the principles enunciated in the Declaration of Helsinki [35] and good clinical practice (GCP).

Author contributions

Aisha Gambo developed the idea for the study; Aisha Gambo created the title, designed the study, and selected outcome measures for the study. Aisha Gambo and Nceba Gqaleni were responsible for drafting the initial manuscript. Aisha Gambo and Nceba Gqaleni edited and critically reviewed the manuscript to add value to its intellectual content.

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Data availability

The datasets used will be available on reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests.

References

1. Swaminathan S, Padmapriyadarsini C, Yoojin L, Sukumar B, Iliayas S, Karthipriya J, et al. Nutritional Supplementation in HIV-Infected Individuals in South India: A Prospective Interventional Study. Clin Infect Dis. 2010;51(1):51-7. DOI: 10.1086/653111.
2. Anabwani G, Navario P. Nutrition and HIV/AIDS in sub-Saharan Africa: an overview. Nutrition. 2005;21:96-9. DOI:10.1016/j.nut.2004.09.013.
3. Kimani-Murage EW, Norris SA, Pettifor JM, Tollman SM, Klipstein-Grobusch K, Gómez-Olivé XF, et al. Nutritional status and HIV in rural South African children. BMC Pediatr. 2011;11(23):1-13. http://www.biomedcentral.com/1471-2431/11/23.
4. Contri PV, Berchielli EM, Tremeschin MH, Negrini BV, Saloma˜o RG, Monteiro JP. Nutritional status and lipid profile of HIV-positive children and adolescents using antiretroviral therapy. Clinics. 2011;66(6):997-1002. DOI:10.590/S807-59322011000600013.
5. Blaise Savadogo LG, Donnen P, Kouéta F, Kafando E, Hennart P, Dramaix M. Impact of multivitamin and mineral supplements on mortality and nutritional status of hospitalized severely malnourished HIV/AIDS infected children. Open J Epidemiol. 2013;3:213-9. http://dx.doi.org/10.4236/ojepi.2013.34031.
6. Frank LB, Schall JI, Samuel J, Zemel BS, Dougherty KA, Tuluc F, et al. Dietary and Supplement Intake of HIV Infected Children and Young Adults. ICAN: Infant, Child Adolescent Nutrition. 2014;6(4):221-32.
7. Ndeezi G, Tylleskär T, Ndugwa CM, Tumwine JK. Effect of multiple micronutrient supplementation on survival of HIV-infected children in Uganda: a randomized, controlled trial. JIAS. 2010;13(18):1-9. http://www.jiasociety.org/content/13/1/8.
8. Flax VL, Phuka J, Cheung YB, Ashorn U, Maleta K, Ashorn P. Feeding patterns and behaviors during home supplementation of underweight Malawian children with lipid-based nutrient supplements or corn-soy blend. Appetite. 2010;54:504-11.
9. Dibari F, Bahwere P, Huerga H, Irena AH, Owino V, Collins S, et al. Development of a cross-over randomized trial method to determine the acceptability and safety of novel ready-to-use therapeutic foods. Nutrition. 2013;29:107-12. DOI:10.1016/j.nut.2012.04.016.
10. Sztam KA, Ndirangu M, Sheriff M, Arpadi SM, Hawken M, Rashid J, et al. Rationale and design of a study using a standardized locally procured macronutrient supplement as adjunctive therapy to HIV treatment in Kenya. AIDS Care 2013;25(9):1138-44. DOI: 10.080/09540121.2012.752564.
11. Thurber MD, Fahey JW. Adoption of Moringa oleifera to combat under-nutrition viewed through the lens of the “Diffusion of Innovations” theory. Ecol Food Nutr. 2009;48(3):212-25. DOI: 10.1080/03670240902794598.
12. Oduro I, Ellis WO, Owusu D. Nutritional potential of two leafy vegetables: Moringa oleifera and Ipomoea batatas leaves. Scientific Res Essay. 2008;3(2):57-60.

13. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: A Food Plant with Multiple Medicinal Uses. Phytother Res. 2007;21:17-25. DOI: 10.1002/ptr.2023.

14. Monera TG, Maponga CC. Prevalence and patterns of Moringa oleifera use among HIV positive patients in Zimbabwe: a cross-sectional survey. JPHiA. 2012;3:e6:22-4. DOI:10.4081/jphia.2012.e6.

15. Monera TG, Wolfe AR, Maponga CC, Benet LZ, Guglielmo J. Moringa oleifera leaf extracts inhibit 6β-hydroxylation of testosterone by CYP3A4. J Infect Dev Ctries. 2008;2(5):379-83. DOI:10.3855/jidc.201.

16. Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. . Behav. 2009;41:1149-60. DOI: 10.3758/BRM.41.4.1149.

17. Cohen J. Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, N J: Lawrence Earlbaum Associates. 1988.

18. Bahadoran Z, Tohidi M, Nazeri P, Mehran M, Azizi F, Mirmiran P. Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. Int J Food Sci Nutr. 2012;63(7): 767–71. DOI: 10.3109/09637486.2012.665043.

19. Idohou-Dossou N, Diouf A, Gueye AL, Guiro AT, Wade S. Impact of daily consumption of Moringa (Moringa Oleifera) dry leaf powder on iron status of senegalese lactating women. AJFAND. 2011;11 (4):4985-99.

20. Tété–Bénissan A, Quashie MIA, Lawson–Evi K, Gnandi K, Kokou K, Gbéassor M. Influence of Moringa oleifera leaves on atherogenic lipids and glycaemia evolution in HIV-infected and uninfected malnourished patients. J Appl Biosci. 2013;62:4610-9. DOI: 10.314/jab.v62i0.86072.

21. Ruel MT. Operationalizing Dietary Diversity: A Review of Measurement Issues and Research Priorities. J Nutr. 2003;133:3911S-26S.

22. Ajani SR. An Assessment of Dietary Diversity in Six Nigerian States. Afr J Biomed Res. 2010;13:161-7.

23. SOP Nigeria. ENUMERATION OF CD4+ T LYMPHOCYTE. STANDARD OPERATING PROCEDURE for Nigeria PreP Study. Available at: https://wwwprepwatchorg/wp-content/uploads/2016/09/NNACA_SOP_304docx 2015;LP-304. [Accessed 2022 January 5].

24. SOP Nigeria. HIV -1 VIRAL LOAD ASSAY. STANDARD OPERATING PROCEDURE for Nigeria PreP Study. Available at: https://wwwprepwatchorg/wp-content/uploads/2016/09/NNACA_SOP_305docx 2015;LP-305. [Accessed 2022 January 5].

25. Roche. COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 20 manuals. 2007; Available at: https://www.fda.gov/media/73824/download. [Accessed 2022 January 5].

26. Fatiregun AA, Mofolorunsho KC, Osagbemi KG. Quality of life of people living with HIV/AIDS in Kogi State, Nigeria. BJPM. 2009;11 (1):21-7.

27. Adedapo AA, Mogbojuri OM, Emikpe BO. Safety evaluations of the aqueous extract of the leaves of Moringa oleifera in rats. J Med Plant Res. 2009;3(8):586-91.

28. Asiedu-Gyekye IJ, Frimpong-Manso S, Awortwe C, Antwi DA, Nyarko AK. Micro- and Macroelemental Composition and Safety Evaluation of the Nutraceutical Moringa oleifera Leaves. J Toxicol. 2014;2014:1-13. http://dx.doi.org/0.1155/2014/786979.

29. Avert. HIV and AIDS in East and Southern Africa regional overview. Available at: https://wwwavertorg/professionals/hiv-around-world/sub-saharan-africa/overview. 2018. [Accessed 2022 January 5].

30. Avert. HIV and AIDS in Nigeria. Available at: https://wwwavertorg/professionals/hiv-around-world/sub-saharan-africa/nigeria. 2019. [Accessed 2022 January 5].

31. UNICEF. Nutrition. Available at: https://wwwuniceforg/nigeria/nutrition. 2018. [Accessed 2022 January 5].

32. Guerra EML, Porbén SS. T-Cell Number, Nutritional Status, and HIV: The Cuban Experience in the Provision of Food and Nutrition Care to People with HIV/AIDS. Health HIV Infected People. 2015;2 Chapter 21:367-87.

33. Bepe N, Madanhi N, Mudzviti T, Gavi S, Maponga CC, Morse GD. The impact of herbal remedies on adverse effects and quality of life in HIV-infected individuals on antiretroviral therapy. J Infect Dev Ctries. 2011;5(1):48-53.

34. RMRDC. Moringa oleifera- A National Crop for Economic Growth and Development. Proceedings of National Summit on Moringa Development. Available at: https://wwwacademiaedu/6686903/RAW_MATERIALS_RESEARCH_AND_DEVELOPMENT_COUNCIL_Moringa_oleifera_A_National_Crop_for_Economic_Growth_and_Development_PROCEEDINGS_OF_THE_FIRST_NATIONAL_SUMMIT_ON_MORINGA_DEVELOPMENT. 2010. [Accessed 2022 January 5].

35. WMA. World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. Clinical Review & Education - Special Communication. Available at: https://wwwwmanet/wp-content/uploads/2016/11/DoH-Oct2013-JAMApdf. 2013;310(20):2191-4. [Accessed 2022 January 5].

1.8 Overview of the thesis

This thesis consists of 6 chapters:

Chapter 1: This is the introduction to the thesis, giving the rationale for the research study, problem statement, research question, aim, and objectives. This chapter presents a review of recent literature on HIV and AIDS and nutritional supplements for HIV. It also presented a detailed methodology. It comprises a manuscript submitted to the journal, Journal of Public Health Research, entitled: ‘*’Evaluation of the addition of Moringa oleifera as a nutritional supplement on the anthropometric; viral load, CD4 cell counts and quality of life of adult HIV patients on antiretroviral therapy in Nigeria: A study protocol”.*

Chapter 2: This chapter presents the study results to determine the type of dietary diversity consumed by the study participants and the effect of *Moringa oleifera* Lam. leaves supplemented – diet on the nutritional status and CD4. This manuscript was presented in a publication in Heliyon. The publication is entitled: ‘*’Dietary diversity and impact of Moringa oleifera Lam. leave supplemented - diet on the nutritional status and CD4 cell counts of patients receiving antiretroviral therapy in Nigeria: A double-blind, randomized trial”.*

Chapter 3: This chapter presents the results of the effects of *Moringa oleifera* Lam. leaves supplementation on the anthropometric status of PLHIV that are on ART. This manuscript was presented in a publication in the Journal of Public Health in Africa. The publication is entitled: *“Does Moringa oleifera Lam. leaves supplementation have an impact on the weight and BMI of people living with HIV? A double-blind, randomized control trial”.*

Chapter 4: This chapter presents the results of the effects of *Moringa oleifera* Lam. leaves supplementation on the immunological and anthropometric status of PLHIV on ART in a resource-constrained setting. This manuscript was presented in a publication in PLOS ONE. The publication is entitled: ‘*’A double-blind randomised control trial to examine the effect of Moringa oleifera leaf powder supplementation on the immune status and anthropometric parameters of adult HIV patients on antiretroviral therapy in a resource-limited setting”*.

Chapter 5: This chapter offers the effects of *Moringa oleifera* Lam. leaves supplementation on the various domains of quality of life (QoL) of PLHIV. The manuscript was presented in a publication in the Quality of Life Research (Springer) journal. The publication is entitled: ‘*’Impact of Moringa oleifera leaves supplementation on the quality of life of people living with HIV: A double-blind randomised control trial”.*

Chapter 6: This chapter synthesises the above. It presents a summary of the research study findings and their strengths and limitations. It also includes a formal conclusion and makes recommendations for future research.

1.9 References

1. UNAIDS. FACT SHEET – WORLD AIDS DAY 2021. UNAIDS. Available: https://wwwunaidsorg/sites/default/files/media_asset/UNAIDS_FactSheet_enpdf. 2021. [Accessed 2022 February 20].

2. Avert. HIV and AIDS in East and Southern Africa regional overview. Available: https://wwwavertorg/professionals/hiv-around-world/sub-saharan-africa/overview. 2018. [Accessed 2022 February 20].

3. Avert. HIV and AIDS in Nigeria. Available: https://wwwavertorg/professionals/hiv-around-world/sub-saharan-africa/nigeria. 2019. [Accessed 2022 February 20].

4. UNAIDS. UNAIDS Data. Available: https://wwwunaidsorg/sites/default/files/media_asset/unaids-data-2018_enpdf. 2018. [Accessed 2022 February 20].

5. NAIIS. Northwest Zone Summary sheet. Nigeria HIV/AIDS Indicator and Impact Survey. 2019:1-4.

6. Contri PV, Berchielli EM, Tremeschin MH, Negrini BV, Saloma˜o RG, Monteiro JP. Nutritional status and lipid profile of HIV-positive children and adolescents using antiretroviral therapy. Clinics. 2011;66(6):997-1002. DOI:10.590/S807-59322011000600013.

7. WHO. Treat all people living with HIV, offer antiretrovirals as additional prevention choice for people at "substantial" risk. Available: https://wwwwhoint/mediacentre/news/releases/2015/hiv-treat-all-recommendation/en/. 2015. [Accessed 2022 February 20].

8. UNAIDS. Press release. New survey results indicate that Nigeria has an HIV prevalence of 1.4%. Available: https://wwwunaidsorg/en/resources/presscentre/pressreleaseandstatementarchive/2019/march/20190314_nigeria. 2019. [Accessed 2022 February 20].

9. Health Reporters. Only 35% Kano HIV positive patients are on medication. Available: http://healthreportersinfo/only-35-kano-hiv-positive-patients-are-on-medication/. 2019. [Accessed 2022 February 20].

10. Magadi MA. Household and community HIV/AIDS status and child malnutrition in sub-Saharan Africa: Evidence from the demographic and health surveys. Soc Sci Med. 2011;73:436-46. DOI:10.1016/j.socscimed.2011.05.042.

11. Swaminathan S, Padmapriyadarsini C, Yoojin L, Sukumar B, Iliayas S, Karthipriya J, et al. Nutritional Supplementation in HIV-Infected Individuals in South India: A Prospective Interventional Study. Clin Infect Dis. 2010;51(1):51-7. DOI: 10.1086/653111.

12. Anabwani G, Navario P. Nutrition and HIV/AIDS in sub-Saharan Africa: an overview. Nutrition. 2005;21:96-9. DOI:10.1016/j.nut.2004.09.013.

13. Kimani-Murage EW, Norris SA, Pettifor JM, Tollman SM, Klipstein-Grobusch K, Gómez-Olivé XF, et al. Nutritional status and HIV in rural South African children. BMC Pediatr. 2011;11(23):1-13. http://www.biomedcentral.com/1471-2431/11/23.

14. McHenry MS, Dixit A, Vreeman RC. A Systematic Review of Nutritional Supplementation in HIV-Infected Children in Resource-Limited Settings. JIAPAC. 2014:1-11. DOI: 0.1177/2325957414539044.

15. FAO. Regional Overview of Food Security and Nutrition in Africa 2017. The food security and nutrition–conflict nexus: building resilience for food security, nutrition and peace. Accra. 2017:1-108.

16. SANHANES-1. South African National Health and Nutrition Examination Survey (SANHANES-1). Available: http://wwwhsrcacza/uploads/pageNews/72/SANHANES-launch%20edition%20(online%20version)pdf. 2013. [Accessed 2022 February 20].

17. FAO. Over 9 million people in Nigeria face food insecurity. Available: https://wwwaacomtr/en/africa/over-9-million-people-in-nigeria-face-food-insecurity/2236509 2021. [Accessed 2022 February 20].

18. UNICEF. Nutrition. Available: https://wwwuniceforg/nigeria/nutrition. 2018. [Accessed 2022 February 20].

19. USAID. Nigeria: Nutrition Profile. Available: https://wwwusaidgov/sites/default/files/documents/1864/Nigeria-Nutrition-Profile-Mar2018-508pdf. 2018. [Accessed 2022 February 20].

20. UNICEF. National Nutrition and Health Survey (NNHS) 2018. Report on the Nutrition and Health Situation of Nigeria. Available: https://wwwuniceforg/nigeria/media/2181/file/Nigeria-NNHS-2018pdf. 2018:1-153. [Accessed 2022 February 20].

21. Banwat ME, Yakubu NW, Olalude EO, Ogunsakin JA. An assessment of the nutritional knowledge, practice and status of adult HIV/AIDS patients attending an art centre in Jos, north central Nigeria. Afr J Food Sci Technol. 2014;5(7):156-62. DOI: http:/dx.doi.org/10.14303/ajfst.2014.054.

22. MartõÂn-Cañavate R, Sonego M, Sagrado MJ, Escobar G, Rivas E, Ayala S, et al. Dietary patterns and nutritional status of HIV-infected children and adolescents in El Salvador: A cross-sectional study. PLOS ONE 2018;13(5):1-14 DOI:org/0.1371/journal.pone.0196380

23. Michels KB, Wolk A. A prospective study on variety of healthy foods and mortality in women. Int. 2002;31:847-54.

24. Masa R, Chowa G, Nyirenda V. Socioeconomic correlates of dietary diversity and its association with adherence and psychosocial functioning of people living with HIV in rural Zambia. Nutr Health. 2018;24(2):93–102. DOI: 10.1177/0260106018761282.

25. NBS. 2019 Poverty and Inequality in Nigeria: Executive Summary. Available: http://nigerianstatgovng/download/1092 2020. [Accessed 2022 February 20].

26. James A, Zikankuba V. Moringa oleifera a potential tree for nutrition security in sub-Sahara Africa. Am J Res Commun. 2017;5(4):1-14.

27. Monera TG, Maponga CC. Prevalence and patterns of Moringa oleifera use among HIV positive patients in Zimbabwe: a cross-sectional survey. JPHiA. 2012;3:e6:22-4. DOI:10.4081/jphia.2012.e6.

28. RMRDC. Moringa oleifera- A National Crop forEconomic Growth and Development. Proceedings of National Summit on Moringa Development. Available: https://wwwacademiaedu/6686903/RAW_MATERIALS_RESEARCH_AND_DEVELOPMENT_COUNCIL_Moringa_oleifera_A_National_Crop_for_Economic_Growth_and_Development_PROCEEDINGS_OF_THE_FIRST_NATIONAL_SUMMIT_ON_MORINGA_DEVELOPMENT._2010. [Accessed 2022 February 20].

29. Said HA, Tsega G, Tesfaye TD. Dietary diversity and associated factors among HIV-positive adults attending the anti-retroviral therapy clinic at Felege Hiwot Comprehensive Specialized Hospital, Northwest Ethiopia: a cross-sectional study. J Nutr Sci. 2021;10(e18):1 -10. DOI:.1017/jns.2021.5.

30. Sirotin N, Hoover D, Segal-Isaacson CJ, Shi Q, Adedimeji A, Mutimura E, et al. Structural determinants of food insufficiency, low dietary diversity and BMI: a cross-sectional study of HIVinfected and HIV-negative Rwandan women. BMJ Open 2012;2:e000714 DOI:10.1136/bmjopen-2011-000714.

31. Ajani SR. An Assessment of Dietary Diversity in Six Nigerian States. Afr J Biomed Res. 2010;13:161-7.

32. Gambo A, Gqaleni N, Babalola TK. 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. Heliyon 2022; 8; e09524:https://doi.org/10.1016/j.heliyon.2022.e09524.

33. Gambo A, Moodley I, Babashani M, Babalola TK, Gqaleni N. A double blind randomised control trial to examine the effect of Moringa oleifera leaf powder supplementation on the immune status and anthropometric parameters of adult HIV patients on antiretroviral therapy in a resource-limited setting. PLoS ONE. 2021;16(12):e0261935.:https://doi.org/10.1371/journal.pone.0261935.

34. Peltzer K, Preez NF, Ramlagan S, Anderson J. Antiretroviral treatment adherence among HIV patients in KwaZulu-Natal, South Africa. BMC Public Health. 2010;10(111):1-10. http://www.biomedcentral.com/1471-2458/10/111.

35. Olisah VO, Baiyewu O, Sheikh TL. Depression underdiagnosis and the effects on quality of life in outpatients with HIV at a Nigerian university teaching hospital. AJAR. 2011;10(3):247-54. DOI: 10.2989/16085906.2011.626294.

36. WHO. WHOQOL-HIV Instrument. Users Manual. Scoring and Coding for the WHOQOL-HIV Instrument. wwwwhoint/mental_health/media/en/613pdf. 2002. [Accessed 2022 February 20].

37. da Silva J, Bunn K, Bertoni RF, Neves OA, Traebert J. Quality of life of people living with HIV. AIDS Care: Psychological and Socio-medical Aspects of AIDS/HIV. 2013;25(1):71-6. DOI:10.1080/09540121.2012.686594.

38. Gambo A, Moodley I, Babashani M, Babalola TK. Impact of Moringa Oleifera leaves supplementation on quality of life of people living with HIV: A double-blind randomized controlled trial. Qual Life Res. 2021:2563–71. https://doi.org/10.1007/s11136-021-02842-0.

39. Khatri S, Amatya A, Shrestha B. Nutritional status and the associated factors among people living with HIV: an evidence from cross-sectional survey in hospital based antiretroviral therapy site in Kathmandu, Nepal. BMC Nutr. 2020;6(22): 1-13. https://doi.org/0.1186/s40795-020-00346-7.

40. Hu W, Jiang H, Wei Chen W, He S-H, Deng B, Wang W-Y, et al. Malnutrition in hospitalized people living with HIV/AIDS: evidence from a cross-sectional study from Chengdu, China. Asia Pac J Clin Nutr 2011;20(4):544-50.

41. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 2013;382:427-51. http://dx.doi.org/10.1016/S0140-6736(13)60937-X.

42. Nunnari G, Coco C, Pinzone MR, Pavone P, Berretta M, Di Rosa M, et al. The role of micronutrients in the diet of HIV-1-infected individuals. Front (Elite edition). 2012;4(7):2442-56.

43. Ndeezi G, Tylleskär T, Ndugwa CM, Tumwine JK. Effect of multiple micronutrient supplementation on survival of HIV-infected children in Uganda: a randomized, controlled trial. JIAS. 2010;13(18):1-9. http://www.jiasociety.org/content/13/1/8.

44. Bunupuradah T, Ubolyam S, Hansudewechakul R, Kosalaraksa P, Ngampiyaskul C, Kanjanavanit S, et al. Correlation of Selenium and Zinc Levels to Antiretroviral Treatment Outcomes in Thai HIV-infected Children without Severe HIV Symptoms. Eur J Clin Nutr 2012;66(8):900-5. DOI:10.1038/ejcn.2012.57.

45. Chege PM, Muthamia OG. Level of micronutrient supplements uptake among people living with HIV/AIDS in Kayole, Nairobi County,Kenya. J AIDS HIV Res. 2017;9(4):74-80. DOI: 10.5897/JAHR2017.0414.

46. WHO. Essential Prevention and care interventions for adults and adolescents living with HIV in resource-limited settings WHO. 2008.

47. Evans D, McNamara L, Maskew M, Selibas K, Amsterdam D, Baines N, et al. Impact of nutritional supplementation on immune response, body mass index and bioelectrical impedance in HIV-positive patients starting antiretroviral therapy. Nutr J. 2013;12(111):1-14. DOI: http://www.nutritionj.com/content/2/1/111.

48. Olsen MF, Abdissa A, Kæstel P, Tesfaye M, Yilma D, Girma T, et al. Effects of nutritional supplementation for HIV patients starting antiretroviral treatment: randomised controlled trial in Ethiopia. BMJ 2014;348(g3187):1-13. DOI: 0.1136/bmj.g3187.

49. Amlogu AM, Tewfik S, Wambebe C, Tewfik I. A comparative study: long and short term effect of a nutrition sensitive approach to delay the progression of HIV to AIDS among people living with HIV (PLWH) in Nigeria. Funct food health dis 2016;6(2):79-90. DOI:10.31989/ffhd.v6i2.235.

50. Sunguya BF, Poudel KC, Mlunde LB, Otsuka K, Yasuoka J, Urassa DP, et al. Ready to Use Therapeutic Foods (RUTF) improves undernutrition among ART-treated, HIV-positive children in Dar es Salaam, Tanzania. Nutr 2012;11(60):1-8. http://www.nutritionj.com/content/11/1/60.

51. UNICEF. Ready-to-Use Therapeutic Food: Current Outlook. UNICEF Supply Division. 2013.

52. Dibari F, Bahwere P, Huerga H, Irena AH, Owino V, Collins S, et al. Development of a cross-over randomized trial method to determine the acceptability and safety of novel ready-to-use therapeutic foods. Nutrition. 2013;29:107-12. DOI:10.1016/j.nut.2012.04.016.

53. Sztam KA, Ndirangu M, Sheriff M, Arpadi SM, Hawken M, Rashid J, et al. Rationale and design of a study using a standardized locally procured macronutrient supplement as adjunctive therapy to HIV treatment in Kenya. AIDS Care 2013;25(9):1138-44. DOI: 10.080/09540121.2012.752564.

54. Thurber MD, Fahey JW. Adoption of Moringa oleifera to combat under-nutrition viewed through the lens of the “Diffusion of Innovations” theory. Ecol Food Nutr. 2009;48(3):212-25. DOI: 10.1080/03670240902794598.

55. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: A Food Plant with Multiple Medicinal Uses. Phytother Res. 2007;21:17-25. DOI: 10.1002/ptr.2023.

56. Pandey A, Pandey RD, Tripathi P, Gupta PP, Haider J, Bhatt S, et al. Moringa Oleifera Lam. (Sahijan) - A Plant with a Plethora of Diverse Therapeutic Benefits: An Updated Retrospection. Med Aromat Plants 2012;1(1):1-8. DOI: 10.4172/2167-0412.1000101.

57. Kumssa DB, Joy EJM, Young SD, Odee DW, Ander EL, Broadley MR. Variation in the mineral element concentration of Moringa oleifera Lam. and M. stenopetala (Bak. f.) Cuf.: Role in human nutrition. PLoS ONE. 2017;12(4):1-26. https://doi.org/10.1371/journal.pone.0175503.

58. Fahey JW. Moringa oleifera: A Review of the Medical Evidence for Its Nutritional,Therapeutic, and Prophylactic Properties. Trees for Life Journal. 2005;1(5):1-24. http://www.tfljournal.org/article.php/20051201124931586.

59. Williams FE, Animashaun JO, Ibrahim H, Toye AA. A preliminary survey on consumption of moringa products for nutraceutical benefits in ilorin, kwara state, nigeria. Agrosearch. 2013;13(1):165-75. DOI: 10.4314/agrosh.v13i1.15.

60. Asiedu-Gyekye IJ, Frimpong-Manso S, Awortwe C, Antwi DA, Nyarko AK. Micro- and Macroelemental Composition and Safety Evaluation of the Nutraceutical Moringa oleifera Leaves. J Toxicol. 2014;2014:1-13. http://dx.doi.org/0.1155/2014/786979.

61. Saini RK, Sivanesan I, Keum Y. Phytochemicals of Moringa oleifera: a review of their nutritional, therapeutic and industrial significance. 3 Biotech. 2016;6(203):1-14. DOI:0.1007/s13205-016-0526-3.

62. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. Antioxidants 2017;6(91):1-13. DOI:0.3390/antiox6040091.

63. Dhakad AK, Ikram M, Sharma S, Khan S, Pandey VV, Singh A. Biological, nutritional, and therapeutic significance of Moringa oleifera Lam. Phytother Res. 2019;33:2870-903 DOI: 10.1002/ptr.6475.

64. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: A review on nutritive importance and its medicinal application. Food Sci Hum Well. 2016;5:49-56. http://dx.doi.org/10.1016/j.fshw.2016.04.001.

65. Falowo AB, Mukumbo FE, Idamokoro EM, Lorenzo JM, Afolayan AJ, Muchenje V. Multi-functional application of Moringa oleifera Lam. in nutrition and animal food products: A review. Food Res Int. 2018;106:317-34. https://doi.org/10.1016/j.foodres.2017.12.079.

66. Kou X, Li B, Olayanju JB, Drake JM, Chen N. Nutraceutical or Pharmacological Potential of Moringa oleifera Lam. Nutrients 2018;10(343):1-12. DOI:0.3390/nu10030343.

67. Alegbeleye OO. How Functional Is Moringa oleifera? A Review of Its Nutritive, Medicinal, and Socioeconomic Potential. Food Nutr Bull 2018;39(1):149-70. DOI: 10.1177/0379572117749814.

68. Koul B, Chase N. Moringa oleifera Lam.: Panacea to several maladies. J Chem Pharm Res. 2015; 7(6):687-707.

69. Tshingani K, Donnen P, Mukumbi H, Duez P, Dramaix-Wilmet M. Impact of Moringa oleifera lam. Leaf powder supplementation versus nutritional counseling on the body mass index and immune response of HIV patients on antiretroviral therapy: a single-blind randomized control trial. BMC Complement Altern Med. 2017;17(420):1-13. DOI: 0.1186/s12906-017-1920-z.

70. Monera TG, Wolfe AR, Maponga CC, Benet LZ, Guglielmo J. Moringa oleifera leaf extracts inhibit 6β-hydroxylation of testosterone by CYP3A4. J Infect Dev Ctries. 2008;2(5):379-83. DOI:10.3855/jidc.201.

71. Nations Encyclopedia. Nigeria - Location, size, and extent. Available: https://wwwnationsencyclopediacom/Africa/Nigeria-LOCATION-SIZE-AND-EXTENThtml. 2020. [Accessed 2022 February 20].

72. World Population Review. Population of Cities in Nigeria. Available: https://worldpopulationreviewcom/countries/cities/nigeria. 2020. [Accessed 2022 February 20].

73. Encyclopaedia Britannica. Kano city. Available: https://wwwbritannicacom/place/Kano-state-Nigeria. 2010. [Accessed 2022 February 20].

74. Sanusi MM, Denis DM. Geographies of poverty in Kano State: The role of GIS in identifying and mapping multidimensionally deprived households. JGG Photon 2015;119:200-13.

75. NBS. Demographic Statistics Bulletin. Available: https://nigerianstatgovng/download/775. 2017:1-26. [Accessed 2022 February 20].

76. Galadanci HS, Idris SA, Sadauki HM, Yakasai IA. Programs and Policies for Reducing Maternal Mortality in Kano State, Nigeria: A Review. Afr J Reprod Health 2010;14(2):31-6.

77. Daily Trust. Nigeria: Kano Partners Niger Republic On Cross Border Trade. Available: https://allafricacom/stories/201209120921html. 2012. [Accessed 2022 February 20]

CHAPTER TWO

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

Chapter 1 of this thesis introduced the research conducted by presenting the rationale, overall review of literature on HIV and AIDS, and nutritional supplements in HIV. It also presented the detailed methodology used in evaluating the addition of *Moringa oleifera* Lam. leaves as a nutritional supplement on the anthropometric and immunological status of adult HIV patients on ART in AKTH, Kano State Nigeria.

Chapter 2 assessed the dietary diversity pattern of the participants over the study period. The study evaluated the different dietary behaviours of the study participants and further examined how *Moringa oleifera* Lam. leaves – supplemented diet affects their nutritional status and CD4 counts. This study ascertained the need for nutritional supplementation to complement the regular diets of the study participants.

The chapter is presented as a published article.

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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 ($p = 0.024$; 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 HIV-positive 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

☆ The clinical trial described in this paper was registered at Pan African Clinical Trial Registry under the registration number PACTR201811722056449.

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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® on the immune response, body mass index (BMI), and bioelectrical impedance in HIV infected individuals commencing ART in South Africa. FutureLife porridge®, 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^3; 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-β [type 2 error probability]), and 95% confidence (or 5% α 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

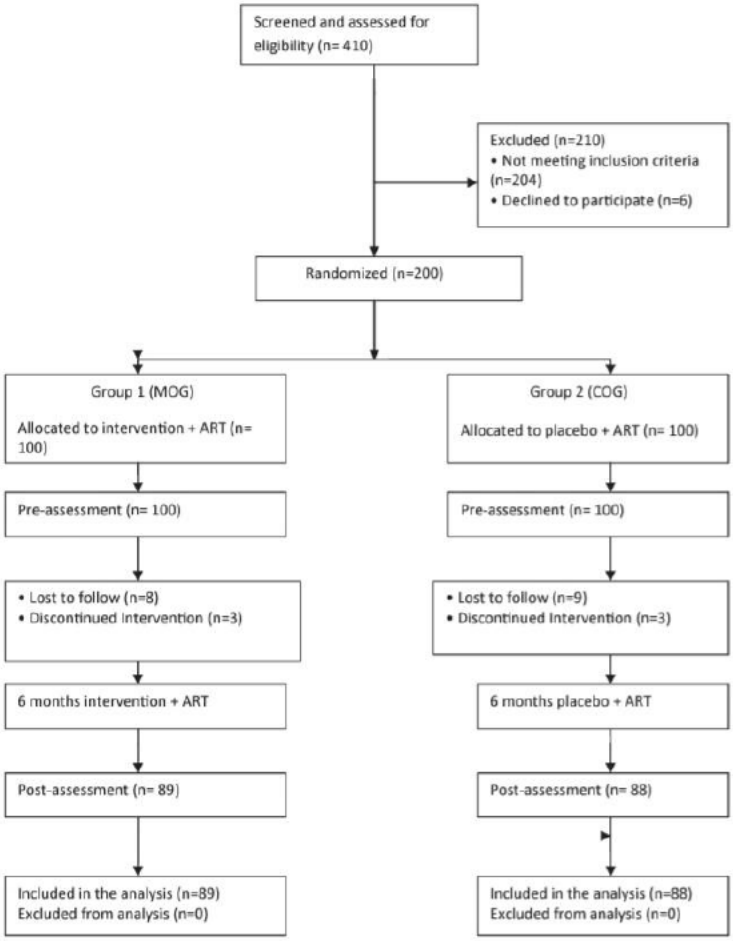

Figure 1. Flow Chart of participants.

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 face-to-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 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].

Table 1. Nutrient composition of *Moringa oleifera* Lam. leaf powder.

Nutrient	100 g	15 g
Energy (kcal)	981	147.15
Protein (g)	28.2	4.23
Fat (g)	3.9	0.59
Carbohydrate (g)	22	3.3
Soluble minerals		
Calcium (mg)	1791.82	268.77
Potassium (mg)	4879.26	731.89
Sodium (mg)	24	3.6
Trace elements		
Zinc (mg)	2.88	0.43
Iron (mg)	37.78	5.67

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 ± SD age of 41.57 ± 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 (±14.8); COG = 61.9 (±12.5). The mean BMIs for MOG and COG was 24.84 (±4.8) and 23.75 (±3.82), respectively. The MUAC means for MOG and COG were 26.3 (±2.1) and 25.9 (±1.8), respectively. At baseline, the mean CD4 cell count for MOG and COG were 341.8 (±106.1) and 352.34 (±126.0), respectively (Table 4).

Table 2. Demographic description of study participants.

Variables	MOG (%) (N = 89)	COG (%) (N = 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-square test; absolute frequency and percentage in parentheses.

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. (%)	P value	Odds ratio (OR)	OR 95%CI		MOG n. (%)	COG n. (%)	P value	Odds ratio (OR)	OR 95%CI	
					Lower	Upper					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												
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 sample 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 ≥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 (±4.09) at 6th month (Table 5).

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 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

Table 5. Comparison of participants' nutritional status (using categorized BMI) at baseline and 6th month between MOG and COG.

BMI Category	Baseline					6th month				
	MOG n. (%)	Mean [±SD] (Kg/m^2)	COG n. (%)	Mean [±SD] (Kg/m^2)	P value	MOG n. (%)	Mean [±SD] (Kg/m^2)	COG n. (%)	Mean [±SD] (Kg/m^2)	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 deviation.

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

Estimates of Fixed Effects[a]

	Parameters	Estimate	Std. Error	t	P value	Estimate 95% CI	
						Lower Bound	Upper Bound
	Intercept	61.92	1.47	42.02	0.0001	61.43	63.65
Weight	[Group = 1 (MOG)]	-0.05	0.08	-0.59	0.5556	-0.20	-0.11
	[Group = 2 (COG)]	0[b]	0	.	.	.	.
	Intercept	23.73	0.47	50.98	0.0001	23.61	24.32
BMI	[Group = 1 (MOG)]	-0.02	0.03	-0.65	0.5145	-0.08	-0.04
	[Group = 2 (COG)]	0[b]	0	.	.	.	.
	Intercept	25.14	.68	69.56	0.0001	22.94	23.52
MUAC	[Group = 1(MOG)]	-0.04	0.06	-0.73	0.6456	-0.09	-0.02
	[Group = 2 (COG)]	0[b]	0	.	.	.	.
	Intercept	356.35	13.10	27.20	0.0001	354.29	376.09
CD4	[Group = 1(MOG)]	10.33	2.65	3.89	0.0001*	5.12	15.54
	[Group = 2 (COG)]	0[b]	0				

* = statistically significant;[a] = Dependent variable (weight, BMI, MUAC, CD4);[b] = 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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References

[1] F. Weldegebreal, T. Digaffe, F. Mesfin, H. Mitiku, Dietary diversity and associated factors among HIV positive adults attending antiretroviral therapy clinics at Hiwot Fana and Dilchora Hospitals, eastern Ethiopia, HIV AIDS (Auckl) 10 (2018) 63–72.

[2] T.R. Ziegler, G.A. McComsey, J.K. Fredlani, E.C. Millson, V. Tangpricha, A.R. Eckard, Habitual nutrient intake in HIV-infected youth and associations with HIV-related factors, AIDS Res. Hum. Retrovir. 30 (9) (2014) 888–889.

[3] R. MartÍÁn-Cañavate, M. Sonego, M.J. Sagrado, G. Escobar, E. Rivas, S. Ayala, et al., Dietary patterns and nutritional status of HIV-infected children and adolescents in El Salvador: a cross-sectional study, PLoS One 13 (5) (2018) 1–14.

[4] G. Nunnari, C. Coco, M.R. Finzone, P. Pavone, M. Berretta, M. Di Rosa, et al., The role of micronutrients in the diet of HIV-1-infected individuals, Front. Times (Elite Ed.) 4 (7) (2012) 2442–2456.

[5] R. Masa, G. Chowa, V. Nyirenda, Socioeconomic correlates of dietary diversity and its association with adherence and psychosocial functioning of people living with HIV in rural Zambia, Nutr. Health 24 (2) (2018) 93–102.

[6] FAO, Guidelines for Measuring Household and Individual Dietary Diversity. Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, 2011. Available at: http://www.fao.org/fileadmin/user_upload/wa_workshop/docs/FAO-guidelines-dietary-. (Accessed 20 February 2022).

[7] G.L. Kennedy, M.R. Pedro, C. Seghieri, G. Nantel, I. Brouwer, Dietary diversity score is a useful indicator of micronutrient intake in non-breast-feeding Filipino Children1,2, J. Nutr. 137 (2007) 472–477.

[8] A. Tesfaw, D. Jara, H. Temesgen, Dietary diversity and associated factors among HIV positive adult patients attending public health facilities in Motta Town, East Gojjam Zone, Northwest Ethiopia, 2017, Adv Publ. Health 2018 (2018) 1–8.

[9] M. Arimond, M.T. Ruel, Dietary diversity is associated with child nutritional status: evidence from 11 demographic and health Surveys[1,2], J. Nutr. 134 (2004) 2579–2585.

[10] NBS, Poverty and Inequality in Nigeria: Executive Summary, 2019. Available at: http://nigerianstatgovng/download/1092. 2020. (Accessed 20 February 2022).

[11] FRAC, The Impact of Poverty, Food Insecurity, and Poor Nutrition on Health and Well-Being, Food Research & Action Center, 2017, pp. 1–14. Available at: www.frac.org. (Accessed 20 February 2022).

[12] A.M. Amlogu, S. Tewfik, C. Wambebe, I. Tewfik, A comparative study: long and short term effect of a nutrition sensitive approach to delay the progression of HIV to AIDS among people living with HIV (PLWH) in Nigeria, Func. Food Health Dis. 6 (2) (2016) 79–90.

[13] D. Evans, L. McNamara, M. Maskew, K. Selibas, D. Amsterdam, N. Baines, et al., Impact of nutritional supplementation on immune response, body mass index and bioelectrical impedance in HIV-positive patients starting antiretroviral therapy, Nutr. J. 12 (111) (2013) 1–14. http://www.nutritionj.com/content/2/1/111.

[14] M.F. Olsen, A. Abdissa, P. Kæstel, M. Tesfaye, D. Yilma, T. Girma, et al., Effects of nutritional supplementation for HIV patients starting antiretroviral treatment: randomised controlled trial in Ethiopia, BMJ 348 (g3187) (2014) 1–13.

[15] T.G. Monera, C.C. Maponga, Prevalence and patterns of Moringa oleifera use among HIV positive patients in Zimbabwe: a cross-sectional survey, JPHIA 3 (e6) (2012) 22–24.

[16] J.O. Animashaun, F.E. Williams, A.A. Toye, Towards validating moringa's nutraceutical benefits: an examination of consumers' perspectives vis-à-vis health benefits efficacy and willingness to pay, Agris on-line Paper. Econ. Inform. V (2) (2013) 11–21.

[17] A.K. Dhakad, M. Ikram, S. Sharma, S. Khan, V.V. Pandey, A. Singh, Biological, nutritional, and therapeutic significance of Moringa oleifera Lam. Phytother Res. 33 (2019) 2870–2903.

[18] L.A. Farinola, O.O. Famuyide, F. Awe, A.F. Adio, A.S. Ewolor, Households' perception, awareness and willingness to pay for Moringa oleifera Lam powder in Oyo State, J. Agric. Crop Res. 2 (6) (2014) 94–103.

[19] F.E. Williams, J.O. Animashaun, H. Ibrahim, A.A. Toye, A preliminary survey on consumption of moringa products for nutraceutical benefits in Ilorin, kwara state, Nigeria, Agrosearch 13 (1) (2013) 165–175.

[20] M.D. Thurber, J.W. Fahey, Adoption of Moringa oleifera to combat under-nutrition viewed through the lens of the "Diffusion of Innovations" theory, Ecol. Food Nutr. 48 (3) (2009) 212–225.
[21] A. Pandey, R.D. Pandey, P. Tripathi, P.P. Gupta, J. Haider, S. Bhatt, et al., Moringa oleifera Lam. (Sahijan) - a plant with a plethora of diverse therapeutic benefits: an updated retrospection, Med. Aromat. Plants 1 (1) (2012) 1–8.
[22] V.R. Rakotosaimimanana, D. Valentin, G. Arvisenet, How to use local resources to fight malnutrition in Madagascar? A study combining a survey and a consumer test, Appetite 95 (2015) 533–543.
[23] A. Gambo, I. Moodley, M. Babashani, T.K. Babalola, Impact of Moringa Oleifera leaves supplementation on quality of life of people living with HIV: a double-blind randomized controlled trial, Qual. Life Res. (2021) 2563–2571.
[24] WMA. World Medical Association, Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects, Clin. Rev. Educ. - Spec. Commun. 310 (20) (2013) 2191–2194. Available at: https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2013-JAMApdf. (Accessed 20 February 2022).
[25] F. Faul, E. Erdfelder, A. Buchner, A.-G. Lang, Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses, Beyond Behav. 41 (2009) 1149–1160.
[26] J. Cohen, Statistical Power Analysis for the Behavioral Sciences, second ed., Lawrence Earlbaum Associates, Hillsdale, N J, 1988.
[27] S.P. Mishra, P. Singh, S. Singh, Processing of moringa oleifera leaves for human consumption, Bull Env. Pharmacol. Life Sci. 2 (1) (2012) 28–31.
[28] Merck, Particle size conversion table, Available at: https://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/particle-size-conversion.html, 2021. (Accessed 20 February 2022).
[29] Z. Bahadoran, M. Tohidi, P. Nazeri, M. Mehran, F. Azizi, P. Mirmiran, Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial, Int. J. Food Sci. Nutr. 63 (7) (2012) 767–771.
[30] N. Idohou-Dossou, A. Diouf, A.L. Gueye, A.T. Guiro, S. Wade, Impact of daily consumption of Moringa (Moringa Oleifera) dry leaf powder on iron status of senegalese lactating women, AJFAND 11 (4) (2011) 4985–4999.
[31] A. Téré-Bénissan, M.I.A. Quashie, K. Lawson-Evi, K. Gnandi, K. Kokou, M. Gbéassor, Influence of Moringa oleifera leaves on atherogenic lipids and glycaemia evolution in HIV-infected and uninfected malnourished patients, J. Appl. Biosci. 62 (2013) 4610–4619.
[32] ASPIRATA, Nutritional Analysis of Moringa Oleifera Leaves Powder, ASPIRATA Food and Beverage Laboratory, 2017. Available at: http://aspiratacoza/. (Accessed 20 February 2022).
[33] SANAS, South African national accreditation System (SANAS), Available at: https://www.sanascoza/. 2017. (Accessed 20 February 2022).
[34] FAO, Dietary Diversity Questionnaire. FAO/Nutrition and Consumer Protection Division, 2007 version of May.
[35] S.R. Ajani, An assessment of dietary diversity in six Nigerian states, Afr. J. Biomed. Res. 13 (2010) 161–167.
[36] FANTA, Nutrition assessment, counseling, and support (NACS) A user's guide module 2, version 2, Available at: https://www.fantaproject.org/sites/default/files/resources/NACS-Users-Guide-Module2-May2016pdf. 2016. (Accessed 20 February 2022).
[37] SOP Nigeria. ENUMERATION OF CD4+ T LYMPHOCYTE, Standard operating procedure for Nigeria PrEP study, Available at: https://www.prepwatch.org/wp-content/uploads/2016/09/NNACA_SOP_304docx 2015;LP-304. (Accessed 20 February 2022).
[38] O.K. Achi, Traditional fermented protein condiments in Nigeria, Afr. J. Biotechnol. 4 (13) (2005) 1612–1621.
[39] M. Maillot, N. Darmon, M. Darmon, L. Lafay, A. Drewnowski, Nutrient-dense food groups have high energy costs: an econometric approach to nutrient Profiling1,2, J. Nutr. 137 (2007) 1815–1820.
[40] J.D. Finger, T. Tylleskä, T. Lampert, G.B.M. Mensink, Dietary behaviour and socioeconomic position: the role of physical activity patterns, PLoS One 8 (11) (2013) 1–10, e78390.
[41] A.A. Abdulrahaman, O.M. Kolawole, Traditional preparations and uses of maize in Nigeria, Ethnobot. Leaflet. 10 (2006) 219–227.
[42] E.S. Otong, S.A. Musa, Antioxidant potentials of miyan kuka (baobab leaves), Ann. Afr. Med. 2 (62) (2019) 11–15.
[43] M.O. Agada, E.M. Igbokwe, Dietary diversity of rural households in North Central Nigeria, EJNFS 5 (3) (2015) 150–155. Article no.EJNFS.2015.014.
[44] Trading Economics, Nigeria national minimum wage 2018-2020 data | 2021-2023 forecast | historical, Available at: https://tradingeconomics.com/nigeria/minimum-wages 2020. (Accessed 20 February 2022).
[45] A.F.A. Razis, M.D. Ibrahim, S.B. Kntayya, Health benefits of moringa oleifera, Asian Pac. J. Cancer Prev. APJCP 15 (20) (2014) 8571–8576.
[46] E.N. Ogbuagu, S. Ufearo, C.N. Ogbuagu, R. Okonkwo, CD4 pattern in HIV positive patients on HAART exposed to moringa oleifera leaf powder in south east Nigeria, in: Poster session 17th International Congress on Infectious Diseases/Int. J. Infect. Dis. 455, 2016, pp. 1–477.
[47] A. Gambo, I. Moodley, M. Babashani, T.K. Babalola, N. Gqaleni, A double blind randomised control trial to examine the effect of Moringa oleifera leaf powder supplementation on the immune status and anthropometric parameters of adult HIV patients on antiretroviral therapy in a resource-limited setting, PLoS One 16 (12) (2021), e0261935.
[48] S. Sidibé, A. Delamou, M.L. Kaba, A.S. Magassouba, A.T. Samake, Y.S.A. Dongo, et al., The effects of nutritional supplementation on body mass index and CD4 count among adult people living with HIV AIDS on antiretroviral treatment in Conakry, Guinea, J. Publ. Health Afr. 9 (2018) 708.

CHAPTER THREE

DOES *MORINGA OLEIFERA* LAM. LEAVES SUPPLEMENTATION HAS AN IMPACT ON THE WEIGHT AND BMI OF PEOPLE LIVING WITH HIV THAT ARE ON ANTIRETROVIRAL THERAPY? A DOUBLE-BLIND RANDOMIZED CONTROL TRIAL

Chapter 2 of this thesis assessed the dietary diversity pattern of the participants and how the *Moringa oleifera* Lam. leaves – supplemented diet affects the various nutritional statuses observed and CD4 counts of the study participants.

Chapter 3 is a study conducted to determine the impact of six months *Moringa oleifera* Lam. leaves supplementation on the weight and BMI of adult HIV patients receiving ART at the study centre. The manuscript addressed the various limitations of the study design that could result in the participants' findings on anthropometric parameters (nutritional status).

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ORIGINAL ARTICLE

Does *Moringa oleifera* Lam. leaves supplementation have an impact on the weight and bone mass index of people living with HIV that are on antiretroviral therapy? A double-blind randomized control trial

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Abstract

Background. HIV-related weight loss and wasting were the most common malnutrition and AIDS-defining conditions before HAART. HAART has led to more obese PLHIV. HIV-positive patients should eat micro- and macronutrient-rich foods to maintain optimal nutrition. This study examined whether *Moringa oleifera* Lam. leaf supplementation affects PLHIV receiving ART.

Methods. A randomized, double-blind, controlled trial was conducted. Two hundred patients with informed consent were randomly assigned to either the *Moringa oleifera* Lam. (MOG) group or the control group (COG). From baseline to six months of *Moringa oleifera* Lam. leaf supplementation, anthropometric parameters [weight; BMI] of the participants were assessed.

Results. One hundred seventy-seven patients completed the 6-month follow-up (89 MOG versus 88 COG). During the study period, the MOG and COG had similar weights and BMIs ($p>0.05$). At baseline and six months, most participants in both study groups had a healthy BMI (18.5). Many participants were overweight; few were underweight (BMI18.5). MOG and COG BMI differences at baseline and six months were not significant ($p> 0.05$). All experiments were 95CI.

Conclusions . *Moringa oleifera* Lam. leaf powder had no effect on HIV-positive adults receiving antiretroviral therapy, in accordance with this study.

Keywords: Moringa oleifera Lam., HIV, BMI, antiretroviral therapy.

INTRODUCTION

According to the 2019 UNAIDS report, there were 1,9 million HIV-positive individuals in Nigeria, with a prevalence of 1.5% among adults aged 15-49 [1]. Malnutrition and HIV/AIDS are inextricably linked [2]. During the pre-highly active antiretroviral therapy (pre-HAART) era, HIV-related weight loss and wasting were the most prevalent forms of malnutrition and AIDS-defining conditions [3]. This was marked by a significant decrease in body fat, lean mass, and bone mass [4]

The introduction of HAART has proven effective in reducing morbidity and mortality associated with HIV infection [5, 6] and in ameliorating the syndrome of wasting associated with HIV [5]. On the other hand, it has been documented that the prevalence of overweight and obesity increases among HIV-positive individuals receiving HAART [7], which may increase the risk of cardiovascular and metabolic diseases [8].

Diet is of the utmost importance for maintaining optimal nutrition and preventing these complications [4]. Patients infected with HIV are urged to consume diets rich in micro- and macronutrients necessary for normal body function [4]. Unfortunately, the diet and food consumption of PLHIV in low-income settings, such as Nigeria, need improvement [9].

The powdered leaves of Moringa oleifera Lam., which has been shown to have a high nutritional value [10-13], could be a valuable addition to the diets of PLHIV. Moringa oleifera Lam. is a member of the Moringaceae family [14]. The leaves are a rich source of both macro- and micronutrients [16] and act as a good source of natural antioxidants [17] due to their high concentration of minerals, vitamins, and other vital phytochemicals.

We have already conducted a study demonstrating the effect of Moringa oleifera Lam. leaves on the immune status [18] and the quality of life of PLHIV on ART [19], which have been reported elsewhere. Consequently, the purpose of this manuscript is to determine if supplementation with Moringa oleifera Lam. leaves affects the weight and BMI of PLHIV on ART compared to placebo at the S.S Wali Virology Centre, Aminu Kano Teaching Hospital, Kano State, Nigeria.

MATERIALS AND METHODS

Study location

The study location was the Sadiq Suleiman Wali, known as S.S Wali Virology Centre at Aminu Kano Teaching Hospital, Kano State (AKTH), Nigeria.

AKTH is a tertiary health institution and referral center that operates a daily HIV clinic (5 days a week). It also serves as a center for clinical evaluation, laboratory tests, HIV counseling and testing (HCT), treatment, and care supported by the Federal Government and the Institute of Human Virology, Nigeria (IHVN) in partnership with its global partners, including the Centers for Disease Control and Prevention (CDC) and the Global Fund to Fight AIDS, Tuberculosis, and Malaria. The center attends to all patients with HIV infection diagnosed within the hospital or referred from outside the health facility.

Research design

The study was a double-blind, randomized controlled trial conducted from December 2017 to November 2018. All research participants and research team members, including the principal investigator (PI), were blinded to the allocation of participants to study groups.

Ethical consideration

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

Supplementary information The online version of this article (Figures/Tables) contains supplementary material, which is available to authorized users.

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(identification number PACTR201811722056449).

Participants were informed about the aims of the study, and the study protocol was explained to them. This study was conducted in compliance with the principles enunciated in Helsinki's Declaration. All the study participants provided signed written informed consent or thumbprint if unable to sign. They were also informed of their right to withdraw from the study at any time.

Participants

Participants were approached to participate in this study as they presented themselves to the clinic for any HIV services offered. The participants comprised of patients diagnosed with HIV infection receiving medical care at the S. S Wali Virology Center at the Aminu Kano Teaching Hospital (AKTH) Kano State, Nigeria.

Inclusion criteria: the inclusion criteria were an HIV diagnosis with CD4 counts $\leq$ 500 cells/mm^3; 18 years or older; commenced ART at least for three months; on Tenofovir + Lamivudine + Efavirenz ART drug combination; male and female, and those who gave consent and complied with the study protocol.

Exclusion criteria: participants who had known allergy or intolerance to *Moringa oleifera* Lam. or placebo (cornstarch powder); pregnant women; active opportunistic infection; participants that took micronutrient or natural health product supplements within 30 days of screening and those that lived outside Kano State where the study was conducted were excluded.

Sample size estimation

The sample size was calculated to be able to detect a medium effect size (Cohen's d=0.5) or 0.5 standard deviation in mean weight or CD4 by randomised control trial (RCT) arm with 90% power (1-β [type 2 error probability]), and 95% confidence (or 5% α error probability [type 1]) assuming a balanced 1:1 study design. A sample size of 172 patients was calculated, which was increased to 200 to give room for attrition.

Out of the 410 participants available for assessment of eligibility, 204 of the participants did not meet the inclusion criteria, 6 participants declined to participate, 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 study (Figure 1). The sample si
calculated using G*Power version 3.1.9.2 [2(

Randomization

Block randomization was used to balance the groups throughout the enrolment period. The randomization sequence was generated using PASS 12.0 software (Wei's Urn algorithm) by an independent person that was not part of the research team. As the recruited participants fulfilled the inclusion criteria and consented, a number was assigned to the patient and were randomly allocated into the MOG and COG groups. All research team members were blinded to the allocation of study participants to the respective study groups.

Intervention - *Moringa oleifera* Lam. nutritional supplement

The fresh *Moringa oleifera* Lam. leaves were obtained from Prime Global Agricultural Industries Limited, Kano State, Nigeria. The fresh leaves were identified and authenticated by a Botanist at the Department of Biological Science, Bayero University Kano (BUK), Nigeria. Confirmation of the taxonomic identity of the plant was achieved by comparison with voucher specimens kept at the Herbarium of the Department of Biological Sciences BUK. It was processed, manufactured, and packaged at Dala Foods Nigeria Limited Kano State, Nigeria.

Placebo

The placebo was obtained by colouring corn starch powder with chlorophyll [21]. It was also processed, manufactured, and packaged at Dala Foods Nigeria Limited Kano State, Nigeria.

Both the *Moringa oleifera* Lam. leaf powder and the placebo were similar in presentation and were identically packaged to be indistinguishable. The interventions were packaged into small (15 g) sachets each. Thirty (30) individual sachets were further packaged in a bigger green-colored plastic bag to be used as supplements taken together with meals for one month. It was sealed, labelled with the study code, visit no, subject no, and instructions on how

to take it, and stored in a dry place away from heat and humidity.

Patients were randomly assigned and were either given Moringa oleifera Lam. or the placebo. They were instructed to divide each sachet three times daily (5 g) and add it to their foods before eating [22,23].

All the above procedure was performed by independent persons not part of the research. All the study participants were advised to maintain their regular diet from inception throughout the study. Additionally, they were also instructed not to consume Moringa oleifera Lam. in any form from other external sources throughout the study.

Compliance was monitored to evaluate adherence by self-report during biweekly telephone calls to the participants and monthly hospital visits.

Nutritional contents of Moringa oleifera Lam. leaves powder

The nutritional content of a 100 g Moringa oleifera Lam. leaf powder (Nigerian ecotype) was analyzed by a South African National Accreditation System (SANAS) [24] accredited laboratory ASPIRATA Food and Beverage Laboratory [25].

Accordingly, the nutritional content of Moringa oleifera Lam. leaf powder used in this study includes; 100 g contained an average of 28 g protein, 3.9 g total fat content (total saturated, monosaturated, and polysaturated fat), and 22 g carbohydrate. It contained 1791.82 mg calcium; 4879.26 mg potassium; 24 mg sodium; 2.88 mg Zinc and 37.78 mg iron.

Data collection

Socio-demographic information

At the beginning of the study, a questionnaire to determine each study participant's socio-demographic information was administered by a trained nurse at the virology clinic. This included age, marital status, educational level, occupation, family size, and monthly income.

Anthropometric measurements

Weight was measured to the nearest 0.1 kg using a digital weighing scale (Tanita HD-372, Tanita Corporation, Tokyo, Japan), with participants wearing light clothing and without shoes. Height was measured to the nearest centimeter using a stadiometer (Seca 217, Seca Gmbh and co. KG., Hamburg, Germany). Body mass index (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) [26]. A trained nurse conducted it at the virology centre at baseline and each monthly h[illegible] visit under the supervision of the PI. Anthrop[illegible] measurements from baseline to the sixth mo[illegible] used.

Study outcomes

The outcomes assessed were changes in anthropometric parameters (weight and body mass index [BMI]).

Data processing and analysis

All filled questionnaires were checked for completeness and consistency. The data input was done in Microsoft Excel and exported into SPSS version 26.0 and SAS version 9.4 statistical analysis software for further analysis. Continuous variables were described using the mean, standard deviation, and range, while categorical variables were reported mainly as frequency and percentage. Normality tests for the data were conducted using Kolmogorov-Smirnov and Shapiro-Wilk tests. The data that were not normally distributed were transformed through Box-Cox transformation. The weight was transformed through a lambda value -0.1 and BMI by lambda value -0.2. Repeated measures analysis of variance was deployed to determine significant changes in the mean of anthropometric parameters from baseline to the sixth month within each group. An independent t-test was used to determine the significant mean difference in anthropometric parameters between the two groups at each experiment stage. A linear mixed effect model analysis was deployed to determine the difference in anthropometrics parameters between the treatment groups over time to confirm variability between the two groups further. All statistical tests were performed at a 95% confidence level.

Data Quality Control

Data were collected by a trained nurse and trained research assistants after undergoing two-day intensive

training to all data collectors and supervisors at the virology clinic. Calibration of anthropometric measuring weight scale was checked at zero before and after each measurement following standard protocol.

RESULTS

Figure 1 shows the flow chart of the participants' progress in both study groups. Four hundred and ten patients were screened and assessed for eligibility. Two hundred and ten patients were excluded [204 did not meet the inclusion criteria for the study, and six refused to participate]. Two hundred patients were randomized into two groups. One hundred patients were randomly selected and allocated to the group receiving *Moringa oleifera* Lam. (MOG), and 100 patients were randomly allocated to the group receiving the placebo (COG). In the MOG, eight patients were lost to follow-up, and three discontinued taking the intervention. In the COG, nine patients were lost to follow-up, and three discontinued taking the intervention. One hundred and seventy-seven (177) patients with the mean±SD age of 41.57±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).

Demographic characteristics of the participants

Table 1 shows the baseline demographic assessments of the 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 30 to 39 years, with a predominantly 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 predominant religion [MOG= 64 (71.9%); COG= (66 (75%)]. More than half of participants belong to Hausa/Fulani ethnicity [MOG= 55 (61.8%0; COG= 47 (53.4%)]. A few of the participants in both groups were without any form of education [MOG= 15 (16.9%); COG= 16 (18.2%)] and majority earned a monthly income below the minimum wage of □ 30,000 (US $ 78.23) [MOG= 67 (75.3%); COG= 66 (75%)] (Table 1).

Table 2 shows the baseline anthropometric parameters [weight; BMI] of the study participants in MOG and COG. The means of weight (kg) for both groups were [MOG= 63.8 (±14.8); COG= 61.9 (12.5)]. The mean BMIs for MOG and COG were 24.84 (±4.76) and 23.75 (±3.82), respectively. More than half of the study participants had BMI within the normal range of 18.5- 24.9 in both groups [MOG= (51.7%); COG= (58%)] while a considerable number were overweight with BMI values of 25.0- 29.9 [MOG= (30.3%); COG= (31.8%)] for both study groups (Table 2).

Table 3 shows the comparison of BMI of part[illegible] at baseline and sixth month between MOG and [illegible]. At both baseline and sixth month, most of the participants in both study groups had normal weight (BMI = 18.5-24.9). Only a few of the participants were underweight (BMI<18.5) or obese (BMI≥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) at 6th month while that of the COG was 23.75 (±3.82) and 24.19 (±4.09) at 6th month (Table 3).

Table 4 shows the result of repeated measures ANOVA to determine changes in anthropometric parameters within each study group. At six months follow-up, the difference in mean weight and BMI observed in the MOG was not statistically significant (P>0.05). However, a statistically significant (P<0.05) increase was observed in both the mean of weight and BMI of participants in the COG at the fifth and sixth months of the study (Table 4). Nonetheless, no significant (P>0.05) difference was observed in both the weight and BMI when compared between the MOG and COG using the independent-sample test from study inception to the 6^{th} month (Table 5).

A linear mixed-effect model was used to further assess for variability in anthropometric parameters between the MOG and COG. Table 6 shows the

linear mixed effect model results showing the differences in weight and BMI between the two groups over the study period.

An unstructured correlation matrix was assumed for the model analysis. Estimate of fixed effects between MOG and COG showed that both the weight ($p=0.5556$) and BMI ($p=0.5145$) were not significantly ($p>0.05$) different over the study period.

Figure 2 shows the mean measurements of BMI and weight of study participants in both MOG and COG groups over the study period. The mean BMI and weight of both study groups were relatively constant throughout the study (Figure 2).

DISCUSSION

This manuscript reports a double-blinded randomized controlled trial conducted to determine the six-month impact of *Moringa oleifera* Lam. leaf powder supplementation on the weight and BMI of adult HIV patients receiving ART care at S. S Wali virology centre, AKTH, Kano State, Nigeria.

Over the study period, the *Moringa oleifera* Lam. leaf powder supplementation intervention did not impact the weight and BMI of the patients compared to the COG. However, a significant increase in weight and BMI was observed within the COG at the fifth and sixth months of study. The lack of significant change in weight and BMI observed in MOG could be attributed to the type of participants included in the study. At study inception, we observed that most of our study participants had a normal BMI. A considerable number were overweight, while only a few were underweight. As inclusion criteria, the study should have recruited underweight participants with BMI<18.5 kg/m^2. This class of patients would probably have benefitted more in terms of improvement in weight and BMI from the *Moringa oleifera* Lam. leaves supplementation due to the vast amounts of nutrients constituted [27]. This is a limitation of our study. Alternatively, *Moringa oleifera* Lam. has been reported to control weight gain due to its low calorific content; as such, its use is advocated in obese individuals [28]. This factor could have also been attributed to the lack of change in weight and BMI observed in the MOG.

On the other hand, Tshingani et al. reported an opposite effect of *Moringa oleifera* Lam. leaves. According to their findings, *Moringa oleifera* Lam. increased BMI in patients with physical activity. They reported a significant increase in BMI in patients with physical activity in the third and sixth months of taking *Moringa oleifera* Lam. leaves supplementation [29].

Although *Moringa oleifera* Lam. leaves intervention did not affect the weight and BMI of the participants in this study, previous research done by the same author reported a significant increase in the CD4 cell counts [18] and quality of life domains [19] of the participants in the MOG after six months of *Moringa oleifera* Lam. leaves supplementation.

The introduction of HAART has increased the prevalence of overweight and obesity in PLHIV [7] as seen in our study participants. This may increase the risk of cardiovascular and metabolic diseases [18] Additionally, Takarinda et al. reported a study conducted to determine the prevalence of malnutrition among HIV – positive patients that are enrolled in HIV treatment in Zimbabwe. The study reported that the majority of their study participants had a normal BMI (63.6%). Also, a high prevalence of overweight and obesity was observed amongst the study participants. The study suggested that HIV may change from a highly fatal infectious disease into a chronic manageable disease due to the high prevalence of obesity observed in the HIV population on ART. This is associated with an increased risk of cardiovascular-related conditions such as hypertension and diabetes mellitus [30]. A similar result was reported in a hospital-based cross-sectional study conducted to assess the nutritional status among people living with HIV in Nepal, where most of the study participants were overweight or obese. The study also highlights the importance of nutritional programs being an integral part of the HIV/AIDS continuum of care [31]

Therefore, to address the burden of malnutrition, the use of *Moringa oleifera* Lam. could be advocated in PLHIV that are taking ART to control weight gain and reduce the risk of cardiovascular and metabolic diseases.

This being a double-blind, randomized placebo trial, a gold standard of intervention studies, gives strength and credence to the findings of this study. Some limitations of the study have been noted. The lack of inclusion of participants that were underweight as well as the sixth month duration of the study and not longer for any significant observable differences. *Moringa oleifera* Lam's distinguishable taste could be a source of bias, including patients who were only on one ART regimen (tenofovir + lamivudine + efavirenz drug regimen) limits the generalizability of our study findings. Lastly, compliance, which was monitored by the self-reporting of the study participants, is a further limitation of the study.

CONCLUSIONS

This study revealed that *Moringa oleifera* Lam. leaf supplementation did not have impact on the weight and BMI of our study participants at sixth month.

INFORMATION

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Conflict of Interest Statement. The authors declare they have no conflict of interest.

Data Availability. Data will be made available on reasonable request.

REFERENCES

1. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: A Food Plant with Multiple Medicinal Uses. Phytother Res. 2007;21:17–25.
2. Mangili A, Murman DH, Zampini AM, Wanke CA. Nutrition and HIV Infection: Review of Weight Loss and Wasting in the Era of Highly Active Antiretroviral Therapy from the Nutrition for Healthy Living Cohort. Clin Infect Dis. 2006;42:836–878.

3. Rakotosamimanana VR, Valentin D, Arvisenet G. How to use local resources to fight malnutrition in Madagascar? A study combining a survey and a consumer test. Appetite. 2015;95:533–576.

4. Takarinda KC, Mutasa-Apollo T, Madzima B, Nkomo B, Chigumira A, Banda M. Malnutrition status and associated factors among HIV-positive patients enrolled in ART clinics in Zimbabwe. BMC Nutr. 2017;3(15):1–11.

5. Nutritional Analysis of Moringa oleifera leaves powder. ASPIRATA Food and Beverage Laboratory. 2022;p. 12–12. Available from: http://aspirata.co.za/ .2017.

6. Khatri S, Amatya A, Shrestha B. Nutritional status and the associated factors among people living with HIV: an evidence from cross-sectional survey in hospital based antiretroviral therapy site in Kathmandu. Nepal BMC Nutr. 2020;6(22):1–13.

7. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: A review on nutritive importance and its medicinal application. Food Sci Hum Well. 2016;5:49–56.

8. Masa R, Chowa G, Nyirenda V. Socioeconomic correlates of dietary diversity and its association with adherence and psychosocial functioning of people living with HIV in rural Zambia. Nutr Health. 2018;24(2).

9. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav. 2009;41.

10. Magadi MA. Household and community HIV/AIDS status and child malnutrition in sub-Saharan Africa: Evidence from the demographic and health surveys. Soc Sci Med. 2011;73:436–482.

11. Tshingani K, Donnen P, Mukumbi H, Duez P, Dramaix-Wilmet M. Impact of Moringa oleifera lam. Leaf powder supplementation versus nutritional counseling on the body mass index and immune response of HIV patients on antiretroviral therapy: a single-blind randomized control trial. BMC Complement Altern Med. 2017;17(420).

12. Bahadoran Z, Tohidi M, Nazeri P, Mehran M, Azizi F, Mirmiran P. Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. Int J Food Sci Nutr. 2012;63(7).

13. Williams FE, Animashaun JO, Ibrahim H, Toye AA. A preliminary survey on consumption of moringa products for nutraceutical benefits in Ilorin, Kwara state. Nigeria Agrosearch. 2013;13(1).

14. Dhakad AK, Ikram M, Sharma S, Khan S, Pandey VV, Singh A. Biological, nutritional, and therapeutic significance of Moringa oleifera Lam. Phytother Res. 2019;33:2870–903.

15. Tété-Bénissan A, Quashie M, Lawson-Evi K, Gnandi K, Kokou K, Gbéassor M. Influence of Moringa oleifera leaves on atherogenic lipids and glycaemia evolution in HIV-infected and uninfected malnourished patients. J Appl Biosci. 2013;62:4610–4619.

16. Idohou-Dossou N, Diouf A, Gueye AL, Guiro AT, Wade S. Impact of daily consumption of Moringa (Moringa Oleifera) dry leaf powder on iron status of senegalese lactating women. AJFAND. 2011;11(4):4985–99.

17. Nutrition Assessment, Counseling, and Support (NACS): A User's Guide-Module 2: Nutrition Assessment and Classification. FANTA Food and Nutrition Technical Assistance III Project (FANTA). 2016;2:1–12.

18. Sax PE, Erlandson KM, Lake JE, Mccomsey GA, Orkin C, Esser S. Weight Gain Following Initiation of Antiretroviral Therapy: Risk Factors in Randomized Comparative Clinical Trials. Clin Infect Dis. 2020;71(6):1380–1389.

19. Moyo B, Masika PJ, Hugo A, Muchenje V. Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves. Afr J Biotechnol. 2011;10(60):12925–12958.

20. Farinola LA, Famuyide OO, Awe F, Adio AF, Ewolor AS. Households' perception, awareness and willingness to pay for Moringa oleifera Lam powder in Oyo State. J Agric Crop Res. 2014;2(6):94–103.

21. Animashaun JO, Williams FE, Toye AA. Towards Validating Moringa's Nutraceutical Benefits: An Examination of Consumers' Perspectives vis-à-vis Health Benefits Efficacy and Willingness to Pay. Agris on-line Papers in. Economics and Informatics. 2013;(2):11–21.

22. SANAS. South African National Accreditation System (SANAS). . Available at. 2017;p. 12–12. Available from: https://www.sanasco.za.

23. Contri PV, Berchielli EM, Tremeschin MH, Negrini BV, o and J P Monteiro RGS. Nutritional status and lipid profile of HIV-positive children and adolescents using antiretroviral therapy. Clinics. 2011;66(6).

24. Martín-Cañavate R, Sonego M, Sagrado MJ, Escobar G, Rivas E, Ayala S. Dietary patterns and nutritional status of HIV-infected children and adolescents in El Salvador: A cross-sectional study. PLOS ONE. 2018;13(5).

25. Gambo A, Moodley I, Babashani M, Babalola TK, Gqaleni N. A double blind randomised control trial to examine the effect of Moringa oleifera leaf powder supplementation on the immune status and anthropometric parameters of adult HIV patients on antiretroviral therapy in a resource-limited setting. PLoS ONE. 2021;16(12):261935–261935.

26. N A. HIV and AIDS in Nigeria; 2022. Available from: https://www.avert.org/professionals/hiv-around-world/sub-saharan-africa/nigeria.2019.

27. Thurber MD, Fahey JW. Adoption of Moringa oleifera to combat under-nutrition viewed through the lens of the "Diffusion of Innovations" theory. Ecol Food Nutr. 2009;48(3):212–237.

28. Gambo A, Moodley I, Babashani M, Babalola TK. Impact of Moringa Oleifera leaves supplementation on quality of life of people living with HIV: A double-blind randomized controlled trial. Qual Life Res. 2021;.

29. Pandey A, Pandey RD, Tripathi P, Gupta PP, Haider J, Bhatt S. Sahijan) - A Plant with a Plethora of Diverse Therapeutic Benefits: An Updated Retrospection. Moringa Oleifera Lam. 2012;1(1).

30. Haffner SM. Relationship of Metabolic Risk Factors and Development of Cardiovascular Disease and Diabetes. Obesity. 2006;14:121–128.

31. Koethe JR, Heimburger DC. Nutritional aspects of HIV-associated wasting in sub-Saharan Africa1-4. Am J Clin Nutr. 2010;91.

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TABLE 1: Socio-demographic characteristics of participants.

Variables	MOG (%) (N=89)	COG (%) (N=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 90,001-120,000	1 (1.1) 3 (3.4)	1 (1.1) 2 (2.3)	
>120,000	1 (1.1)	3 (3.4)	

Statistical test = Chi-square test.

TABLE 2: Description of baseline anthropometric parameters between MOG and COG.

	Baseline		
Parameters	**MOG** (n = 89) Freq. (%)	**COG** (n = 88) Freq. (%)	**p value**
Anthropometrics			
Weight (Kg)			
Mean (±SD)	63.8 (±14.8)	61.9 (±12.5)	0.361
BMI (Kg/m^2)			
Underweight (<18.5)	5 (5.6)	5 (5.7)	
Normal (18.5 – 24.9)	46 (51.7)	51 (58.0)	
Overweight (25.0 – 29.9)	27 (30.3)	28 (31.8)	
Obese (> 30.0)	11 (12.4)	4 (4.5)	
Mean (±SD)	24.84 (±4.8)	23.75 (±3.8)	0.093

Statistical test = independent sample t-test.

TABLE 3: Changes in BMI of participants at baseline and 6th month in MOG and COG.

BMI	**Baseline**			**6th month**		
	MOG n. (%)	**COG** n. (%)	**P**	**MOG** n. (%)	**COG** n. (%)	**P**
Underweight (<18.5)	5 (5.6)	5 (5.7)	0.357	5 (5.6)	7 (8.0)	0.549
Normal Weight (18.5 – 24.9)	46 (51.7)	51 (58.0)	0.781	41 (46.1)	46 (52.3)	0.600
Overweight (25.0 – 29.9)	27 (30.3)	28 (31.8)	0.576	29 (32.6)	27 (30.7)	0.872
Obese (≥ 30.0)	11 (12.4)	4 (4.5)	0.130	14 (15.7)	8 (9.1)	0.248
Mean [±SD] (Kg/m^2)	24.84 [±4.78]	23.75 [±3.82]		25.16 [±4.93]	24.19 [±4.09]	
Minimum (Kg/m^2)	16.82	16.03		15.62	16.44	
Maximum (Kg/m^2)	37.97	34.68		39.84	35.67	

Statistical test = independence sample t-test; SD=standard deviation.

TABLE 4: Repeated measures ANOVA analysis of anthropometric parameters within each study group.

			MOG			COG		
			Mean Diff (I-J)	Std. Error	P	Mean Diff (I-J)	Std. Error	P
BMI	Baseline (I)	1st month	-0.018	0.072	0.999	-0.028	0.051	0.999
		2nd month	-0.148	0.097	0.999	-0.179	0.073	0.336
		3rd month	-0.150	0.118	0.999	-0.215	0.089	0.366
		4th month	-0.212	0.128	0.999	-0.293	0.102	0.106
		5th month	-0.313	0.143	0.665	-0.390*	0.106	0.009*
		6th month	-0.317	0.141	0.568	-0.446*	0.113	0.003*
Weight	Baseline (I)	1st month	-0.045	0.181	0.999	-0.091	0.133	0.999
		2nd month	-0.427	0.224	0.999	-0.500	0.193	0.238
		3rd month	-0.483	0.282	0.999	-0.602	0.233	0.238
		4th month	-0.640	0.306	0.827	-0.784	0.267	0.089
		5th month	-0.899	0.342	0.213	-1.045*	0.283	0.008*
		6th month	-0.876	0.343	0.259	-1.216*	0.301	0.002*

**Statistically significant.*

TABLE 5: Independent – sample analysis showing the differences in anthropometric parameters between MOG and COG.

Study groups					**Mean weight (SD)**			
	n	**Admission**	**1st month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
MOG	89	63.83 (14.77)	63.88 (14.89)	64.26 (14.76)	64.31 (14.93)	64.47 (14.93)	64.73 (15.00)	64.71 (15.07)
COG	88	61.94 (12.54)	62.03 (12.92)	62.44 (13.26)	62.55 (13.36)	62.73 (13.37)	62.99 (13.38)	63.16 (13.49)
Diff (CL at 95%)		1.89	1.85	1.82	1.76	1.74	1.74	1.55
p (interaction)	> 0.001							
					Mean BMI (SD)			
MOG	89	24.84 (4.76)	24.86 (4.84)	24.99 (4.82)	24.99 (4.88)	25.06 (4.87)	25.16 (4.93)	25.16 (4.93)
COG	88	23.75 (3.82)	23.78 (3.93)	23.92 (4.02)	23.96 (4.04)	24.04 (4.10)	24.14 (4.08)	24.19 (4.09)
Diff (CL at 95%)		1.09	1.08	1.07	1.03	1.02	1.02	0.97
p (interaction)	> 0.001							

TABLE 6: Linear mixed effects model showing the differences in anthropometric parameters between MOG and COG over the study period.

	Estimates of Fixed Effects						
						95% CI	
	Parameter	Estimate	Std. error	t	Sig.	Lower Bound	Upper Bound
	Intercept	61.92	1.47	42.02	0.0001	61.43	63.65
Weight	MOG	-0.05	0.08	-0.59	0.5556	-0.20	-0.11
	COG	0	0	-	-	-	-
	Intercept	23.73	0.47	50.98	0.0001	23.61	24.32
BMI	MOG	-0.02	0.03	-0.65	0.5145	-0.08	-0.04
	COG	0	0	-	-	-	-

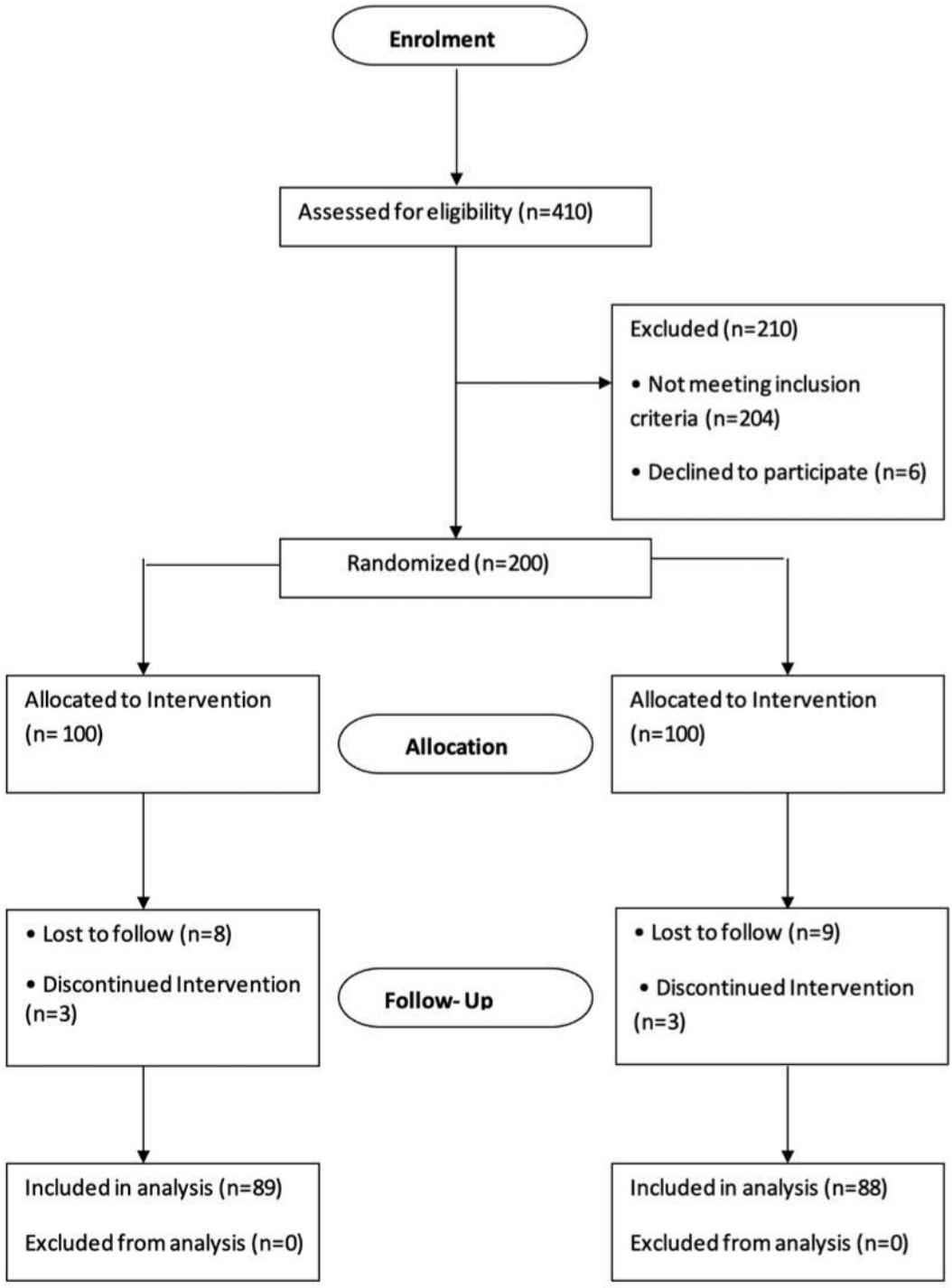

FIGURE 1: Flow chart of participants.

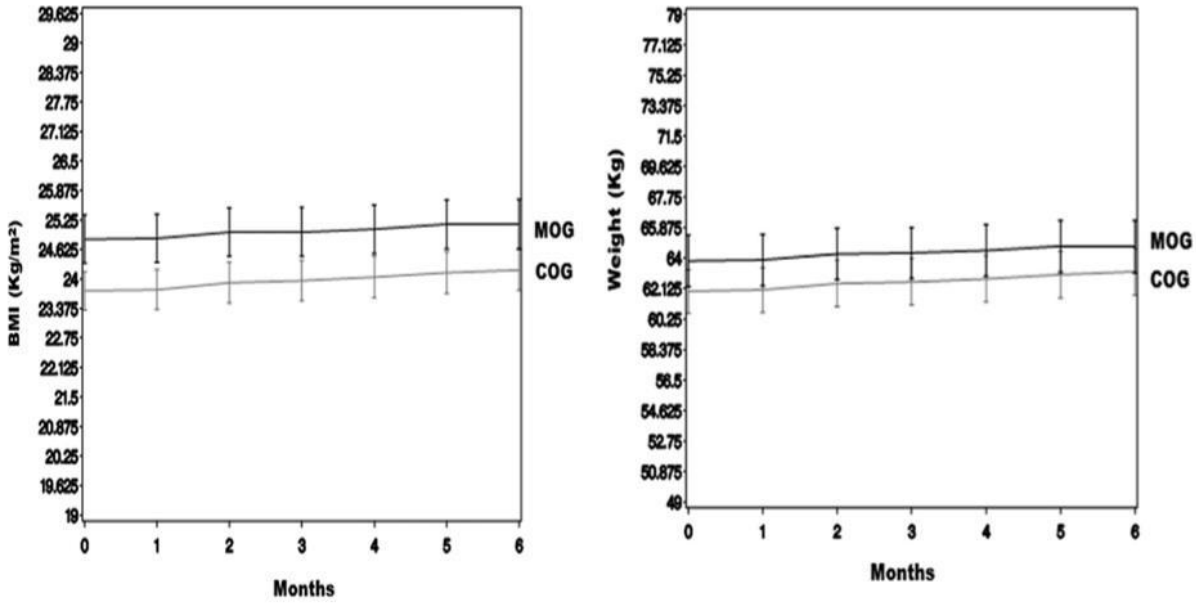

FIGURE 2: Charts showing mean measurements of BMI andweight in MOG and COG over the study period.

CHAPTER FOUR

A DOUBLE-BLIND, RANDOMIZED CONTROLLED TRIAL TO EXAMINE THE EFFECT OF *MORINGA OLEIFERA* LEAF POWDER SUPPLEMENTATION ON THE IMMUNE STATUS AND ANTHROPOMETRIC PARAMETERS OF ADULT HIV PATIENTS ON ANTIRETROVIRAL THERAPY IN A RESOURCE-LIMITED SETTING

Chapter 3 of this thesis examined the impact of *Moringa oleifera* leaf supplementation intervention on the nutritional status of the participants and the limitations of the study design concerning the anthropometric parameters assessed.

Chapter 4 is a detailed report of a study conducted to examine the effect of *Moringa oleifera* leaf powder supplementation on the CD4 cell counts, viral load, weight, and BMI of adult HIV patients that are on ART. The manuscript further highlights the challenges of conducting a randomized clinical trial in a resource-limited setting.

The chapter is presented as a published article.

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RESEARCH ARTICLE

A double-blind, randomized controlled trial to examine the effect of *Moringa oleifera* leaf powder supplementation on the immune status and anthropometric parameters of adult HIV patients on antiretroviral therapy in a resource-limited setting

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Abstract

Background

People living with HIV (PLHIV) in resource-limited settings are vulnerable to malnutrition. Nutritional interventions aimed at improving food insecurity and malnutrition, together with antiretroviral therapy (ART), could improve treatment outcomes. In Nigeria, there is a high awareness of the nutraceutical benefits of *Moringa oleifera*. Thus, this study aimed to evaluate the effects of *Moringa oleifera* leaf supplementation on the CD4 counts, viral load and anthropometric of HIV-positive adults on ART.

Methods

This was a double-blind, randomized study. Two hundred HIV-positive patients were randomly allocated to either the *Moringa Oleifera* group (MOG) given *Moringa oleifera* leaf powder or the control group (COG) given a placebo. Changes in anthropometric parameters [weight; body mass index (BMI)] and CD4 cell counts were measured monthly for six months, while HIV-1 viral loads were measured at baseline and the end of the study for both groups.

Results

Over the study period, the treatment by time interaction shows a significant difference in CD4 counts by treatment group ($p<0.0001$). A further estimate of fixed effects showed that the CD4 counts among MOG were 10.33 folds greater than COG over the study period.

Data Availability Statement: Data cannot be shared publicly due to the ethical restrictions regarding patient confidentiality imposed by the ethics committee of Aminu Kano Teaching Hospital, Kano state, Nigeria and University of Kwazulu- Natal, Durban, South Africa. Interested and qualified researchers who meet the criteria for access of data can request data access from the: Biomedical Research Ethics Committee of University of Kwazulu-Natal, Durban South Africa. Westville Campus, Govan Mbeki Building. Postal address: Private Bag x54001, Durban 4000. Tel: +27(0) 31 260 2486, Facsimille: +27(0) 31 260 4609. Email: brec@ukzn.ac.za.

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Competing interests: The authors have declared that no competing interests exist.

However, the viral load (p = 0.9558) and all the anthropometric parameters (weight; p = 0.5556 and BMI; p = 0.5145) between the two groups were not significantly different over time. All tests were conducted at 95CI.

Conclusion

This study revealed that *Moringa oleifera* leaf supplementation was associated with increased CD4 cell counts of PLHIV on ART in a resource-limited setting. Programs in low-resource settings, such as Nigeria, should consider nutritional supplementation as part of a comprehensive approach to ensure optimal treatment outcomes in PLHIV.

Introduction

The HIV and AIDS epidemic is a major pandemic that affects millions of people globally. The UNAIDS Global AIDS Update 2019 reported that 74.9 million people have become infected with HIV since the start of the epidemic, with 32.0 million deaths from AIDS-related illnesses [1]. Nigeria has the second-largest HIV epidemic worldwide [2]. In 2018, 130,000 new infections and 53,000 AIDS-related deaths were recorded. Nigeria alone accounts for more than half of the new infections and deaths from AIDS-related illnesses in the western and eastern Africa region in 2017 [3]. This high mortality is probably attributed to the large population size of Nigeria compared to other countries in the region [3].

Considerable progress has been made in providing global access to antiretroviral therapy (ART), with 23.3 million people accessing therapy worldwide [4]. ART has greatly reduced AIDS-related mortality and morbidity and increased the life expectancy of people living with HIV and AIDS (PLHIV) [5]; however, it has led to other consequences, including malnutrition [6]. PLHIV are vulnerable to malnutrition due to intestinal damage, which causes impaired nutrient absorption and reduced food intake from vomiting and painful swallowing [7]. Furthermore, malnutrition could result from food insecurity and the side effects of ART, such as appetite loss and abdominal pain [7]. The adverse effects of HIV and malnutrition on the immune system are similar in that they both reduce CD4 and CD8 T-lymphocyte numbers [8], which eventually increase susceptibility to opportunistic infections. Opportunistic infections and malnutrition can affect intake, absorption, and metabolism of food, worsen disease progression [7, 9] and increase HIV-related mortality [6].

PLHIV are encouraged to consume healthy diets rich in essential amino acids, unsaturated fats, and micronutrients at the recommended daily allowance (RDA) to achieve an adequate nutritional status vital for health and survival [10]. Unfortunately, several studies reported a poor diet intake with inadequate nutrients among PLHIV in Sub-Saharan Africa, including Nigeria [11, 12]. A study conducted in Nigeria reported significant malnutrition in early HIV infection before ART initiation [13].

Malnutrition is a public health challenge in Nigeria; available data showed that the country has the second-highest burden of stunted children worldwide [14]. Two million children and 7% of women of childbearing age were also reported to suffer from severe acute malnutrition [14].

Moringa oleifera Lam (syn. M. ptreygosperma Gaertn.) is a species of the monogeneric family Moringaceae [15, 16]. It has been documented to contain many nutrients and bioactive compounds in literature [17, 18]. The leaves are the part of the plant mostly used and with several nutrients often deficient in malnourished PLHIV. It is a rich source of both macro and

micronutrients and natural antioxidants source [19]. *Moringa oleifera* leaf powder is a novel, cheap, culturally acceptable, efficacious, and regionally produced plant and can reduce the malnutrition burden in Sub-Saharan Africa [19]. Furthermore, in Nigeria, *Moringa oleifera* use is promoted based on the commendation of its nutraceutical benefits, and the Nigerian Federal Government Raw Materials Research and Development Council (RMRDC) has been actively encouraging farming and consumption of *Moringa oleifera* [20]. Monera *et al.* reported the *in vitro* CYP3A4 inhibitory activity of *Moringa oleifera* leaf extracts, suggesting the potential for interaction with antiretroviral drugs. However, the *in vitro* data alone is insufficient to conclude the clinical significance of concomitant administration of *Moringa oleifera* with ART in PLHIV [21]. Moreover, the interaction between tenofovir/lamivudine/efavirenz and *Moringa oleifera* leaf powder has not been reported. No adverse clinical effects have been reported in the literature despite its widespread use and concomitant use by PLHIV.

Therefore, this double-blind, randomized study aimed to evaluate the effects of six months of *Moringa oleifera* leaf supplementation on the CD4 counts, viral load and anthropometric parameters of HIV-positive adults who were on ART in Kano State, Nigeria.

Methods

Study location

The study was conducted at the S. S Wali Virology Center at the Aminu Kano Teaching Hospital, Kano State (AKTH), Nigeria. AKTH is a tertiary health institution and referral center that operates a daily HIV clinic (5 days a week). It also serves as a center for clinical evaluation, laboratory tests, HIV counseling and testing (HCT), treatment, and care supported by the Federal Government and the Institute of Human Virology, Nigeria (IHVN) in partnership with its global partners, including the Centers for Disease Control and Prevention (CDC) and the Global Fund to Fight AIDS, Tuberculosis, and Malaria. The center attends to all patients with HIV infection diagnosed within the hospital or referred from outside the health facility.

Type of study and participants

The study was a double-blind, randomized control trial conducted between December 2017 and November 2018. Registered HIV-infected individuals receiving treatment and care at the S.S Wali Virology Center were invited to participate. Inclusion criteria for the study were: being HIV sero-positive, $\geq$ 18 years old, CD4 counts $\leq$ 500 cells/mm^3, ART for at least three months (tenofovir + lamivudine + efavirenz combination), informed consent, and compliance with the study protocol. Exclusion criteria for the study were: known allergy or intolerance to *Moringa oleifera* or placebo (cornstarch powder), pregnancy, CD4 counts $>$ 500 cells/mm^3, presence of active opportunistic infection, and intake of micronutrient or natural health product supplements within 30 days of screening. For ease of monitoring, patients who lived outside Kano State, where the study was conducted, were excluded.

Sample size

The sample size was calculated to ensure detection of medium effect size (Cohen's d = 0.5) [22] or 0.5 standard deviation in mean weight or CD4 by randomized control trial (RCT) arm with 90% power (1-β [type 2 error probability]) and 95% confidence (or 5% α error probability [type 1]), assuming a balanced 1:1 study design. A sample size of 172 patients was calculated, rounded up to 200 to give room for attrition. The sample size was calculated using G*Power version 3.1.9.2 [23].

Randomization

Block randomization was used to balance the groups throughout the enrollment period. PASS 12.0 software was used to develop the randomization list using Wei's Urn algorithm by an independent statistician who held the randomization code. A random allocation sequence was generated to allocate and assign each patient to either the *Moringa Oleifera* group (MOG) or the control group (COG) when participants fulfilled the inclusion criteria and consented, with 100 patients in each group. All the research team members, including the principal investigator (PI) and the study participants, were blinded to the allocation of patients to the study groups.

Preparation for study

Fresh *Moringa oleifera* leaves were obtained from Prime Global Agricultural Industries Limited, Kano State, Nigeria. Fresh leaves were identified and authenticated by a botanist at the Department of Biological Science, Bayero University Kano (BUK), Nigeria. Confirmation of the taxonomic identity of the plant was achieved by comparison with voucher specimens kept at the Herbarium of the Department of Biological Sciences, BUK. The leaves were processed by HOMIP Spices and Foods Limited, Kano State, Nigeria. The procedure involved washing and drying the fresh leaves in a clean environment on a net mesh away from direct sun for days until it was completely dried. The dried leaves were cleaned, and the small branches were removed. The dried leaves were ground using a grinder and sieved using a 0.500 mm standard sieve (No. 35 mesh size) [24, 25] to obtain a fine powder. Fine Moringa leaf powder was stored in airtight containers.

The placebo was obtained by coloring cornstarch powder with chlorophyll [26]. It was manufactured and processed at Dala Foods Nigeria Limited, Kano State, Nigeria. Both the *Moringa oleifera* and the placebo were similar in presentation and were identically packaged to be indistinguishable. The interventions were packaged into small (15 g) sachets each. Thirty (30) individual sachets were further packaged in a bigger green-colored plastic bag to be used as supplements taken together with meals for one month. It was sealed, labeled, and stored in a dry place away from heat and humidity. Patients could simply put a sachet in the pocket or bag while going out for their daily activities. The supplements were taken together with meals.

Intervention

The interventions were provided in 15 g Moringa leaf powder sachets. Thirty sachets were given to the participants to represent one month prescription, and they were directed to divide each sachet into three and use it thrice daily (5 g), adding it into meals [27, 28]. They were asked to maintain their regular diet and not consume *Moringa oleifera* in any form from other sources during the study period.

In Kano State, home visits of participants by research members to ensure adherence was not convenient for fear of stigmatization by family members. Thus, adherence was monitored by biweekly phone calls to the patients and interviewing them during their monthly visits to evaluate compliance.

Data collection

At the first visit, the research team interviewed the patients to obtain socio-demographic information, patient history, and other relevant information, including dietary information. A trained nurse at the virology clinic and a trained research assistant were responsible for all anthropometric measurements and data collection under the supervision of the PI. Weight was measured to the nearest 0.1 kg using a digital weighing scale (Tanita HD-372, Tanita

Corporation, Tokyo, Japan), with participants wearing light clothing and without shoes. Height was measured to the nearest centimeter using a stadiometer (Seca 217, Seca Gmbh and co. KG., Hamburg, Germany). Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Anthropometric parameters were measured at baseline and each monthly visit.

All laboratory evaluations were performed by a trained phlebotomist at the President's Emergency Plan for AIDS Relief (PEPFAR) laboratory of the S. S Wali Virology Center at AKTH. The CD4 count was tested using a Partec flow cytometer (Partec, Munster, Germany). Five (5 ml) venous blood samples were aseptically collected from each study participant. Briefly, equal volumes (20 μL) of CD4 PE antibody and ethylene diamine tetraacetic acid blood were mixed and incubated for 15 min, and 800 μL of CD4 buffer was added before reading in the cell counter [29]. The viral load was quantified by polymerase chain reaction (PCR). Ten (10 ml) of venous blood samples were aseptically collected from each study participant. The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 2.0, manuals (Roche Diagnostics GmbH) was used as the standard operating procedure [30, 31]. The CD4 test was conducted at baseline and each subsequent monthly visit for each study participant, while the viral load test was conducted twice, at baseline and after the sixth month.

Study outcomes

The outcomes assessed were changes in immune status (CD4 cell count and viral load) and changes in anthropometric parameters (weight and body mass index [BMI]) and from baseline to the sixth month.

Data analysis

The data input was done in Microsoft excel and exported into SPSS and SAS statistical software for analysis. Findings from the analysis were reported in frequency tables, charts and descriptive analysis was done to estimate mean and standard deviation. Normality test for the data was conducted using Kolmogorov-Simonov and Shapiro-Wilk tests. Data which were not normally distributed were transformed through Box-Cox transformation. Independent t-test was used to determine the significance of mean difference in immunological and anthropometric parameters between the two groups at each stage of the experiment. To further confirm variability between the two groups, a repeated measure linear mixed effect model analysis was deployed to determine the difference in immunological and anthropometrics outcomes between the treatment groups overtime. An exploratory analysis was done to evaluate the influence of socio-demographic characteristics on the immunological and anthropometrics outcomes of the treatment groups over the study period. All statistical tests were carried out at 95% Confidence Interval.

Ethical considerations

This study was reviewed and approved by the ethics committee of Aminu Kano Teaching Hospital (AKTH) Kano, Nigeria (reference number NHREC/21/08/2008/AKTH/EC/2012), and the Biomedical Research Ethics Committee of the University of Kwazulu-Natal Durban, South Africa (reference number BFC294/16). The study was registered with the Pan African Clinical Trial Registry (identification number PACTR201811722056449). The study complied with the principles outlined in the Declaration of Helsinki [32]. All participants provided oral or written informed consent before enrolling them in the study. The procedures of the study, together with the aims, were explained to the participants. Participants were also informed of their right to withdraw from the study at any time.

Results

Participants flow

Fig 1 shows the flow chart of participants in the study. Four hundred and ten patients were screened and assessed for eligibility. Two hundred and ten patients were excluded (204 did not meet the inclusion criteria for the study, and 6 refused to participate). Two hundred patients were randomized into two groups. One hundred patients were randomly selected and allocated to the group receiving MOG, and 100 patients were randomly assigned to the group receiving COG. In the MOG, 8 patients were lost to follow-up, and 3 discontinued the intervention. In the COG, 9 patients were lost to follow-up, and 3 discontinued the intervention. Overall, 177 patients (89 and 88 in the MOG and COG, respectively) completed the 6-month study.

Nutritional contents of *Moringa oleifera* leaves powder

The nutritional content of a 100 g *Moringa oleifera* leaf powder (Nigerian ecotype) was analyzed using a South African National Accreditation System (SANAS) [33] accredited laboratory ASPIRATA Food and Beverage Laboratory [34]. Each 100 g contained an average of 28 g protein, 3.9 g total fat content (total saturated, monounsaturated, and polyunsaturated fatty acids), and 22 g carbohydrate. It contained 1791.82 mg calcium; 4879.26 mg potassium; 24 mg sodium; 2.88 mg Zinc and 37.78 mg iron (S1 & S2 Files).

Characteristics of participants at study inception

Table 1 shows the socio-demographic characteristics of the study participants at baseline. Participants in the MOG's socio-demographic, socioeconomic, nutritional status, and immunological characteristics were similar to those in the COG at baseline. Females were predominant in both groups [MOG = 70 (78.7%); COG = 67 (76.1%)]. The majority were between 30 to 39 years of age in both groups [MOG = 37 (41.6%); COG = 36 (40.9%)]. The majority were married [MOG = 42 (47.2%); COG = 38 (43.2%)]. Islam was the predominant religion of participants in both groups [MOG = 64 (71.9%); COG = 66 (75%)] with more than half of participants belonging to the Hausa/Fulani ethnicity [MOG = 55 (61.8%); COG = 47 (53.4%)]. A few of the participants were without any form of education in either group [MOG = 15 (16.9%); COG = 16 (18.2%)]. The majority of the participants in both groups earned a monthly income below the minimum wage of ₦30,000 ($78.23) [MOG = 67 (75.3%); COG = 66 (75%)].

The baseline anthropometric and immunological characteristics of the study participants in both study groups showed a similar trend. The means of weight (kg) for both groups were [MOG = 63.8 (± 14.8); COG = 61.9 (±12.5)]. The mean BMIs for MOG and COG was 24.84 (± 4.76) and 23.75 (± 3.82), respectively. More than half of the patients had BMI within the normal range of 18.5–24.9 in both groups [MOG = (51.7%); COG = (58%)] while a significant number were overweight with BMI values of 25.0–29.9 [MOG = (30.3%); COG = (31.8%)] for both study groups (Table 2).

At baseline, the mean CD4 cell counts were statistically similar for both MOG and COG with values of 341.78 (± 106.06) and 352.34 (± 125.99) cells/μL, respectively (Table 2). The majority of the patients in both groups had an undetected viral load at baseline (MOG = 76.4% and COG = 71.6%) (Table 2).

A test of normality for the dependent variables was carried out using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The data that were not normally distributed were transformed through Box-Cox transformation. The CD4 count was transformed through a lambda value of 0.5, weight by lambda value -0.1 and BMI by lambda value -0.2.

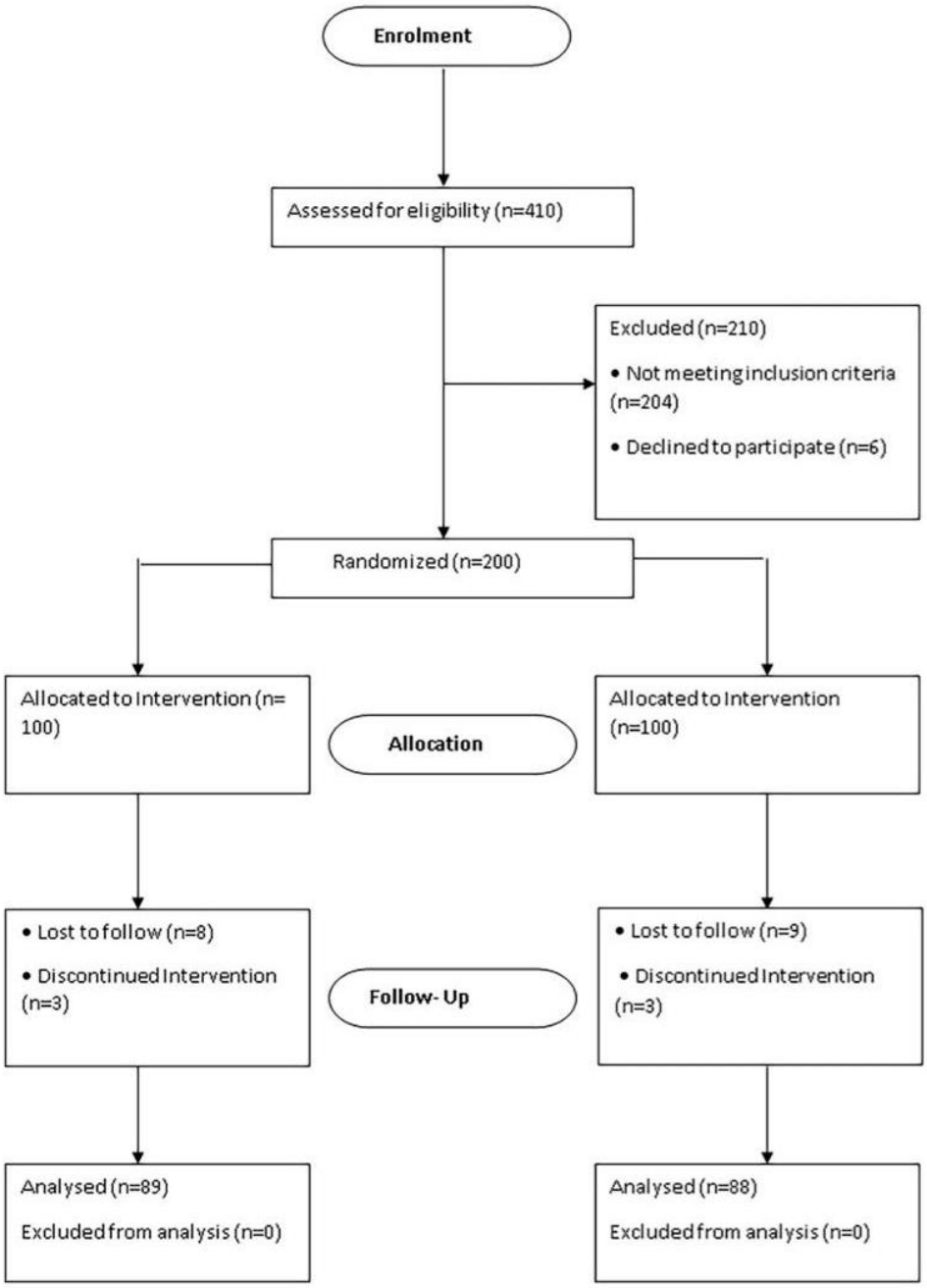

Fig 1. Flow chart of participants.

https://doi.org/10.1371/journal.pone.0261935.g001

Table 1. Socio-demographic characteristics of participants.

Variables	MOG (%) (N = 89)	COG (%) (N = 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)	

https://doi.org/10.1371/journal.pone.0261935.t001

Table 2. Description of baseline anthropometric and immunological parameters between the two groups.

	Baseline		
Parameters	**MOG (n = 89)**	**COG (n = 88)**	**P-value**
	Freq. (%)	**Freq. (%)**	
Anthropometric			
Weight (Kg)			**0.361**
Mean (±SD)	63.8 (±14.8)	61.9 (±12.5)	
BMI (Kg/m^2)			**0.093**
Underweight (<18.5)	5 (5.6)	5 (5.7)	
Normal (18.5–24.9)	46 (51.7)	51 (58.0)	
Overweight (25.0–29.9)	27 (30.3)	28 (31.8)	
Obese (> 30.0)	11 (12.4)	4 (4.5)	
Mean (±SD)	24.84 (±4.8)	23.75 (±3.8)	
Immunological Parameters			
CD4 Counts (Cells/μl)			**0.547**
< 350	46 (51.7)	38 (43.2)	
≥ 350	43 (48.3)	50 (56.8)	
Mean (±SD)	341.8 (±106.1)	352.34 (±126.0)	
Viral load (RNA copies/ml)			
<1000 (Undetected)	68	63	**0.497**
≥1000(Detected)	21	25	

https://doi.org/10.1371/journal.pone.0261935.t002

Effect of nutritional supplement intervention on immunological and anthropometric parameters

Table 3 shows the results of independent samples test for the difference in immunological parameters anthropometric between the MOG and COG. The mean CD4 count between the two groups was not significantly different throughout the period of measurement except at the 6th month. From baseline to the 6th month, there was no significant ($P>0.05$) difference in all the anthropometric parameters [weight; BMI] between the MOG and COG (Table 3).

In addition to the bivariate analysis test above, a linear mixed-effect model was used to examine the differences in anthropometric and immunological parameters between the MOG and COG. Table 4 shows the linear mixed effect model results showing the differences in the CD4 counts, viral load, weight and BMI between the two groups over the study period. An unstructured correlation matrix was assumed for the model analysis. For CD4 counts, the treatment by time interaction shows a significant difference in CD4 counts by treatment group over time ($p<0.0001$). A further estimate of fixed effects showed that the CD4 counts among MOG were 10.33 folds greater than COG over the study period. On the other hand, viral load ($p = 0.9558$) and the anthropometric parameters (BMI; $p = 0.5145$ and weight; $p = 0.5556$) between the two groups were not significantly different over time (Table 4).

Fig 2 shows the chart depicting CD4 cell count mean measurements by treatment group over the study period. Over the six months study period, there was significant increase in mean CD4 cell counts for MOG while the mean CD4 cell counts for the COG was relatively constant.

An exploratory analysis of the influence of socio-demographic characteristics on the changes in immunological and anthropometric parameters by treatment groups over time was computed. The analysis of the impact of socio-demographic characteristics on the changes in CD4 counts by treatment group over time showed that ethnicity ($p = 0.0491$) and family size

Table 3. Bivariate analysis showing the differences in anthropometric and immunological parameters between the two study groups.

Parameters	Period	MOG (n = 89)	95% Confidence Interval		COG (n = 88)	95% Confidence Interval		F	P-value
		Mean (SD)	Lower	Upper	Mean (SD)	Lower	Upper		
CD4 Counts	Baseline	341.78 (106.06)	319.43	364.12	352.34 (125.99)	325.64	379.03	4.88	0.55
	1st month	363.06 (127.91)	336.11	390.00	361.14 (130.28)	333.53	388.74	0.27	0.92
	2nd month	373.74 (130.79)	346.19	401.29	366.40 (144.47)	335.78	397.01	0.50	0.72
	3rd month	387.29 (134.61)	358.94	415.65	367.51 (142.24)	337.37	397.65	0.98	0.34
	4th month	401.51 (138.50)	372.33	430.68	368.78 (150.89)	336.81	400.76	0.24	0.14
	5th month	414.79 (144.02)	384.45	445.13	375.26 (152.18)	343.01	407.51	0.08	0.08
	6th month	425.75 (153.76)	393.36	458.14	373.44 (157.31)	340.11	406.77	0.02	0.03*
Weight	Baseline	63.83 (14.77)	60.64	66.73	61.94 (12.54)	59.45	64.82	1.62	0.36
	1st month	63.88 (14.89)	60.80	66.89	62.03 (12.92)	59.41	64.88	1.58	0.38
	2nd month	64.26 (14.76)	61.13	67.17	62.44 (13.26)	59.68	65.37	0.75	0.39
	3rd month	64.31 (14.93)	61.16	67.33	62.55 (13.36)	59.83	65.49	0.96	0.41
	4th month	64.47 (14.93)	61.29	67.44	62.73 (13.37)	60.04	65.65	0.92	0.41
	5th month	64.73 (15.00)	61.59	67.76	62.99 (13.38)	60.27	65.91	1.18	0.42
	6th month	64.71 (15.07)	61.54	67.82	63.16 (13.49)	60.48	66.19	1.09	0.47
BMI	Baseline	24.84 (4.76)	23.84	25.85	23.75 (3.82)	22.94	24.56	3.52	0.09
	1st month	24.86 (4.84)	23.84	25.88	23.78 (3.93)	22.94	24.61	3.25	0.10
	2nd month	24.99 (4.82)	23.98	26.01	23.92 (4.02)	23.08	24.78	2.06	0.11
	3rd month	24.99 (4.88)	23.97	26.02	23.96 (4.04)	23.11	24.82	1.76	0.13
	4th month	25.06 (4.87)	24.03	26.08	24.04 (4.10)	23.17	24.91	1.45	0.14
	5th month	25.16 (4.93)	24.12	26.20	24.14 (4.08)	23.27	25.00	1.78	0.14
	6th month	25.16 (4.93)	24.12	26.20	24.19 (4.09)	23.33	25.06	2.40	0.16

* Statistically significant difference between two groups.

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Table 4. Linear mixed effects model framework showing the differences in immunological and anthropometric parameters between the treatment groups overtime.

	Estimates of Fixed Effects[a]						
	Parameter	Estimate	Std. Error	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	Intercept	356.35	13.10	27.20	0.0001	354.29	376.09
CD Counts	MOG	10.33	2.65	3.89	0.0001*	5.12	15.54
	COG	0[b]	0				
	Intercept	-1.07	0.31	-3.47	0.0007	-2.02	-0.99
Viral load	MOG	-0.005	0.09	-0.06	0.9558	-0.19	0.18
	COG	0[b]	0	.	.	.	.
	Intercept	61.92	1.47	42.02	0.0001	61.43	63.65
Weight	MOG	-0.05	0.08	-0.59	0.5556	-0.20	-0.11
	COG	0[b]	0	.	.	.	.
	Intercept	23.73	0.47	50.98	0.0001	23.61	24.32
BMI	MOG	-0.02	0.03	-0.65	0.5145	-0.08	-0.04
	COG	0[b]	0	.	.	.	.

* = statistically significant.

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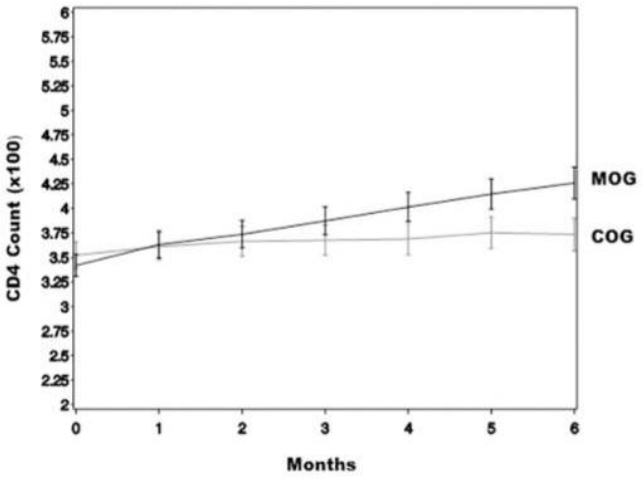

Fig 2. Chart depicting CD4 cell count mean measurements by treatment group over the study period.

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(p = 0.0483) had a significant influence. However, the changes in CD4 counts by treatment group remained significantly different over time (p = 0.0001). None of the socio-demographic characteristics explored significantly influenced the viral load, BMI, and weight overtime. The changes in these parameters between the treatment groups over time were not significant after controlling for socio-demographic characteristics.

Discussion

This study examined the effect of the sixth month's consumption of *Moringa oleifera* leaf powder supplement on the immunological profile (CD4 cell count and viral load) and anthropometric parameters (weight and BMI) of PLHIV that are on ART in Kano State, Nigeria. We studied 200 patients randomly divided into MOG (100 patients) and COG (100 patients). Our sample size was larger than that reported by Tshingani *et al.* (60 patients) in the Democratic Republic of Congo and Ogbuagu *et al.* (40 patients) conducted in Anambra State, Southeast Nigeria [10, 35].

Over the study period, a significant increase was observed in CD4 cell counts of the MOG participants than the COG using the linear mixed effect model. There was no significant difference in viral load between the two study groups. This improvement observed in the CD4 counts in the MOG was influenced by some socio-demographic characteristics of the study participants that include ethnicity and family size. However, the improvement was still detected regardless of their influence. This result suggests that *Moringa oleifera* leaf powder supplementation and ART effectively improved the CD4 cell counts of the study participants.

Conversely, the *Moringa oleifera* leaf powder supplementation intervention was not effective in improving the weight and BMI of the patients when compared to the COG over the study period. Furthermore, none of the socio-demographic characteristics explored was observed to significantly influence any of the anthropometric parameters overtime.

Moringa oleifera nutritional constituents and ART effect could be responsible for the increased CD4 cell counts in the MOG. *Moringa oleifera* leaves (Nigerian ecotype) analysis shows that they are rich sources of vitamins and micro-and macronutrients. Additionally, it contains minerals and trace elements reported to have multiple curative properties, improve

the immune system, and act as strong antioxidants [17–19]. Furthermore, the dried leaves of *Moringa oleifera* have been documented as a good source of polyphenol compounds, such as flavonoids. Flavonoid consumption has been reported to offer body protection against chronic diseases associated with oxidative stress [18].

In addition, ART has proven effective in reducing morbidity and mortality related to HIV infection by reducing HIV viral load. This improved CD4 level is associated with a reduced number of opportunistic infections [36]. Our results agree with a similar study from Eastern Nigeria by Ogbuagu *et al*.. A significant increase in CD4 counts was observed in PLHIV receiving ART and supplemented their local meals with *Moringa oleifera* leaf powder for two months. Local meals were prepared using palm oil. The limited information offered by the study article prevented an in-depth analysis of the results. However, the study demonstrated the potential of *Moringa oleifera* of improving the CD4 count of PLHIV within short period of supplementing it with regular meals prepared using local food items [35]. Palm oil has been documented to contain palmitic acid which is a saturated fatty acid with health benefits [37].

The lack of significant change in BMI observed in MOG in our study could be due to the fact that few participants (n = 5) were underweight with BMI<18.5 kg/m^2 at study inception. This class of people would probably have benefitted more in terms of improvement in BMI from the *Moringa oleifera* leaves supplementation due to the vast amounts of nutrients constituted.

Contrary to our results, Tshingani *et al*. did not report a significant difference in CD4 lymphocyte counts after six months of *Moringa oleifera* leaf powder supplementation between their study groups. This could be due to the small sample size. In addition, the presentation of their intervention in bags of 100 g could have reduced adherence, which resulted in a lack of significant increase in CD4 cell counts [10].

This study's outcomes can be attributed to factors related to the study design. This includes being a double-blinded randomized trial, a larger sample size, and the presentation of the intervention in individual sachets representing daily dose. In addition, the involvement of the virology clinic 'support group' members improved patient monitoring and adherence to the study protocol. Nevertheless, future studies using a more diverse population of PLHIV are recommended.

The *Moringa oleifera* leaf intervention was not effective in decreasing the viral load of HIV-infected individuals accessing ART at the S.S Wali Virology Center. This could be attributed to some of the challenges encountered. The study protocol did not follow the standard operating procedure (SOP) of viral load monitoring conducted at the S. S Wali Virology Center. Viral load was monitored yearly for patients without any medical problems, whereas the study protocol was designed to have a viral load test conducted twice, at baseline and after six months of receiving the intervention. The study plan overcame this challenge. The use of viral load alone without CD4 cell counts to monitor treatment outcomes remains a challenge in resource-limited settings.

Further challenges encountered during the study also include participants' reluctance to keep monthly appointments to the clinic for the study. This is because at the S. S. Wali virology center, ART drugs were dispensed to last for two to three months for patients with stable medical conditions. This challenge was alleviated with the bi-weekly telephone calls performed to monitor the study participants and remind them of their hospital appointments. The stipend given for transport fare after each monthly hospital visit assisted the study participants in keeping their appointments. This is because of the high poverty level in resource-limited settings such as Kano State and the poor socioeconomic status of the study participants. No other incentives or gifts were provided to participants.

Some limitations of this study must be noted. The distinguishable taste of *Moringa oleifera* could be a source of bias. Use of *Moringa oleifera* in capsules could forestall this limitation in future studies. In addition, the inclusion of patients who were only on one ART regimen (tenofovir + lamivudine + efavirenz drug regimen) limits the generalizability of our study findings. Lastly, the short duration of the study and compliance, which was monitored by the self-reporting of the study participants, are further limitations of the study.

Conclusion

This study revealed an association between *Moringa oleifera* leaf nutritional supplementation consumption and increased CD4 cell counts among PLHIV on ART in a limited resource setting. Programs in low-resource settings, such as Nigeria, should consider nutritional supplementation as part of a comprehensive approach to ensure optimal treatment outcomes in PLHIV.

Supporting information

S1 Checklist. CONSORT checklist.
(DOC)

S1 File. Certificate of analysis Moringa oleifera powder.
(PDF)

S2 File. Certificate of analysis Moringa oleifera powder_Minerals.
(PDF)

S3 File. Detailed statistical analysis of data.
(DOCX)

S4 File. Study protocol.
(DOCX)

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References

1. UNAIDS. Press release. New survey results indicate that Nigeria has an HIV prevalence of 1.4%. Available: https://wwwunaidsorg/en/resources/presscentre/pressreleaseandstatementarchive/2019/march/20190314_nigeria. 2019. [Accessed 2021 August 28].
2. Avert. HIV and AIDS in Nigeria. Available: https://wwwavertorg/professionals/hiv-around-world/sub-saharan-africa/nigeria. 2019. [Accessed 2021 August 28].
3. UNAIDS. UNAIDS Data. Available: https://wwwunaidsorg/sites/default/files/media_asset/unaids-data-2018_enpdf. 2018. [Accessed 2021 August 28].
4. UNAIDS. Fact Sheet–Global AIDS update UNAIDS. Available: https://wwwunaidsorg/sites/default/files/media_asset/UNAIDS_FactSheet_enpdf. 2019. [Accessed 2021 August 28].
5. Toh H-S, Yang C-T, Yang K-L, Ku H-C, Liao C-T, Kuo S, et al. Reduced economic burden of AIDS-defining illnesses associated with adherence to antiretroviral therapy. Int J Infec Dis 2020; 91:44–9. https://doi.org/10.1016/j.ijid.2019.11.010 PMID: 31740407
6. Feleke DG, Yemanebrhane N, Gebretsadik D. Nutritional Status and CD4 Cell Counts in HIV/AIDS Patients under Highly Active Antiretroviral Therapy in Addis Ababa, Ethiopia. J AIDS Clin Res. 2017; 8 (4):1–5. https://doi.org/10.4172/2155-6113.1000688
7. Guerra EML, Porbén SS. T-Cell Number, Nutritional Status, and HIV: The Cuban Experience in the Provision of Food and Nutrition Care to People with HIV/AIDS. Health HIV Infected People. 2015;2 Chapter 21:367–87.
8. Colecraft E. HIV/AIDS: nutritional implications and impact on human development. Proc Nutr Soc. 2008; 67:109–13. https://doi.org/10.1017/S0029665108006095 PMID: 18234139
9. Nnyepi MS. The risk of developing malnutrition in people living with HIV/AIDS: Observations from six support groups in Botswana S Afr J Clin Nutr. 2009; 22(2):89–93. https://doi.org/10.1080/16070658.2009.1734224
10. Tshingani K, Donnen P, Mukumbi H, Duez P, Dramaix-Wilmet M. Impact of Moringa oleifera lam. Leaf powder supplementation versus nutritional counseling on the body mass index and immune response of HIV patients on antiretroviral therapy: a single-blind randomized control trial. BMC Complement Altern Med. 2017; 17(420):1–13. https://doi.org/10.1186/s12906-017-1920-z PMID: 28830411
11. Tesfaw A, Jara D, Temesgen H. Dietary Diversity and Associated Factors among HIV Positive Adult Patients Attending Public Health Facilities in Motta Town, East Gojjam Zone, Northwest Ethiopia, 2017. Adv Public Health. 2018; 2018:1–8. https://doi.org/org/10.1155/2018/6135482
12. Akwiwu UN. Nutritional Intake of People Living with HIV/AIDS (PLWHA) in Rural Communities of Benue State, Nigeria. J Agric Food Sci. 2018; 16(2):88–99. https://doi.org/10.4314/jafs.v16i2.8
13. Obiako RO, Akase IE, Hassan A, Babadoko A, Musa BOP, Balogun Y, et al. Malnutrition in HIV: Are patients in early stages of disease and with high CD4 counts spared? Sub-Saharan Afr J Med 2018; 6 (1):43–8.
14. UNICEF. Nutrition. Available: https://wwwuniceforg/nigeria/nutrition. 2018. [Accessed 2021 August 28].
15. Pandey A, Pandey RD, Tripathi P, Gupta PP, Haider J, Bhatt S, et al. Moringa Oleifera Lam. (Sahijan) —A Plant with a Plethora of Diverse Therapeutic Benefits: An Updated Retrospection. Med Aromat Plants 2012; 1(1):1–8. https://doi.org/10.4172/2167-0412.1000101
16. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: A Food Plant with Multiple Medicinal Uses. Phytother Res. 2007; 21:17–25. https://doi.org/10.1002/ptr.2023 PMID: 17089328

17. Dhakad AK, Ikram M, Sharma S, Khan S, Pandey VV, Singh A. Biological, nutritional, and therapeutic significance of Moringa oleifera Lam. Phytother Res. 2019; 33:2870–903 https://doi.org/10.1002/ptr.6475 PMID: 31453658

18. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. Antioxidants 2017; 6(91):1–13. https://doi.org/10.3390/antiox6040091 PMID: 29144438

19. Thurber MD, Fahey JW. Adoption of Moringa oleifera to combat under-nutrition viewed through the lens of the "Diffusion of Innovations" theory. Ecol Food Nutr. 2009; 48(3):212–25. https://doi.org/10.1080/03670240902794598 PMID: 20161339

20. RMRDC. Moringa oleifera- A National Crop forEconomic Growth and Development. Proceedings of National Summit on Moringa Development. Available: https://wwwacademiaedu/6686903/RAW_MATERIALS_RESEARCH_AND_DEVELOPMENT_COUNCIL_Moringa_oleifera_A_National_Crop_for_Economic_Growth_and_Development_PROCEEDINGS_OF_THE_FIRST_NATIONAL_SUMMIT_ON_MORINGA_DEVELOPMENT. 2010. [Accessed 2021 August 28].

21. Monera TG, Wolfe AR, Maponga CC, Benet LZ, Guglielmo J. Moringa oleifera leaf extracts inhibit 6β-hydroxylation of testosterone by CYP3A4. J Infect Dev Ctries. 2008; 2(5):379–83. https://doi.org/10.3855/jidc.201 PMID: 19745507

22. Cohen J. Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, N J: Lawrence Earlbaum Associates. 1988.

23. Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav. 2009; 41:1149–60. https://doi.org/10.3758/BRM.41.4.1149 PMID: 19897823

24. Mishra SP, Singh P, Singh S. Processing of Moringa oleifera Leaves for Human Consumption. Bull Env Pharmacol Life Sci. 2012; 2(1):28–31.

25. Merck. Particle Size Conversion Table. Available: https://wwwsigmaaldrichcom/chemistry/stockroom-reagents/learning-center/technical-library/particle-size-conversionhtml. 2021. [Accessed 2021 August 28].

26. Bahadoran Z, Tohidi M, Nazeri P, Mehran M, Azizi F, Mirmiran P. Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. Int J Food Sci Nutr. 2012; 63(7): 767–71. https://doi.org/10.3109/09637486.2012.665043 PMID: 22537070

27. Idohou-Dossou N, Diouf A, Gueye AL, Guiro AT, Wade S. Impact of daily consumption of Moringa (Moringa Oleifera) dry leaf powder on iron status of senegalese lactating women. AJFAND. 2011; 11 (4):4985–99.

28. Tété–Bénissan A, Quashie MIA, Lawson–Evi K, Gnandi K, Kokou K, Gbéassor M. Influence of Moringa oleifera leaves on atherogenic lipids and glycaemia evolution in HIV-infected and uninfected malnourished patients. J Appl Biosci. 2013; 62:4610–9. https://doi.org/10.314/jab.v62i0.86072

29. SOP Nigeria. ENUMERATION OF CD4+ T LYMPHOCYTE. STANDARD OPERATING PROCEDURE for Nigeria PreP Study. Available: https://wwwprepwatchorg/wp-content/uploads/2016/09/NNACA_SOP_304docx 2015;LP-304. [Accessed 2021 August 28].

30. Roche. COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 20 manuals. 2007; Available: https://www.fda.gov/media/73824/download. [Accessed 2021 August 28].

31. SOP Nigeria. HIV -1 VIRAL LOAD ASSAY. STANDARD OPERATING PROCEDURE for Nigeria PreP Study. Available: https://wwwprepwatchorg/wp-content/uploads/2016/09/NNACA_SOP_305docx 2015;LP-305. [Accessed 2021 August 28].

32. WMA. World Medical Association Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjectss. Clinical Review & Education—Special Communication. Available: https://wwwwmanet/wp-content/uploads/2016/11/DoH-Oct2013-JAMApdf. 2013;310(20):2191–4. [Accessed 2021 August 28].

33. SANAS. South African National Accreditation System (SANAS) Available: https://wwwsanascoza/. 2017. [Accessed 2021 August 28].

34. ASPIRATA. Nutritional Analysis of Moringa oleifera leaves powder. ASPIRATA Food and Beverage Laboratory. Available: http://aspiratacoza/. 2017. [Accessed 2021 August 28].

35. Ogbuagu EN, Ufearo S, Ogbuagu CN, Okonkwo R. CD4 pattern in HIV positive patients on HAART exposed to moringa oleifera leaf powder in south east Nigeria. Poster session 17th International Congress on Infectious Diseases / Int J Infect Dis 2016; 455:1–477. https://doi.org/10.1016/j.ijid.2016.02.595

36. Contri PV, Berchielli EM, Tremeschin MH, Negrini BV, Saloma¯o RG, Monteiro JP. Nutritional status and lipid profile of HIV-positive children and adolescents using antiretroviral therapy. Clinics. 2011; 66 (6):997–1002. https://doi.org/10.1590/s1807-59322011000600013 PMID: 21808865

37. Mancini A, Imperlini E, Nigro E, Montagnese C, Daniele A, Orrù S, et al. Biological and Nutritional Properties of Palm Oil and Palmitic Acid: Effects on Health. Molecules 2015; 20:17339–61. https://doi.org/10.3390/molecules200917339 PMID: 26393565

CHAPTER FIVE

IMPACT OF *MORINGA OLEIFERA* LEAVES SUPPLEMENTATION ON QUALITY OF LIFE OF PEOPLE LIVING WITH HIV: A DOUBLE BLIND RANDOMIZED CONTROLLED TRIAL

Chapter 4 of this thesis examined the effect of *Moringa oleifera* Lam. leaves supplementation on the immune status and anthropometric parameters of adult HIV patients on ART in a resource-constrain setting.

The aim of every intervention is to improve the quality of life (QoL). Therefore, chapter 5 assessed the QoL of the patients before and after taking the intervention. This is vital in understanding the impact of *Moringa oleifera Lam.* leaves supplementation on the various QoL domains of PLHIV.

The chapter is presented as a published article.

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Impact of *Moringa Oleifera* leaves supplementation on quality of life of people living with HIV: A double-blind randomized controlled trial

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Abstract

Purpose Advances in antiretroviral therapy (ART) and increased interest on nutritional interventions have led to improvements in life expectancy of people living with HIV (PLHIV). These have led to greater emphasis on their quality of life (QoL). This study was aimed at exploring the effects of *Moringa oleifera* leaves supplementation on QoL of HIV-positive adults in Nigeria.

Method A double-blind randomized controlled trial was conducted to determine the six months' impact of consuming *Moringa oleifera* leaves powder as a nutritional supplement on the different domains of QoL of PLHIV that are on ART. Two hundred consented patients were randomly allocated to either *Moringa Oleifera* group (MOG) or control group (COG). The WHOQOL-HIV-BREF was used to assess QoL at baseline and at six-month follow-up.

Results One hundred and seventy seven patients completed the study. At six-month follow-up, a significant ($p < 0.05$) increase in the mean scores of all the six domains of QoL was observed in the MOG. However, in the COG, a significant increase was observed in the social relationship, environment, and spirituality/religion/personal beliefs domains. The comparison between the MOG and COG at 6 months' follow-up showed a significant mean score difference in the MOG in the physical, psychological, level of independence, and social relationships domains of QoL.

Conclusion This study shows that supplementation with *Moringa oleifera* leaves for PLHIV that are on ART improves the QoL domains of physical, psychological, level of independence, and social relationships.

Clinical Trial Registry registration number: PACTR201811722056449.

Keywords *Moringa oleifera* · Quality of life · HIV · Antiretroviral therapy · Nutritional supplement

Introduction

Approximately 74.9 million people are living with Human Immunodeficiency Virus (HIV) globally [1]. Nigeria has the second largest HIV epidemic in the world with 130,000 new infections and 53,000 AIDS-related deaths in 2018 [2].

People infected with HIV and AIDS have reduced dietary intake which is often a result of gastrointestinal symptoms like change in taste, smell, nausea, and vomiting [3]. The side effects of antiretroviral therapy (ART) together with the pill burden are all related with a reduced quality of life (QoL) in people living with HIV (PLHIV) [4].

In Nigeria, two million children and 7 per cent of women of childbearing age suffer from severe acute malnutrition [5].

Advances in ART and increased interest on nutritional interventions of PLHIV have led to improvements in their life expectancy. These have led to greater emphasis on the QoL of PLHIV [6].

The World Health Organisation Quality of Life-HIV Brief (WHOQOL-HIV BREF) is a validated tool that has been used in studies conducted in various countries including Nigeria [7, 8]. The assessment of QoL in different domains permits examining the dimensions in which treatments are effective, potentially helping to make decisions about the most appropriate therapeutic measures with the possibility to reduce health care costs [9].

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Studies have shown that PLHIV use complementary and alternative or traditional medicines because they get a feeling of control over their lives and illness [10].

Moringa oleifera plant also known as the 'miracle tree' has been documented to have various pharmacological activities [11–14]. *Moringa oleifera* leaves may be a good supplement as they contain both macro- and micronutrients [15] and natural antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids [16]. Moreover, various studies have established evidence of the use of *Moringa oleifera* as a nutritional supplementation among HIV-positive patients who have commenced ART [17, 18].

This is a secondary outcome in the clinical trial conducted to determine the impact of *Moringa oleifera* supplementation on the nutritional and immunological development of adult HIV patients that are on ART which was reported elsewhere.

Hence, in this study, we examine the impact of six months of *Moringa oleifera* leaves supplementation on the QoL of HIV-positive adults that are on ART in comparison with a matched control group at Aminu Kano Teaching Hospital, Kano State, Nigeria.

Methods and materials

Study setting

The setting of the study was the S. S Wali Virology centre at the Aminu Kano Teaching Hospital, Kano State (AKTH) Nigeria.

Type of study

The study was a double-blinded randomized control trial conducted from December 2017 to November 2018.

Ethical consideration

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

Participants were informed about the aims of the study, and study protocol was explained to them. The informed consent form was translated into Hausa language by an independent translator for those participants that cannot read in English. This study was in compliance with the principles included in the Declaration of Helsinki [19]. All the study participants provided written informed consent or thumbprint if unable to write. They were also informed of their right to withdraw from the study at any time.

Participants

HIV-positive patients aged 18 years and above who were registered and are on ART for at least 3 months at the centre were invited to participate.

Participants were recruited using convenience sampling technique. A total of 410 participants were screened and assessed for eligibility criteria. Among the 410 participants, 210 were excluded [204 did not meet the inclusion criteria for the study and 6 participants declined to participate (see flow chart)]. Finally 200 patients were selected to participate in the study.

Sample size

The sample size of participants needed was determined by the requirements of the primary outcome where a sample of 86 patients in each arm was calculated to be able to detect a medium effect size (Cohen's $d = 0.5$) [20] or 0.5 standard deviation in mean weight or CD4 by RCT arm with 90% power (1-β [type 2 error probability]) and 95% confidence (or 5% α error probability [type 1]) assuming a balanced 1:1 study design, giving a total sample size of 172 patients. This sample size was increased to 200 to give room for attrition. The final sample size was 200 (100 patients in each arm). The sample size was calculated using G*Power version 3.1.9.2 [21].

Randomization

Block randomization with a block size of ten was used to balance the groups throughout the enrolment period. PASS 12.0 software was used to generate the randomization sequence using Wei's Urn algorithm and was kept by an independent person that was not part of the research team. As they fulfilled the inclusion criteria and consented, a number was assigned to the patient and randomly allocated to either *Moringa Oleifera* group (MOG) or control group (COG) by an independent investigator. All research team members were blinded to the allocation of study participants to the respective study groups.

Criteria for inclusion Patients who were HIV sero positive; 18 years or older; with CD4 counts ≤ 500 cells/mm^3; on ART for at least three months; on Tenofovir + Lamivudine + Efavirenz ART drug combination; and patients who gave consent and complied with study protocol were included in the study.

Criteria for exclusion Patients were excluded from participation in the study if they had known allergy or intolerance to *Moringa oleifera* or placebo (cornstarch powder); pregnant women; CD4 counts > 500 cells/mm^3; active opportunistic infection; patients that took micronutrient or natural health product supplements within 30 days of screening and those patients that lived outside Kano State where the study was conducted were excluded for ease of monitoring.

Intervention

Moringa oleifera nutritional supplement

The fresh *Moringa oleifera* leaves were obtained from Prime Global Agricultural Industries Limited, Kano State, Nigeria. It was processed, manufactured, and packaged at Dala Foods Nigeria Limited Kano State, Nigeria.

Placebo

The placebo was obtained by colouring corn starch powder with chlorophyll [22]. It was also processed, manufactured and packaged at Dala Foods Nigeria Limited Kano State, Nigeria.

Both the *Moringa oleifera* leaf powder and the placebo were identical in presentation. They were both packaged to be indistinguishable and sealed in sachets of 15 g representing a daily dose. Thirty (30) individual sachets were further packaged and sealed in a leaf green bag to represent one month's dose. The bag was labelled with the study code, visit number, subject number, and instructions on how to take it. It was stored in a dry place away from heat and humidity.

Patients were randomly assigned and were either given *Moringa oleifera* or the placebo. They were instructed to supplement their daily diet by dividing each sachet three times daily (5 g) [23, 24].

All the above procedures were performed by independent persons not part of the research.

Compliance was monitored by biweekly telephone calls to the patients. Also patients were questioned during their monthly visits to evaluate adherence.

Their diet was monitored by taking their diet history during the biweekly phone calls and on their monthly visits. The participants were told to maintain their regular diet and to avoid consuming *Moringa oleifera* in any form from other sources during the study duration.

Assessments and tools

Study outcome measures

WHO quality of life-HIV bref (WHOQOL- HIV BREF)

The WHOQOL-HIV BREF is considered to be a valid and reliable tool to assess different domains of QoL among PLHIV. It consists of parts A and B. Part A assessed demographic characteristics of the participant that included age, gender, level of education, marital status, health status, HIV serostatus, year first tested positive, expected year the participant was infected, and how the participant thought he/she was infected. Part B assessed participants' QoL and contained 31 items with each item using a 5-point Likert scale. A score of 1 indicated a low mark (negative perceptions), and 5 indicated a high mark (positive perceptions). Domain scores were calculated with a range from 4 to 20 where higher scores indicate better QoL. The six domains of QoL are as follows: physical health, psychological health, level of independence, social relationships, environment, and spirituality/religion/personal beliefs. The physical health domain measured pain and discomfort, energy and fatigue, and sleep and rest of the participant. The psychological health domain measured positive feelings, thinking, learning, memory and concentration, self-esteem, body image and appearance, and negative feelings of the participant. The level of Independence domain measured mobility, daily life activities, dependence on medications or treatments, and work capacity of the participant. The social relationships domain measured personal relationships, social support, and sexual activity of the participant. The environment domain measured physical safety and security, home environment, financial resources, health and social care, accessibility and quality, opportunities for acquiring new information and skills, participation in and opportunities for recreation and leisure activities, and physical environment (pollution, noise, traffic, climate, and transport) of the participant. The spirituality/religion/personal beliefs domain measured forgiveness and blame, concerns about the future, and death and dying of the participant [25].

At the beginning of the study, a questionnaire to determine the socio-demographic information and diet history of each study participant was administered by a trained nurse at the virology clinic. Anthropometric measurements were also taken.

The WHOQOL-HIV BREF instrument was administered by a trained nurse and a trained research assistant under supervision of the principal investigator (PI). Both groups administered the WHOQOL-HIV BREF questionnaire at baseline and after six months of using the study

interventions while anthropometric measurements were taken at baseline and at each monthly visits for 6 months.

Data analysis

The data were imputed into Microsoft excel and exported into SPSS version 26.0 for analysis. Data were subjected to the Shapiro–Wilk normality test and all variables were found to be normally distributed. Descriptive statistics were presented as means and standard deviations. Paired sample t-tests and independent sample t-tests were used to compare the characteristics within-group and between-groups, respectively. P values (significance) of less than 0.05 indicated a statistically significant difference. Effect sizes (d) were calculated using Cohen's d [20] formula with 0.2 indicating small effect; 0.5 moderate effect; and values above 0.8 as large effect.

Results

Participants flow

Figure 1 shows the study flow chart. Four hundred and ten (410) patients were screened and assessed for eligibility. Two hundred and ten (210) were excluded [204 did not meet the inclusion criteria for the study and 6 refused to participate]. Finally, two hundred (200) patients were randomized into two groups. One hundred (100) were randomly selected and allocated to the group receiving *Moringa oleifera* supplementation (MOG) and 100 patients were randomly allocated to the group receiving placebo (COG). In the MOG, five (5) patients were lost to follow up, 3 patients did not respond to their hospital appointment, and 3 discontinued taking the intervention. In the COG, five (5) patients were lost to follow up, 4 patients did not respond to their hospital appointment, and 3 discontinued taking the intervention. One hundred and seventy seven patients (88.5%) completed the 6 months study and were analysed with 89 in the MOG and 88 in COG (Fig. 1).

Table 1 describes the characteristics of participants in both groups. The participants in the MOG (n = 89) were similar to those in the COG (n = 88) in terms of socio-demographic and socioeconomic characteristics.

Females were predominant in both groups [MOG = 70 (78.7%); COG = 67(76.1%)]. The mean age (SD) of participants in both study groups was [MOG = 34.5 (8.7); COG = 35.8 (9.2) years]. The majority were married [MOG = 42 (47.2%); COG = (38 (43.2%)]. Islam was the predominant religion of participants in both groups [MOG = 64 (71.9%); COG = (66 (75%)] with more than half of participants belonging to Hausa/Fulani ethnicity [MOG = 55 (61.8%); COG = 47 (53.4%)]. A few of the participants were

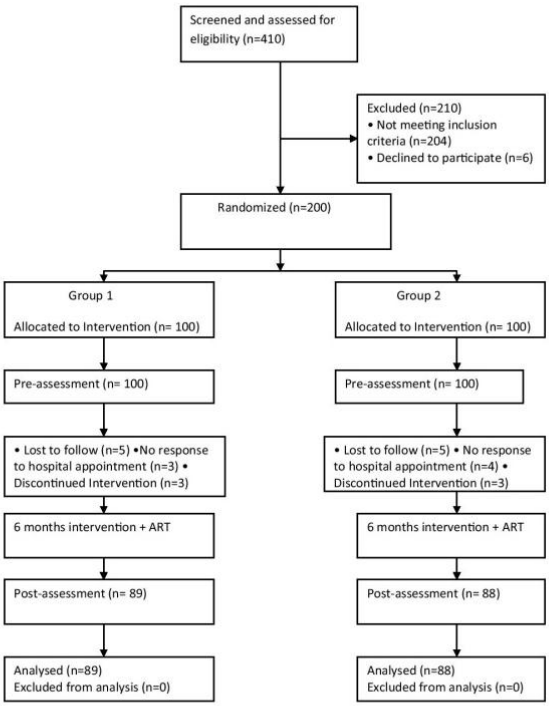

Fig. 1 Flow Chart of participants

without any form of education in both groups [MOG = 15 (16.9%); COG = 16 (18.2%)]. The majority of the participants in both groups earned a monthly income below the minimum wage which is ₦30,000 ($78.23) [MOG = 67 (75.3%); COG = 66 (75%)] (Table 1).

Compliance was high as the intervention was well accepted but at the beginning of taking the intervention, five cases of diarrhoea were recorded from both study groups.

Table 2 shows the QoL domains of MOG and COG at baseline. No statistically significant difference was observed in the mean of the QoL domain scores between the MOG and COG at baseline (Table 2).

QoL comparison within the *Moringa oleifera* group (MOG)

The first section of Table 3 summarizes the comparison between baseline and 6 month QoL domains for the MOG. There was a significant ($p < 0.05$) increase with large effect sizes in all six QoL domains at 6 months [(physical health d = 1.44); (psychological d = 2.05); (level of independence d = 1.84); (social relationship d = 1.01); (environment d = 1.18); (spirituality/religion/personal beliefs d = 0.94)] (Table 3).

Table 1 Socio-demographic characteristics of participants

Variables	MOG (%) (N = 89)	COG (%) (N = 88)
Age (years)		
Mean (SD)	34.5 (8.7)	35.8 (9.2)
Height (cm)	159.42 (8.65)	160.49 (9.67)
Weight (kg)	63.83 (14.77)	64.94 (12.54)
Gender		
Males	19 (21.3)	21 (23.9)
Female	70 (78.7)	67 (76.1)
Marital Status		
Married	42 (47.2)	38 (43.2)
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)
Christianity	25 (28.1)	22 (25.0)
Ethnicity		
Hausa/Fulani	55 (61.8)	47 (53.4)
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)
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)
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 Members		
2–5	38 (42.7)	32 (36.4)
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)
< 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)

QoL comparison within the control group (COG)

The second section of Table 3 summarizes the comparison between baseline and 6-month QoL domains for the COG.

Table 2 QOL domains of MOG and COG at baseline

Variables	MOG ($n = 89$) Mean ± SD	COG ($n = 88$) Mean ± SD
Physical QoL domain	12.19 ± 1.87	11.98 ± 2.03
Psychological QoL domain	11.83 ± 1.56	11.89 ± 1.46
Level of independence QoL domain	11.97 ± 2.08	11.85 ± 2.07
Social relationship QoL domain	12.00 ± 1.64	12.03 ± 1.84
Environment QoL domain	10.73 ± 1.29	10.70 ± 1.34
Spirituality/religion/personal believes QoL domain	12.21 ± 1.83	12.36 ± 1.89

There was an increase in the QoL in all the domains. However, this was found to be statistically significant only in the social relationship, environment, and spirituality/religion/personal beliefs domains ($p < 0.05$). There was a significant difference ($p < 0.05$) with moderate effect size (d = 0.75) observed in the social relationship domain while large effect sizes were observed in the environment (d = 1.12) and spirituality/religion/personal beliefs (d = 0.85) domains, respectively (Table 3).

QoL comparison between the two groups (MOG*COG)

The last section of Table 3 presents the comparison of QoL domains between the two groups. At 6 months, there was statistically significant ($p < 0.05$) mean differences with large effect sizes between MOG and COG in all the QoL domains except the environment and spirituality/religion/personal beliefs domains [(physical health d = 2.39); (psychological d = 2.88); (level of independence d = 2.38); (social relationship d = 0.82)] (Table 3).

Discussion

Despite the widespread use of *Moringa oleifera* leaves by PLHIV, to our knowledge, our study is the first in Nigeria to examine the effect of *Moringa oleifera* leaves supplementation on the QoL of PLHIV that are on ART.

The measurements at baseline were similar between both groups in terms of socio-demographic characteristics and QoL domains.

At six months follow-up, significant differences were observed. In the MOG, a significant increase in the means of all six domains of QoL was observed. The magnitude of the increase (effect sizes) was large for all the six domains. This significant increase observed in all domains of the MOG could be due to the effect of the various bioactive substances and antioxidants present in *Moringa oleifera* leaves [14, 26] together with the effect of ART. Meanwhile in the COG, a

Table 3 Comparison of QoL domains within the groups from baseline to 6 months and between the two groups at 6 months

Domains	MOG				COG				MOG*COG	
	Baseline Mean ± SD	6 month Mean ± SD	*P*-value	Effect Size (d)	Baseline Mean ± SD	6 month Mean ± SD	*P*-value	Effect Size (d)	*P*-value (Between group comparison)	Effect Size (d)
Physical	12.19 ± 1.87	15.84 ± 1.59	< 0.0001*	1.44	11.98 ± 2.03	12.05 ± 1.57	0.788	N/A	< 0.0001*	2.39
Psychological	11.83 ± 1.56	15.82 ± 1.24	< 0.0001*	2.05	11.89 ± 1.46	13.04 ± 1.02	0.320	N/A	< 0.0001*	2.88
Level of independence	11.97 ± 2.08	16.31 ± 1.46	< 0.0001*	1.84	11.85 ± 2.07	12.33 ± 1.94	0.079	N/A	< 0.0001*	2.38
Social relationship	12.00 ± 1.64	14.78 ± 2.02	< 0.0001*	1.01	12.03 ± 1.83	13.30 ± 1.50	< 0.0001*	0.75	< 0.0001*	0.82
Environment	10.72 ± 1.28	12.24 ± 1.18	< 0.0001*	1.18	10.70 ± 1.34	12.27 ± 1.18	< 0.0001*	1.12	0.887	N/A
Spirituality/religion/personal believes	12.21 ± 1.82	14.43 ± 1.58	< 0.0001*	0.94	12.36 ± 1.88	14.51 ± 1.52	< 0.0001*	0.85	0.719	N/A

* = statistically significant

statistically significant increase was found only in the social relationships, environment, and spirituality/religion/personal beliefs domains. The magnitude of the increase was moderate for the social relationships while the environment and spirituality/religion/personal beliefs domains demonstrated large effects. The significant increase seen in some QoL domains in the COG could be attributed to the effect of ART [27–29].

When compared between the MOG and COG groups at 6-month follow-up, the increases observed in the physical, psychological, level of independence, and social relationships domains of the MOG were significant. The magnitude of the increase in the domains was very large.

In the literature, *Moringa oleifera* has been documented to have different therapeutic purposes [26]. The pharmacological activities could be attributed to the high amount of bioactive compounds present [14]. The compounds contained in *Moringa oleifera* leaves include a wide range of vitamin, amino acids, polyphenols, phenolic acids, flavonoids, and saponins [14, 16].

The improvement in the physical health and the level of independence domains in this study could be explained by feelings of improved energy. Some study participants expressed their perceived benefit of consuming *Moringa oleifera* leaves through having improved strength and energy, increased mobility with increases in activities of daily living and work capacity. They claimed it enabled them to perform some house chores easily like clothes washing. Some claimed their ability to walk for longer distance without getting tired easily. This claim could be consistent with the study conducted by Gopi et al., which reported the use of a sport supplement formulation that consists of the extracts of *Moringa oleifera* leaves (50%), black ginger (15%), and pomegranate peel (35%) to evaluate its efficacy for physical endurance in healthy adults. The study concluded that the formulation increases the performance and also reduces oxidative stress to the muscles and tissues during exercise [30]. Although their study included healthy patients during exercise while our study included PLHIV who are on ART. This could limit a conclusive argument on the impact of *Moringa oleifera* on our study participants as claimed.

The beneficial impact of *Moringa oleifera* on the cognitive aspect as demonstrated by the increase in the psychological domain of our patients has been reported by other authors [31–33]. The extracts of *Moringa oleifera* leaves improved the spatial memory and neurodegeneration in male wistar rats when administered orally for 7 days [31]. This suggests the potential of *Moringa oleifera* leaves extract as a cognitive enhancer and neuroprotectant.

The World Health Organisation now recognizes social relationships as an important social determinant of health throughout our lives [34]. Social support plays a valuable role in health [35]. Our study observed a statistically

significant increase in the social relationship domain of the MOG. An improved social interaction could decrease depression and stigmatization which are the most common mental health concerns among PLHIV [35].

Sexual health and intimate partner relationships in PLHIV are an important factor for QoL and emotional well-being [36]. An improvement in social relationships of participants in the MOG could be due to improved sexual desire and thus improvement with the sexual activity of our study participants. This is because the combination of *Moringa oleifera*, Bryophyllum pinnatum, and vitamin C has been reported to increase libido in men [37]. This could be attributed to the antioxidant properties of *Moringa oleifera* and Bryophyllum pinnatum [37]. Furthermore, the use of *Moringa oleifera* as a potential aphrodisiac has been documented [38].

At six months' follow-up, there was no significant difference observed in the environment domain when compared between MOG and COG. This could be due to the poor financial status of our study participants in both groups. The majority of the participants in both groups earned a monthly income below the minimum wage which is ₦30,000 ($ 78). This is most likely responsible for the poor living conditions in their physical environments. It could also be an indication of discrimination experienced due to their HIV sero-positive status [25].

Lastly, an increase in the spirituality/religion/personal beliefs domain was observed at the 6th month in both study groups. This result suggests that the participants in both groups have a spiritual life and consider their spiritual health as important [39]. The majority of PLHIV belonged to an organized religion and probably used their religion to cope with their illness [40]. Generally, in a country like Nigeria, there is a perception that when people are confronted with issues that are beyond them, they tend to be more spiritual and religious [41]. Also in the northern part of Nigeria where the study was conducted, there is a strong belief that life and death are from God. A review by Mueller et al. reported that during illness, people may have greater spiritual needs. Furthermore, the review showed that spirituality and taking part in religious practices are associated with positive health outcomes which includes mortality, physical and mental illness, health-related QoL, and ability to cope with illness (including terminal illness) [39].

Limitation of the study

Our study has some limitations. As the WHOQOL-HIV BREF Questionnaire measures QoL within two weeks prior to the interview, the information provided by the participants may be influenced by recall bias. Also additional assessment of QoL at the third month of the study would have provided better understanding of the results. Lastly, the long-term effect of the intervention was not assessed.

Conclusion

This study shows that *Moringa oleifera* leaves powder increased the physical, psychological, level of independence, and social relationship domains of QoL as compared to the placebo. Nutritional supplementation with *Moringa oleifera* leaves powder has the potential to improve the QoL of PLHIV who are on ART. In conclusion, we recommend that *Moringa oleifera* leaves can be used as an available local solution to improve treatment outcomes by improving the nutritional intake and QoL of PLHIV in resource-constrained settings.

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Data availability Data are available only on reasonable request.

Declarations

Conflict of interest The authors have no conflict of interest.

References

1. UNAIDS. Fact Sheet – Global AIDS update UNAIDS https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_enpdf. 2019. Accessed 17 March 2021.
2. UNAIDS. Press release. New survey results indicate that Nigeria has an HIV prevalence of 1.4%. https://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2019/march/20190314_nigeria. 2019. Accessed 17 March 2021.
3. Folasire, O. F., Folasire, A. M., & Sanusi, R. A. (2015). Measures of Nutritional Status and Quality of Life in Adult People Living with HIV/AIDS at a Tertiary Hospital in Nigeria. *Food and Nutrition Sciences, 6*, 412–420.
4. Bello, S. I., & Bello, I. K. (2013). Quality of Life of Hiv/Aids Patients in a Secondary Health Care Facility, Ilorin. *Nigeria. Proc (Bayl Univ Med Cent), 26*(2), 116–119.
5. UNICEF. Nutrition. https://www.unicef.org/nigeria/nutrition. 2018. Accessed 17 March 2021.
6. Hsiung, P., Fang, C., Wu, C., Sheng, W., Chen, S., Wang, J., et al. (2011). Validation of the WHOQOL-HIV BREF among HIV infected patients in Taiwan. *AIDS Care: Psychological and Socio-medical Aspects of AIDS/HIV., 23*(8), 1035–1042.
7. Peltzer, K., Preez, N. F., Ramlagan, S., & Anderson, J. (2010). Antiretroviral treatment adherence among HIV patients in KwaZulu-Natal. *South Africa. BMC Public Health., 10*(111), 1–10.

8. Olisah, V. O., Baiyewu, O., & Sheikh, T. L. (2011). Depression underdiagnosis and the effects on quality of life in outpatients with HIV at a Nigerian university teaching hospital. *African Journal of AIDS Research, 10*(3), 247–254.
9. da Silva, J., Bunn, K., Bertoni, R. F., Neves, O. A., & Traebert, J. (2013). Quality of life of people living with HIV. *AIDS Care, 25*(1), 71–76.
10. Bepe, N., Madanhi, N., Mudzviti, T., Gavi, S., Maponga, C. C., & Morse, G. D. (2011). The impact of herbal remedies on adverse effects and quality of life in HIV-infected individuals on antiretroviral therapy. *Journal of Infection in Developing Countries, 5*(1), 48–53.
11. Falowo, A. B., Mukumbo, F. E., Idamokoro, E. M., Lorenzo, J. M., Afolayan, A. J., & Muchenje, V. (2018). Multi-functional application of Moringa oleifera Lam. in nutrition and animal food products: A review. *Food Res Int, 106*, 317–34.
12. Kou, X., Li, B., Olayanju, J. B., Drake, J. M., & Chen, N. (2018). Nutraceutical or Pharmacological Potential of Moringa oleifera Lam. *Nutrients, 10*(343), 1–12.
13. Alegbeleye, O. O. (2018). How Functional Is Moringa oleifera? A Review of Its Nutritive, Medicinal, and Socioeconomic Potential. *Food and Nutrition Bulletin, 39*(1), 149–170.
14. Vergara-Jimenez, M., Almatrafi, M. M., & Fernandez, M. L. (2017). Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. *Antioxidants, 6*(91), 1–13.
15. Oduro, I., Ellis, W. O., & Owusu, D. (2008). Nutritional potential of two leafy vegetables: Moringa oleifera and Ipomoea batatas leaves. *Scientific Res Essay., 3*(2), 57–60.
16. Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). Moringa oleifera: A Food Plant with Multiple Medicinal Uses. *Phytotherapy Research, 21*, 17–25.
17. James, A., & Zikankuba, V. (2017). Moringa oleifera a potential tree for nutrition security in sub-Sahara Africa. *Am J Res Commun., 5*(4), 1–14.
18. Monera, T. G., & Maponga, C. C. (2012). Prevalence and patterns of Moringa oleifera use among HIV positive patients in Zimbabwe: a cross-sectional survey. *J Public Health Afr., 3*(e6), 22–24.
19. WMA. (2013). World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjectss. *Clinical Review & Education—Special Communication., 310*(20), 2191–4.
20. Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. (2nd ed.). Hillsdale, N J: Lawrence Earlbaum Associates.
21. Faul, F., Erdfelder, E., Buchner, A., & Lang, A.-G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav., 41*, 1149–60.
22. Bahadoran, Z., Tohidi, M., Nazeri, P., Mehran, M., Azizi, F., & Mirmiran, P. (2012). Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. *International Journal of Food Sciences and Nutrition, 63*(7), 767–771.
23. Tété-Bénissan, A., Quashie, M. I. A., Lawson-Evi, K., Gnandi, K., Kokou, K., & Gbéassor, M. (2013). Influence of Moringa oleifera leaves on atherogenic lipids and glycaemia evolution in HIV-infected and uninfected malnourished patients. *J Appl Biosci., 62*, 4610–4619.
24. Idohou-Dossou, N., Diouf, A., Gueye, A. L., Guiro, A. T., & Wade, S. (2011). Impact of daily consumption of Moringa (Moringa Oleifera) dry leaf powder on iron status of senegalese lactating women. *AJFAND*. https://doi.org/10.4314/ajfand.v11i4.69176.
25. Fatiregun, A. A., Mofolorunsho, K. C., & Osagbemi, K. G. (2009). Quality of life of people living with HIV/AIDS in Kogi State. *Nigeria. BJPM., 11*(1), 21–27.
26. Dhakad, A. K., Ikram, M., Sharma, S., Khan, S., Pandey, V. V., & Singh, A. (2019). Biological, nutritional, and therapeutic significance of Moringa oleifera Lam. *Phytotherapy Research, 33*, 2870–2903.
27. Jelsma, J., MacLean, E., Hughes, J., Tinise, X., & Darder, M. (2005). An investigation into the health-related quality of life of individuals living with HIV who are receiving HAART. *AIDS Care, 17*(5), 579–588.
28. Louwagie, G. M., Bachmann, M. O., Meyer, K., Booysen, F. I. R., Fairall, L. R., & Heunis, C. (2007). Highly active antiretroviral treatment and health related quality of life in South African adults with human immunodeficiency virus infection: A cross-sectional analytical study. *BMC Public Health*. https://doi.org/10.1186/1471-2458-7-244.
29. Rasoolinajad, M., Abedinia, N., Noorbala, A. A., Mohraz, M., Badie, B. M., Hamad, A., & Sahebi, L. (2018). Relationship Among HIV.Related Stigma, Mental Health and Quality of life for HIV.Positive Patients in Tehran. *AIDS Behav., 22*, 3773–82.
30. Gopi, S., Jacob, J., Varma, K., Amalraj, A., Sreeraj, T. R., Kunnumakkara, A. B., et al. (2017). Natural sports supplement formulation for physical endurance: a randomized, double-blind, placebo-controlled study. *Sport Sci Health., 13*, 183–194.
31. Sutalangka, C., Wattanathorn, J., Muchimapura, S., & Thukham-mee, W. (2013). Moringa oleifera Mitigates Memory Impairment and Neurodegeneration in Animal Model of Age-Related Dementia. *Oxid Med Cell Longev*. https://doi.org/10.1155/2013/695936.
32. Mohamed, A. A., Metwally, M. M. M., Khalil, S. R., Salem, G. A., & Ali, H. A. (2019). Moringa oleifera extract attenuates the CoCl2 induced hypoxia of rat's brain: Expression pattern of HIF-1α. *NF-kB MAO and EPO. Biomed Pharmacother., 109*, 1688–1697.
33. Jamari, H., Rofiee, M. S., Johari, R. J., Salleh, M. Z., & Kek, T. L. (2020). Standardised Extracts of Moringa Oleifera and Centella Asiatica Enhanced the Antioxidant Activity, Learning and Memory Effects by Inhibiting Acetylcholinesterase Activity in D-Galactose Induced Ageing Rats. *Pertanika J Sci & Technol., 28*(1), 293–310.
34. NIH. The importance of social relationships over the life course. National Institutes of Health, Office of Behavioral and Social Sciences research 2016;https://obssr.od.nih.gov/the-importance-of-social-relationships-over-the-life-course/. Accessed 17 March 2021.
35. Matsumoto, S., Yamaoka, K., Takahashi, K., Tanuma, J., Mizushima, D., Do, C. D., et al. (2017). Social Support as a Key Protective Factor against Depression in HIV Infected Patients: Report from large HIV clinics in Hanoi. *Vietnam. Sci Rep., 7*, 15489. https://doi.org/10.1038/s41598-017-15768-w:1-12.
36. Stanton, A. M., Goodman, G., Looby, S. E., Robbins, G. K., & Psaros, C. (2019). Sexuality and Intimacy Among Older Women Living with HIV: a Systematic Review. *Curr Sex Health Rep*. https://doi.org/10.1007/s11930-019-00227-6:1-12.
37. Sparman, A., & Thompson, K. (2017). Safety Profile and Effect on Libido of a Combined Bryophyllum pinnatum, Moringa oleifera and Vitamin C Phytotherapeutic Agent. *Free Radicals and Antioxidants., 7*(2), 156–159.
38. Prabsattroo, T., Wattanathorn, J., Iamsaard, S., Somsapt, P., Sritragool, O., Thukhummee, W., et al. (2015). Moringa oleifera extract enhances sexual performance in stressed rats. *J Zhejiang Univ-Sci B (Biomed & Biotechnol), 16*(3), 179–190.
39. Mueller, P. S., Plevak, D. J., & Rummans, T. A. (2001). Religious Involvement, Spirituality, and Medicine: Implications for Clinical Practice. *Mayo Clinic Proceedings, 76*, 1225–1235.
40. Cotton, S., Puchalski, C. M., Sherman, S. N., Mrus, J. M., Peterman, A. H., Feinberg, J., et al. (2006). Spirituality and Religion

in Patients with HIV/AIDS. *Journal of General Internal Medicine, 21*, S5-13.
41. Suleiman, B. A., Yahaya, M., Olaniyan, F. A., Sule, A. G., & Sufiyan, M. B. (2020). Determinants of health-related quality of life among human immunodeficiency virus positive (HIV-positive) patients at Ahmadu Bello University teaching hospital, Zaria, Nigeria- 2015. *BMC Public Health, 20*(531), 1–9.

CHAPTER SIX

SYNTHESIS: SUMMARY, CONCLUSION, AND RECOMMENDATION

Chapter 6 provides a summary of our findings and assesses the strengths and limitations of this study. It presents our conclusions, and recommendations and offers suggestions for further research.

6.1 Summary and Discussion

Globally, Nigeria has the second-largest HIV endemic. It has one of the highest rates of new infection in sub-Saharan Africa. Similarly, it has the second-highest burden of stunted children in the world [1]. Available data shows that almost 83 million people in Nigeria, live below the poverty line of 137,430 naira ($381.75) per year [2]. Maintaining good health and consuming a nutritious diversified diet can be a challenge while coping with an existing HIV infection [3].

Moringa oleifera Lam. known as the ‘’miracle tree’’ has been documented in the literature to contain many nutrients and bioactive compounds [4, 5]. It is a cost-effective and culturally acceptable plant with several nutritional and medicinal applications and can thus be used to reduce the malnutrition burden in Sub-Saharan Africa [6].

This thesis reports on the various sub-projects conducted to answer the overall objective of evaluating the addition of *Moringa oleifera* Lam. leaves powder as a nutritional supplement on the anthropometric, viral load, and CD4 cell counts of adult HIV patients on ART in AKTH, Kano State, Nigeria. It showed that PLHIV in low-income settings like Nigeria has diet and food consumption of marginal states that require improvement. The studies also showed that malnutrition is still a challenge amongst PLHIV on ART as it has been shown to affect their nutritional status, immune status, and disease progression. Furthermore, it revealed that the QoL of PLHIV on ART could be improved with nutritional supplementation.

The following sections discuss the key findings reported in this thesis in the context of the objectives of the study, which are stated in Chapter 1, section 1.5

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

There is a significant overlap of food insecurity in communities with a high prevalence of poverty and HIV. This contributes to the consumption of foods that are less diverse and nutritious [7]. In Nigeria, diets are primarily plant-based starchy staples that are low in micronutrients. The diets contain few or no animal products with few fruits and vegetables in season [8]. This study revealed a poor dietary diversity amongst PLHIV that are on ART. The study suggests the need for effective nutritional supplementation to improve the general well-being of PLHIV to enable them to realise their full potential (chapter 2).

Does *Moringa oleifera* Lam. leaves supplementation have impact on the weight and BMI of people living with HIV that are antiretroviral therapy? A double-blind, randomized control trial

PLHIV experiences lower rates of HIV-related weight loss and wasting as a result of the beneficial effects of highly active antiretroviral therapy (HAART) [9]. This has increased the prevalence of overweight and obesity [10]. HIV-infected patients are encouraged to consume healthy foods rich in both micro and macronutrients to maintain an optimal nutritional status [11] and prevent medical comorbidities associated with excess weight [9]. As most of the participants in both study groups were of normal weight with a considerable number with overweight and only a few with underweight, the study showed that *Moringa oleifera* Lam. leaf supplementation did not have any impact on the weight and BMI of our participants over the study period (chapter 3).

A double-blind, randomised control trial to examine the effect of *Moringa oleifera* leaf powder supplementation on the anthropometric and immune status of adult HIV patients on antiretroviral therapy in a resource-limited setting

PLHIV that are in resource-limited settings are vulnerable to malnutrition [12] due to intestinal damage which causes impaired nutrient absorption and reduced food intake [13]. This increases susceptibility to opportunistic infections that worsen disease progression [13, 14] and increase HIV-related mortality [15]. Consumption of diets with adequate nutrients is encouraged to support their weakened immune system and improve health and survival [12]. *Moringa oleifera* leaf can reduce the malnutrition burden in Sub-Saharan Africa and Nigeria. This study revealed that *Moringa oleifera* leaf supplementation was associated with increased CD4 cell counts of PLHIV on ART in a resource-limited setting (chapter 4).

Impact of *Moringa oleifera* leaves supplementation on the quality of life of people living with HIV: A double-blind, randomised control trial

PLHIV are more vulnerable to malnutrition due to reduced dietary intake, food insecurity, side effects of ART, and the pill burden [12, 16]. All these factors are related to a reduced QoL in PLHIV. This study is the first in Nigeria to examine the impact of *Moringa oleifera* Lam. leaves supplementation on the QoL of PLHIV that are on ART despite its widespread use. *Moringa oleifera* Lam. leaves nutritional supplementation has shown to improve the QoL domains of physical, psychological, level of independence, and social relationships for PLHIV that are on ART. PLHIV could experience better social interaction and improved intimate partner relationships which could decrease depression and stigmatization which are the most common mental health concerns among PLHIV (chapter 5).

General Discussion

Nutritional supplementation studies play a vital role in establishing causality between diet or intake of nutrients and health outcome measures. As nutritional supplementation studies are performed with humans, it often has many challenges and considerations which include the baseline nutritional status of study participants, defining appropriate control groups, and effective blinding of participants and investigators [17]. Further methodological challenges of implementing nutritional trials include ascertaining adherence, sharing nutritional supplements in families, the timing of taking the interventions as well as knowing the right dose to consume. As most of the participants in our study were of normal weight with a considerable number with overweight and only a few with underweight, the study reported the ineffectiveness of *Moringa oleifera* Lam. leaf supplementation in improving the weight and BMI. Our study was conducted on out-patients, therefore home visits to monitor adherence were inappropriate due to issues of confidentiality and fear of stigmatization by family members. Nutritional supplementation studies that reported an impact on nutritional status [12, 18, 19] recruited a considerable number of patients that presented with weight loss. Despite these challenges, the information to be gained in nutritional supplementation research remain high.

6.2 Strength of the study

This study is a double-blind, randomized placebo trial which is a gold standard of intervention studies. This gives strength and credence to the findings of the study. During study preparation, effort was exerted to present the interventions in the daily-dose prescription sachet. Patients could put a sachet in their pocket or bag while going out for their daily activities. This is to improve adherence to study protocol.

6.3 Limitation of the study

Although the study has presented important information and evidence on nutritional supplementation in PLHIV in low-resource settings, the following limitations of the study are worth noting. The inability to recruit participants with appropriate nutritional status. Presentation of *Moringa oleifera* Lam*.* leaves in powdered form. The inability to present it in capsule form, which could mask its distinguishable taste, could be a source of bias. Also, the inclusion of patients who were only on one ART regimen (tenofovir + lamivudine + efavirenz drug regimen) limits the generalizability of our study findings. A longer follow-up period would also have demonstrated more clearly the differences in the study outcomes (nutritional status, CD4 counts, Viral loads) between the study groups. Lastly, compliance which was monitored by the self-reporting of the study participants, recall and social desirability biases, and lack of diet control and diet documentation of participant's nutrient intakes during the study are further limitations of this study.

6.4 Conclusion

The work reported in this thesis provided important information on nutritional supplementation in PLHIV that are on ART. *Moringa oleifera* Lam. leaves have provided an available local solution to solve nutritional deficiency-related issues in PLHIV. In resource-limited settings, programs and policymakers should consider nutritional supplementation as part of a comprehensive approach to ensure optimal treatment outcomes in PLHIV.

6. 5 Recommendations

The studies reported in this thesis identified the following recommendations:

• Inclusion of nutritional supplements as part of comprehensive therapy alongside ART medications for PLHIV.

• Awareness of available dietary choices should be emphasised as part of routine counselling for PLHIV.

• Nutritional status of PLHIV on ART needs to be monitored to prevent overweight/obesity and its consequences.

• Inclusion of WHOQOL-BREF as an additional periodic qualitative tool for monitoring and evaluation of health outcomes in PLHIV on ART.

6.6 Future Studies

Based on our findings and experience obtained while conducting this present study, the following factors should be considered in future studies:

• Recruitment of participants with differing nutritional status: underweight, normal weight, overweight and obese, to determine the use of *Moringa oleifera* Lam. in improving weight and BMI in underweight subjects as well as control weight gain in overweight/ obese individuals.

• Due to the problem of increasing overweight and chronic diseases in people with HIV, markers of chronic diseases should be measured.

• The adoption of the Diet Quality Questionnaire (DQQ) designed by the Global Diet Quality Project is recommended. The DQQ is especially helpful for programs that have limited finances and technical capacity. The dietary assessment is a low-burden, simple, rapid, and reliable tool that has been designed to capture information on nutrient adequacy and healthy and unhealthy food consumption. It is standardized and adapted for use in over 100 countries with detailed lists of food groups unique to every country.

References

1. UNICEF. National Nutrition and Health Survey (NNHS) 2018. Report on the Nutrition and Health Situation of Nigeria. Available: https://wwwuniceforg/nigeria/media/2181/file/Nigeria-NNHS-2018pdf. 2018:1-153. [Accessed 2022 February 20].
2. NBS. 2019 Poverty and Inequality in Nigeria: Executive Summary. Available: http://nigerianstatgovng/download/1092 2020. [Accessed 2022 February 20].
3. FRAC. The Impact of Poverty, Food Insecurity, and Poor Nutrition on Health and Well-Being. Food Research & Action Center. 2017:1-14. Available: www.frac.org. [Accessed 2022 February 20].
4. Dhakad AK, Ikram M, Sharma S, Khan S, Pandey VV, Singh A. Biological, nutritional, and therapeutic significance of Moringa oleifera Lam. Phytother Res. 2019;33:2870-903 DOI: 10.1002/ptr.6475.
5. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. Antioxidants 2017;6(91):1-13. DOI:0.3390/antiox6040091.
6. Koul B, Chase N. Moringa oleifera Lam.: Panacea to several maladies. J Chem Pharm Res. 2015; 7(6):687-707.
7. Masa R, Chowa G, Nyirenda V. Socioeconomic correlates of dietary diversity and its association with adherence and psychosocial functioning of people living with HIV in rural Zambia. Nutr Health. 2018;24(2):93–102. DOI: 10.1177/0260106018761282.

8. Arimond M, Ruel MT. Dietary Diversity Is Associated with Child Nutritional Status: Evidence from 11 Demographic and Health Surveys[1,2]. J Nutr. 2004;134:2579–85.

9. Crum-Cianflone N, Roediger MP, Eberly L, Headd M, Marconi V, Ganesan A, et al. Increasing Rates of Obesity among HIV-Infected Persons during the HIV Epidemic. PLoS ONE 2010;5; 4:e10106. doi:10.1371/journal.pone.0010106.

10. Sax PE, Erlandson KM, Lake JE, McComsey GA, Orkin C, Esser S, et al. Weight Gain Following Initiation of Antiretroviral Therapy: Risk Factors in Randomized Comparative Clinical Trials. Clin Infect Dis. 2020;71(6):1380–9. DOI: 10.093/cid/ciz999.

11. MartõÂn-Cañavate R, Sonego M, Sagrado MJ, Escobar G, Rivas E, Ayala S, et al. Dietary patterns and nutritional status of HIV-infected children and adolescents in El Salvador: A cross-sectional study. PLOS ONE 2018;13(5):1-14 DOI:org/0.1371/journal.pone.0196380

12. Evans D, McNamara L, Maskew M, Selibas K, Amsterdam D, Baines N, et al. Impact of nutritional supplementation on immune response, body mass index and bioelectrical impedance in HIV-positive patients starting antiretroviral therapy. Nutr J. 2013;12(111):1-14. DOI: http://www.nutritionj.com/content/2/1/111.

13. Guerra EML, Porbén SS. T-Cell Number, Nutritional Status, and HIV: The Cuban Experience in the Provision of Food and Nutrition Care to People with HIV/AIDS. Health HIV Infected People. 2015;2 Chapter 21:367-87.

14. Nnyepi MS. The risk of developing malnutrition in people living with HIV/AIDS: Observations from six support groups in Botswana S Afr J Clin Nutr. 2009;22(2):89-93. DOI: 10.1080/16070658.2009.1734224.

15. Feleke DG, Yemanebrhane N, Gebretsadik D. Nutritional Status and CD4 Cell Counts in HIV/AIDS Patients under Highly Active Antiretroviral Therapy in Addis Ababa, Ethiopia. J AIDS Clin Res. 2017;8(4):1-5. DOI: 10.4172/2155-6113.1000688.

16. Bello SI, Bello IK. Quality of Life of Hiv/Aids Patients in a Secondary Health Care Facility, Ilorin, Nigeria. Proc (Bayl Univ Med Cent) 2013;26(2):116–9. DOI: 10.1080/08998280.2013.11928933.

17. Weaver CM, Miller JW. Challenges in conducting clinical nutrition research. Nutr Rev VR 2017;75(7):491–9; doi: 10.1093/nutrit/nux026.

18. Tshingani K, Donnen P, Mukumbi H, Duez P, Dramaix-Wilmet M. Impact of Moringa oleifera lam. Leaf powder supplementation versus nutritional counseling on the body mass index and immune response of HIV patients on antiretroviral therapy: a single-blind randomized control trial. BMC Complement Altern Med. 2017;17(420):1-13. DOI: 0.1186/s12906-017-1920-z.

19. Amlogu AM, Tewfik S, Wambebe C, Tewfik I. A comparative study: long and short term effect of a nutrition sensitive approach to delay the progression of HIV to AIDS among people living with HIV (PLWH) in Nigeria. Funct food health dis 2016;6(2):79-90. DOI:10.31989/ffhd.v6i2.235.

APPENDICES

Appendix 1: University Research Ethical Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

23 May 2017

Ms A Gambo (213574503)
Discipline of Public Health Medicine
School of Nursing and Public Health Medicine
gamboaishatu@yahoo.com

Dear Ms Gambo

Protocol: Evaluation of the addition of Moringa Oleifera as a nutritional supplement on the anthropometric viral load and CD4 counts of HIV patients on antiretroviral therapy.
Degree: PhD
BREC reference number: BFC294/16

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application at a meeting held on 14 June 2016.

The study was provisionally approved by BREC pending appropriate responses to queries raised. Your responses received on 19 May 2017 to queries raised on 10 May 2017 have been noted and approved by a sub-committee of the Biomedical Research Committee. **Please forward final approval form Nigeria as soon as available.**

This approval of the study is valid for one year from **23 May 2017.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

Pg. 2/...

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2486 **Facsimile:** +27 (0) 31 260 4609 **Email:** brec@ukzn.ac.za

Appendix 2: Aminu Kano Teaching Hospital, Kano State Nigeria Ethical Approval

AMINU KANO TEACHING HOSPITAL

P. M. B. 3452, ZARIA ROAD, KANO.

(☎:07068297399)www.akth.info/www.akth.gov.ng, E-mail: enquiries@akth.info/akthkano@yahoo.com

CHIEF MEDICAL DIRECTOR
PROF. AMINU ZAKARI MOHAMMED,
MBBS, FMCPath

CHAIRMAN M.A.C
Dr. ABDURRAHMAN ABBA SHESHE
MBBS, FMCS,FICS

DIRECTOR OF ADMINISTRATION
ADAMU HUSSAINI ALIYU

NHREC/21/08/2008/AKTH/EC/2012

AKTH/MAC/SUB/12A/P-3/VI/2112 **24th July, 2017**

Aisha Gambo
Sch. of Public Health Medicine
University of Kwazulu-Natal
Durban, South Africa.

ETHICS APPROVAL

Further to your application in respect of your research proposal titled "Evaluation of the Addition of Moringa Oleifera as a Nutritional Supplement on the Anthropometric, Viral Load and CD4 Counts of Adult HIV Patients on Antiretroviral Therapy in Nigeria", the Committee reviewed the proposal and noted same as a prospective randomized study.

In view of the above, Ethics approval is hereby granted to conduct the research.

However, the approval is subject to periodic reporting of the progress of the study and its completion to the Research Ethics Committee.

Regards,

Abubakar S. Mahmud
Secretary, Research Ethics Committee
For: Chairman

Appendix 3: University Research Recertification Ethical Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

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02 May 2018

Ms A Gambo (213574503)
Discipline of Public Health Medicine
School of Nursing and Public Health Medicine
gamboaishatu@yahoo.com

Dear Ms Gambo

Protocol: Evaluation of the addition of Moringa Oleifera as a nutritional supplement on the anthropometric viral load and CD4 counts of HIV patients on antiretroviral therapy.
Degree: PhD
BREC reference number: BFC294/16

We wish to advise you that your application for recertification received on 09 March 2018 for the above study has been **noted and provisionally approved** by a sub-committee of the Biomedical Research Ethics Committee subject to the submission of:

- A copy of the current Info Sheet and Consent Form.

Yours sincerely

Mrs A Marimuthu
Senior Admin Officer: Biomedical Research Ethics Committee

cc postgraduate administrator: ramialm@ukzn.ac.za cc: Supervisors: moodleyi15@ukzn.ac.za, babashanim@yahoo.com

Appendix 4: Patient Informed Consent Form

UKZN BIOMEDICAL RESEARCH ETHICS COMMITTEE

Information Sheet and Consent to Participate in Research

Date:

Dear Sir/ Madam,

My name is Aisha Gambo, a PhD student in the Discipline of Public Health Medicine, University of Kwazulu-Natal, Durban, South Africa. My contact number is +234035392408 and my email address is gamboaishatu@yahoo.com

You are being invited to consider participating in a study titled

"Evaluation of the addition of Moringa oleifera as a nutritional supplement on the anthropometric, viral load; CD4 counts and quality of life of adult HIV patients on antiretroviral therapy in Nigeria"

The purpose of this research is to find out if Moringa oleifera as a dietary supplement will have any effect on the body measurements (weight, BMI; MUAC) and HIV status (CD4 and viral load) of people living with HIV and AIDS who are on anti-retroviral therapy (ART).

We know that people living with HIV and AIDS have better health outcomes if they have good nutrition. We also know that the leaves of Moringa oleifera is packed with nutrients that includes all amino acids (proteins), many vitamins, minerals and anti-oxidants. All of these nutrients are essential for daily functioning. We need to evaluate and determine if adding Moringa oleifera supplement to your normal meals will make a difference to you in terms of your weight, mid-upper arm circumference and also your immune status (i.e. CD4 counts and viral loads). To do this in a scientific manner, we will put people taking part in this study into two groups. These groups are selected purely by chance to eliminate any bias. One group will be given Moringa oleifera and the other group, the control will be given cornstarch powder which will be similar in look and presentation (placebo). If you choose to participate in this study, you should not have any known allergy or intolerance to Moringa oleifera or cornstarch. You should be on Tenofovir + Lamivudine + Efavirenz drug combination. You should not be pregnant. Also, patients with active opportunistic infection and also those taking micronutrient or natural health product supplements within 30days of randomization cannot participate in the study. The study is expected to have a total number of 200 people.

Participants will be given Moringa oleifera supplement or the placebo to take together with their meals three times a day. All participants will be observed for the same measures of weight, mid-upper arm circumference and immune status. The research team will be looking after all participants closely during the study.

During the research, you are expected to make seven visits to the clinic.

- In the first visit, we will ask you a few questions about yourself, your general health, and the types of food eaten. We will measure your height and weight. Then a small amount of blood, equal to about a teaspoon, will be taken from your arm with a syringe. This blood will be tested for the number of cells that help your body to fight HIV infections (CD4 cells). The test that tells the amount of the HIV virus in your body (viral load) will also be conducted.
- At the next visit, which will be two weeks later, you will again be asked some questions about your health and then you will be divided into two groups and be given either the Moringa oleifera supplement or placebo with your usual prescription of ART drugs.
- At all subsequent visits to the clinic thereafter your height, weight and other body measurements will be taken. A blood sample will be taken as explained earlier and the participant will be examined by the clinician if you have any infections associated with HIV and AIDS and how serious the infection is.

The duration of your participation, if you choose to enroll and remain in the study, is expected to be six months. During this period if you become sick as a result of taking Moringa oleifera or the placebo, you will be medically treated free of charge.

The safety of Moringa oleifera has been evaluated and to our knowledge, there are no notable toxic effects. Patients on anti-coagulants and pregnant women will be excluded as a precautionary measure.

If you participate in the study, you will benefit from free CD4 and viral load tests. We hope that the study will enhance your knowledge about the benefits of good nutrition and also about the nutrients benefits of Moringa oleifera and whether it could have any additional benefits to improve the health status of people living with HIV and AIDS. Also, society will benefit from the study with the scientific information on the use of Moringa oleifera as nutritional supplements in HIV infection.

This study has been ethically reviewed and approved by the UKZN Biomedical Research Ethics Committee (Reference number BFC294/16) and AKTH Ethics Committee (Reference number AKTH/MAC/SUB/12A/P-3/VI/2112). In the event of any problem or concerns/questions, you may contact the researcher at +234035392408 or the research team at HIV clinic AKTH at the addresses provided on this sheet.

Please note that your participation in this study is entirely voluntary. It is your choice whether to participate or not. Whether you participate or not, all the services you receive at this clinic will continue and nothing will change. If you agreed earlier and you later change your mind to stop participating in the study, you will need to inform the researcher on this number +234035392408 or inform the research team at HIV clinic, AKTH. Also if you chose to participate, the researcher can terminate you from participating in the study if you refuse to adhere to the basic protocols of the study.

We will give you [₦] to pay for your travel to the clinic for the purpose of the study on days other than your normal clinic days. You will not be given any other money or gifts to take part in this study.

With this study, it is possible if people are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the study. The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up under lock and key. It will not be shared with or given to anyone except the researcher, the AKTH HIV management team, and your doctors.

At the end of the study, in six months, any leftover blood samples taken during the study will be destroyed. The result of the study will be made known to you at the clinic and thereafter it will be published in local and international journals without disclosing your identity.

Here are the addresses to contact if you have questions or concerns.

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CONSENT

I (Name)……have been informed about the study entitled (provide details) …….. by (provide name of researcher/fieldworker) ……..

I understand the purpose and procedures of the study (add these again if appropriate).

I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

I have been informed about any available compensation or medical treatment if injury occurs to me as a result of study-related procedures.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at +234035392408

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus
Govan Mbeki Building
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Email: enquiries@akth.org.ng; akthkano@yahoo.com

_____ _____

Signature of Participant **Date**

_____ _____

Signature of Witness **Date**

(Where applicable)

_____ _____

Signature of Translator **Date**

(Where applicable)

Appendix 5: DIETARY DIVERSITY QUESTIONNAIRE [1]

Please describe the foods (meals and snacks) that you ate yesterday during the day and night, whether at home or outside the home. Start with the first food eaten in the morning.

Write down all food and drinks mentioned by the respondent. When the respondent has finished, probe for meals and snacks not mentioned.

Breakfast	Snack	Lunch	Snack	Dinner	Snack

[Household level: consider foods eaten by <u>any member of the household</u>, and <u>exclude</u> foods purchased <u>and</u> eaten outside of the home]

When the respondent recall is complete, fill in the food groups based on the information recorded above. For any food groups not mentioned, ask the respondent if a food item from this group was consumed.

Question number	Food group	Examples	YES=1 NO=0
1	CEREALS	corn/maize, rice, wheat, sorghum, millet or any other grains or foods made from these (e.g. bread, noodles, porridge or other grain products) + *insert local foods e.g. ugali, nshima, porridge or pastes or other locally available grains*	
2	VITAMIN A RICH VEGETABLES AND TUBERS	pumpkin, carrots, squash, or sweet potatoes that are orange inside + *other locally available vitamin-A rich vegetables (e.g. red sweet pepper)*	
3	WHITE TUBERS AND ROOTS	white potatoes, white yams, white cassava, or other foods made from roots	
4	DARK GREEN LEAFY VEGETABLES	dark green/leafy vegetables, including wild ones + *locally available vitamin-A rich leaves such as amaranth, cassava leaves, kale, spinach etc.*	
5	OTHER VEGETABLES	other vegetables (e.g. tomato, onion, eggplant) , including wild vegetables	
6	VITAMIN A RICH FRUITS	ripe mangoes, cantaloupe, apricots (fresh or dried), ripe papaya, dried peaches + *other locally available vitamin A-rich fruits*	
7	OTHER FRUITS	other fruits, including wild fruits	
8	ORGAN MEAT (IRON-RICH)	liver, kidney, heart or other organ meats or blood-based foods	
9	FLESH MEATS	beef, pork, lamb, goat, rabbit, wild game, chicken, duck, or other birds	
10	EGGS	chicken, duck, guinea hen or any other egg	
11	FISH	fresh or dried fish or shellfish	

12	LEGUMES, NUTS AND SEEDS	beans, peas, lentils, nuts, seeds or foods made from these	
13	MILK AND MILK PRODUCTS	milk, cheese, yogurt or other milk products	
14	OILS AND FATS	oil, fats or butter added to food or used for cooking	
15	RED PALM PRODUCTS	Red palm oil, palm nut or palm nut pulp sauce	
16	SWEETS	sugar, honey, sweetened soda or sugary foods such as chocolates, candies, cookies and cakes	
17	SPICES, CONDIMENTS, BEVERAGES	spices(black pepper, salt), condiments (soy sauce, hot sauce), coffee, tea, alcoholic beverages OR *local examples*	
			YES=1 NO=0
Individual level only	Did you eat anything (meal or snack) OUTSIDE of the home yesterday?		
Household level only	Did you or anyone in your household eat anything (meal or snack) OUTSIDE of the home yesterday?		

[1] FAO/Nutrition and Consumer Protection Division, version of May, 2007. Please acknowledge FAO in any documents pertaining to use of this questionnaire.

[2] This questionnaire may be used for any individual above the age of three years. For children under three, the dietary diversity questionnaire used in DHS surveys for young children is more appropriate.

Appendix 6: WHOQOL-HIV BREF

WHO/MSD/MER/02.2
English only
Distr.: General

WHOQOL-HIV BREF

MENTAL HEALTH: EVIDENCE AND RESEARCH
DEPARTMENT OF MENTAL HEALTH
AND SUBSTANCE DEPENDENCE
WORLD HEALTH ORGANIZATION
GENEVA

		Raw Score	Transformed Score	
Domain 1	(6-Q3) + (6-Q4) + Q14 + Q21 □ + □ + □ + □			
Domain 2	Q6 + Q11 + Q15 + Q24 + (6-Q31) □ + □ + □ + □ + □			
Domain 3	(6-Q5) + Q20 + Q22 + Q23 □ + □ + □ + □			
Domain 4	Q17 + Q25 + Q26 + Q27			

	□ + □ + □ + □	
Domain 5	Q12 + Q13 + Q16 + Q18 + Q19 + Q28 + Q29 + Q30 □ + □ + □ + □ + □ + □ + □+ □ + □	
Domain 6	Q7 + (6–Q8) + (6-Q9) + (6-Q10) □ + □ + □ + □	

Further copies of this document may be obtained from

Department of Mental Health and Substance Dependence
World Health Organization
CH-1211 Geneva 27
Switzerland

ABOUT YOU

Before you begin we would like to ask you to answer a few general questions about yourself: by circling the correct answer or by filling in the space provided.

What is your **gender**? Male / Female

How old are you? _____ (age in years)

What is the highest level **education** you received? None at all / Primary / Secondary / Tertiary / Quranic

What is your **Ethnicity**? Hausa:Fulani / Yoruba / Igbo / Others

What is your **Marital status**? Single / Married / Divorced / Separated / Widowed

What is your **Occupation**? Farmer / Trader / Civil servant / Artisan / Unemployed / Others

How many members are in your family? _____

What is your **monthly income**?

_____

Apart from being HIV positive, do you have any other health problems: Yes/No . If yes, please indicate what these are

Please respond to the following questions if they are applicable to you:

What is your **HIV serostatus**? Asymptomatic / Symptomatic / AIDS converted

In what year did you first **test positive** for HIV? _____

In what year do you think you were infected? _____

How do you believe you were **infected with HIV**? (circle one only):
Sex with a man / Sex with a woman / Injecting drugs / Blood products / Other (specify)_____

Instructions

This assessment asks how you feel about your quality of life, health, or other areas of your life. **Please answer all the questions.** If you are unsure about which response to give to a question, **please choose the one** that appears most appropriate. This can often be your first response. Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the last two weeks.** For example, thinking about the last two weeks, a question might ask:

		Not at all	A little	A moderate amount	Very much	Extremely
11 (F5.3)	How well are you able to concentrate?	1	2	3	4	5

You should circle the number that best fits how well are you able to concentrate over the last two weeks. So you would circle the number 4 if you were able to concentrate very much. You would circle number 1 if you were not able to concentrate at all in the last two week

Please read each question, assess your feelings, and circle the number on the scale for each question that gives the best answer for you.

		Very poor	Poor	Neither poor nor good	Good	Very good
1(G1)	How would you rate your quality of life?	1	2	3	4	5

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
2 (G4)	How satisfied are you with your health?	1	2	3	4	5

The following questions ask about **how much** you have experienced certain things in the last two weeks.

		Not at all	A little	A moderate amount	Very much	An extreme amount
3 (F1.4)	To what extent do you feel that physical pain prevents you from doing what you need to do?	1	2	3	4	5
4 (F50.1)	How much are you affected by any physical problems related to your HIV infection?	1	2	3	4	5
5 (F11.3)	How much medical treatment do you need to function in your daily life?	1	2	3	4	5
6 (F4.1)	How much do you enjoy life?	1	2	3	4	5
7 (F24.2)	To what extent do you feel your life to be meaningful?	1	2	3	4	5
8 (F52.2)	To what extent are you bothered by people blaming you for your HIV status	1	2	3	4	5
9 (F53.4)	How much do you fear the future?	1	2	3	4	5
10 (F54.1)	How much do you worry about death?	1	2	3	4	5

		Not at all	A little	A moderate amount	Very much	Extremely
11 (F5.3)	How well are you able to concentrate?	1	2	3	4	5
12 (F16.1)	How safe do you feel in your daily life?	1	2	3	4	5
13 (F22.1)	How healthy is your physical environment?	1	2	3	4	5

The following questions ask about **how completely** you experience or were able to do certain things in the last two weeks.

		Not at all	A little	Moderately	Mostly	Completely
14 (F2.1)	Do you have enough energy for everyday life?	1	2	3	4	5
15 (F7.1)	Are you able to accept your bodily appearance?	1	2	3	4	5
16 (F18.1)	Have you enough money to meet your needs?	1	2	3	4	5

17 (F51.1)	To what extent do you feel accepted by the people you know?	1	2	3	4	5
18 (F20.1)	How available to you is the information that you need in your day-to-day life?	1	2	3	4	5

19 (F21.1)	To what extent do you have the opportunity for leisure activities?	1	2	3	4	5

		Very poor	Poor	Neither poor nor good	Good	Very good
20 (F9.1)	How well are you able to get around?	1	2	3	4	5

The following questions ask you how **good or satisfied** you have felt about various aspects of your life over the last two weeks.

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
21 (F3.3)	How satisfied are you with your sleep?	1	2	3	4	5
22 (F10.3)	How satisfied are you with your ability to perform your daily living activities?	1	2	3	4	5
23 (F12.4)	How satisfied are you with your capacity for work?	1	2	3	4	5
24 (F6.3)	How satisfied are you with yourself?	1	2	3	4	5
25 (F13.3)	How satisfied are you with your personal relationships?	1	2	3	4	5
26 (F15.3)	How satisfied are you with your sex life?	1	2	3	4	5
27 (F14.4)	How satisfied are you with the support you get from your friends?	1	2	3	4	5
28 (F17.3)	How satisfied are you with the conditions of your living place?	1	2	3	4	5
29 (F19.3)	How satisfied are you with your access to health services?	1	2	3	4	5
30 (F23.3)	How satisfied are you with your transport?	1	2	3	4	5

The following question refers to **how often** you have felt or experienced certain things in the last two weeks.

		Never	Seldom	Quite often	Very often	Always
31 (F8.1)	How often do you have negative feelings such as blue mood, despair, anxiety, depression?	1	2	3	4	5

Did someone help you to fill out this form? _____

How long did it take to fill this form out? _____

Do you have any comments about the assessment? _____

_____

_____

_____

THANK YOU FOR YOUR HELP