

Full Length Research Paper

Immunomodulatory effect of *Artemisia annua* and *Moringa oleifera* on viral load among PLWH on antiretroviral therapy in Uganda

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Contemporary highly active antiretroviral therapy (HAART) is effective and tolerable for a long time but cannot eradicate human immunodeficiency virus (HIV) infection by either elimination of viral reservoirs or enhancement of HIV-specific immune responses. HIV infects the B cell germinal centers in follicles of lymphoid tissue and induces dysfunction, which may persist during HAART, negatively affecting IgG subclass content and the avidity of IgG antibodies. This study investigated the effect of *Artemisia annua* and *Moringa oleifera* leaf powder supplementation on p24-specific IgG antibody responses, CD4 count, and viral load among people living with HIV (PLWH) on HAART at an HIV clinic in Uganda. Immunomodulatory effects of *A. annua* and *M. oleifera* leaf powder supplementation on viral load, CD4 count, and p24-specific IgG and IgM antibodies among 37 PLWH on antiretroviral therapy were determined by indirect ELISA while chaotrope-based assays were used to determine IgG avidity against the p24 protein. Viral load was determined using the RNA PCR technique. The findings show a statistically significant decrease in the p24-specific IgG antibodies ($p = 0.029$) while the chaotropic-based assays indicated that HAART supplementation with *M. oleifera* and *A. annua* leaf powder was associated with higher IgG antibody avidity against p24 protein ($p = 0.026$) than the control group. These findings were supported by a statistically significant increase in CD4 count ($p < 0.001$) and viral load suppression ($p = 0.035$) in the intervention group compared to the control. No statistically significant difference was found in p24-specific IgM antibody titers between the intervention and control groups. Supplementation with a combination of *M. oleifera* and *A. annua* leaf powder in patients on highly active HAART diminishes virus-specific B cell responses, suppresses viral replication, and increases CD4 count.

Keywords: *Artemisia annua*, *Moringa oleifera*, immunoglobulins, p24, PLWH, CD4, viral load, highly active antiretroviral therapy (HAART).

INTRODUCTION

Acquired Immuno-Deficiency Syndrome (AIDS), caused by the Human Immunodeficiency Virus (HIV), is the most

common immunosuppressive disease and one of the major global health problems (Anywar et al., 2020). Reports released by the Joint United Nations Programme on HIV and AIDS (UNAIDS) revealed that 39.0 million [33.1 to 45.7 million] people globally were living with HIV in 2022, two-thirds of whom (25.6 million) are in the WHO African Region (Gouda, 2023), and about 36.3 million people have died from the endemic by the end of 2020 (Kankara et al., 2022). In HIV infection, CD4 cells are targeted, leading to a progressive reduction in their numbers and dysregulation of their function. Over time, sustained and chronic HIV replication can culminate in AIDS, characterized by significantly low CD4 counts, heightened susceptibility to opportunistic infections, associated morbidity, and eventual mortality (Amlogu et al., 2016; Gunda et al., 2017; Aprioku et al., 2022). In the era of highly active antiretroviral therapy (HAART), CD4 T cell depletion is halted, and their numbers may even increase, provided patients are receiving proper HAART. In a few circumstances, for reasons not fully understood, patients on HAART may fail to recover optimal CD4 T cell numbers and/or function, leading to a situation known as immunological failure. Patients with HIV infection also exhibit a generalized, non-HIV-specific polyclonal B-cell activation, resulting in hypergammaglobulinemia of all immunoglobulin (Ig) isotypes in addition to HIV-specific IgM and IgG. Serum immunoglobulin concentrations increase as one progresses from asymptomatic to symptomatic HIV infection (Lugada et al., 2004; Nair et al., 2009; Onifade et al., 2017).

As is observed with CD4 T cells, B-cell activation is significantly reversed by HAART, leading to associated reductions in total IgG levels, including HIV-1-specific antibodies (Voltersvik et al., 2003). The effectiveness of humoral responses primarily depends on the affinity and avidity of pathogen-specific antibodies. Antibodies with higher affinity or avidity are capable of binding pathogens at lower concentrations, thereby mediating protective functions more effectively. Conversely, antibody avidity is known to be associated with immune complex-mediated disorders in patients (Dimitrov et al., 2011), complicating the landscape of HIV/AIDS disease and its outcomes. Nevertheless, HIV is recognized for its impact on altering follicular T helper cell function, potentially negatively influencing the affinity maturation process in germinal centers, leading to the production of antibodies with low avidity (Kimuda et al., 2018). While contemporary HAART enhances HIV-1-specific and all immune responses in general (French et al., 2017), the synergistic role of a well-balanced nutritional program cannot be underestimated and is encouraged. In resource-limited countries such as Uganda, maintenance of patients on a balanced diet drains family resources and is often not

sustainable for long periods by most families. In Uganda, many PLWH, in addition to HAART, widely use medicinal plants for boosting immunity as well as managing infections (Lubinga et al., 2012; Anywar et al., 2020; Kankara et al., 2022). These medicinal plants represent a source of potential immunomodulating adjuvants that may boost protective HIV-1-specific immune responses (French et al., 2017; Anywar et al., 2020).

Artemisia annua L. (Asteraceae), an annual herb native to Asia and other continents, with deeply grooved branches and leaf color that varies from light green to dark green, has been used for the treatment and prevention of fever, chills, some cancers, and malaria, in traditional and modern medicine (Liu et al., 2013; Mirbehbahani et al., 2020; Ekiert et al., 2021). *A. annua* leaf powder which increases monocyte and lymphocyte levels (Ogwang et al., 2011; Lubbe et al., 2012), has been used widely by PLWH in Uganda to boost the immune system. Macrophages, representing a key component of the innate immune system, can produce both pro-inflammatory cytokines, such as IL-12/23 P40 and TNF α , and anti-inflammatory cytokines, including IL-10 (Hou and Huang, 2016).

Equally, *Moringa oleifera* Lam (Moringaceae), a fast-growing drought-resistant tree, distributed in many countries of the tropics and subtropics, is commonly consumed by PLWH on HAART (Monera-Penduka et al., 2017; Aprioku et al., 2022) as a supplement to enhance immunity and manage opportunistic infections. Besides having anti-inflammatory, antioxidant, anti-hyperglycemic, and hepatoprotective activity, *M. oleifera* has been reported to exhibit a positive influence on leucocytes, lymphocytes, neutrophils, erythrocytes, hemoglobin, and packed cell volume (Aprioku et al., 2022). However, the synergistic immunological and/or virological effect of the combined use of *A. annua* and *M. oleifera* by PLWH in Uganda has not been demonstrated. This study investigated the effect of combined usage of *A. annua* and *M. oleifera* powders on CD4 counts, viral load suppression, and on HIV-specific antibodies among PLWH on HAART.

MATERIALS AND METHODS

Study design and population

This cohort study was nested within a Randomized Controlled Clinical Trial conducted at Mbarara Regional Referral Hospital (MRRH) in Uganda, registered with ClinicalTrials.gov under NCT03366922. The investigation aimed to assess the impact of *A. annua* and *M. oleifera* on CD4 count and viral load among people living with HIV (PLWH) HAART. The parent study enrolled 267 participants aged 18 years or older, who were HIV positive and had

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been on HAART for at least one year, with a CD4 count of fewer than 350 cells/ μ L, and normal haematological and biochemical indices. Exclusion criteria comprised patients with opportunistic infections, those using other herbal medicines, or pregnant individuals. In our nested study, we sampled 37 participants from the parent study. The participants were divided into two groups: a control group consisting of 17 individuals on HAART only and an intervention group comprising 20 participants on *A. annua* and *M. oleifera* in addition to HAART. This pilot study had its sample size determined by the availability of sufficient sample volumes for laboratory analyses.

Preparation of plant materials

A. annua and *M. oleifera* young leaves were obtained from Kabale district, Western Uganda and were authenticated by a botanist from the Faculty of Science at MUST. The leaves were air-dried under the shade, pulverized mechanically, and packaged in 4 and 10 g of *A. annua* and *M. oleifera*, respectively following study-specific standard operating procedures and in a setting of good compounding practices.

Administration of treatments and measurement of study parameters

Participants in the intervention arm were given 10 and 4 g of *M. oleifera* and *A. annua* powders, respectively to mix and take in their drink of choice (cassava, millet, or posho porridges) every morning at 8 a.m. for 12 months. Each parcel supplied to the participant contained *M. oleifera* and *A. annua* packs labelled with participants' study numbers to last one month. Parcels also contained dosing instructions as well as information on storage and safety. To ensure adherence to the *M. oleifera* and *A. annua* and HAART treatments, every participant received a reminder SMS every morning between 7 and 8 a.m. Participants were requested to respond with the message "Taken" or a call prompt as a proxy to confirm adherence/compliance to the intervention. The study team worked closely with the HIV clinic to ensure that participants were reviewed and accessed routine HIV care as prescribed, including HAART. During the study information sessions before informed consent and throughout the study period, study staff emphasized to all participants that these *M. oleifera* and *A. annua* herbal medicines were not replacements for HAART in the treatment of HIV, and as such the study participants remained in the study only while enrolled on HAART as prescribed. This phrase was also added in the consent form, "Please note that these herbal medicines are not being given to you for treatment of HIV, and as such you MUST keep taking your prescribed ARVs as usual and correctly".

Each study participant was followed up for 12 months and reviewed once a month by the study clinician, with additional unscheduled visits allowed in cases of medical emergencies or as needed. The study clinician conducted physical examinations of the participants, documented the findings, and supplemented them with self-reports from the study participants. The evaluation and documentation of performance status and quality of life were also carried out. Case report forms were utilized to capture vital outcome data from participants, alongside laboratory report forms. A blood sample for the measurement of CD4 count, viral load, p24-specific IgG, and IgM antibodies was collected immediately after enrollment but before the initiation of *M. oleifera* and *A. annua* treatments. Subsequent samples were drawn at 6 and 12 months following the initiation of the intervention.

HIV-1 p24 Antigens

Recombinant HIV-1 p24 protein (NBP1-46036) was obtained from

Novus Biologicals, UK, and was aliquoted and stored at -80°C until use.

HIV-1 p4 ELISA procedure

Each well of an Immulon 4 HBX microtitre plate (Thermoscientific, USA) was coated at 4°C overnight with 200 ng of HIV-1 p24 antigen in 50 μ L/well of commercial coating buffer (immunochemistry technologies, USA). The plate wells were then washed five times with wash buffer and blocked with 50 μ L/well of blocking solution (5% w/v skimmed milk in PBS) for 2 h at 37°C . Two-fold serial dilutions of serum samples were prepared starting with a dilution of 1/50 and ending at a dilution of 1/200. Each sample dilution (50 μ L) was incubated in duplicate wells for 1 h at 37°C , washed as before and then 50 μ L/well of anti-human IgG horseradish peroxidase (HRP) conjugate was incubated in the test-well for 1 h at 37°C . The plates were washed again to remove excess conjugate antibodies. Substrate 3,3', 5,5'-tetramethylbenzidine (ImmunoChemistry Technologies, USA) (100 μ L/well), was incubated for 30 min at room temperature in the dark. The reaction was stopped by adding 25 μ L of 2M sulphuric acid. Optical density (OD) was read in an ELISA plate reader (BioTek ELx 80) at a test wavelength of 450 nm and a reference wavelength of 630 nm. The difference between the two readings was computed to control for background optical interference and was taken as the final OD result.

Ammonium thiocyanate ELISA for determination of p24-IgG antibody avidity

Immulon 4 HBX microtitre plates (Thermoscientific, USA) were coated and blocked as described. Samples were individually diluted to give normalized uniform ODs. Then 50 μ L/well of the normalized serum dilutions were incubated in duplicate on the HIV-1 p24 coated plates at 4°C overnight. The next morning the assay plate was washed and 50 μ L/well of 0.6 M ammonium thiocyanate (LOBA CHEMIE PVT) was added and incubated for 10 min at room temperature. The plates were washed five times and then 50 μ L/well of anti-human IgG-HRP conjugate was added followed by incubation for 1 h at 37°C . The plates were washed, 100 μ L of the substrate, 3,3', 5,5'-tetramethylbenzidine (ImmunoChemistry Technologies USA), was incubated in each well for 30 min at room temperature in the dark. The reaction was stopped with 2 M sulfuric acid and ODs were read on a BioTek ELx80 plate reader.

Statistical data analysis

The optical densities (ODs) obtained from the reader for both the standards and assay serum samples were exported to MS Excel and subsequently imported into My Assays, an online ELISA tool. A 4-parameter logistic regression analysis was conducted using the My Assays tool. The output from the tool included the standard graph and slope. The concentrations of HIV-1 p24-specific IgG and IgM in the participants' samples were calculated utilizing the standard graph. Additionally, for the avidity ELISA, the ODs were transformed into an avidity index by dividing the average OD of the wells with chaotrope by the average OD of the wells without chaotrope for the same sample. Data were analyzed using GraphPad Prism software version 8. A paired t-test was used to determine the difference between the intervention and control group means of p24-specific immunoglobulin levels, avidity index, and CD4 count. Viral load data were analyzed using STATAv15. The Mann-Whitney (Ranksun) test was used to check for viral load median differences between the test and control groups, while the Wilcoxon matched-pairs signed-rank test was utilized to detect any median differences between the baseline and later concentrations.

Table 1. Baseline characteristics of the study participants.

Characteristic	Control Group (n=17) Mean (\pm SD) or n (%)	Artemisia + <i>Moringa</i> Group (n =20) Mean (\pm SD) or n (%)	p-value
Age (years)	39.37 (9.39)	38.95 (9.50)	0.891
Gender			
Male	10 (58.8)	11(55)	0.60
Female	7(41.2)	9 (45)	
Mean CD4 (cells/ μ L)	222.2 (92.43)	229.6 (82.80)	0.822
VL (copies/mL)	8636 (33915)	1520 (5222)	0.412
HAART regimen			
AZT +3TC +EFV	3 (15.8)	1 (5)	0.337
3TC + EFV + TDF	9 (47.4)	8 (40)	0.822
3TC + EFV + others	5 (26.3)	9 (45)	0.412

HAART: Highly active antiretroviral therapy; AZT: zidovudine; 3TC: lamivudine; EFV: efavirenz; NVP: nevirapine; TDF: tenofovir; VL: viral load.

A p-value of less than 0.05 was considered statistically significant and hence concluded that the means and medians were different.

RESULTS

Baseline characteristics of study participants

Baseline demographics, CD4, and viral load characteristics of the participants in the control and intervention groups were comparable at enrollment. Table 1 describes the demographic and immunological characteristics of the study participants at enrollment. Equally at baseline, the number of patients on the three categories of HAART regimens: AZT + 3TC + EFV, 3TC + EFV + TDF, and 3TC + EFV + others, was statistically similar for both the intervention and control groups (Table 1).

The effect of *A. annua* and *M. oleifera* was examined on the median viral loads at the 6 and 12-month time points. A gradual decline in viral load was observed from baseline, through the 6 months and up to the 12 months of participant follow-up. However, the viral load was only significantly different between the intervention and control groups at the 12 months' follow-up time point (Table 2).

Effect of *A. annua* and *M. oleifera* supplementation to HAART on CD4 counts

At enrollment, participants were marched for CD4 count in addition to other parameters. Therefore, no significant difference in CD4 count at enrolled was observed (Table 1). However, after 12 months of *A. annua* and *M. oleifera* supplementation of HAART, the intervention arm showed a significantly higher mean CD4 count when compared with the control group (Figure 1).

Data were expressed as Mean \pm SD. The level of statistical significance was calculated by comparing the mean CD4 count of the control (A) and intervention (B) groups at baseline and exit. P values less than 0.05 were taken to be statistically significant.

Effect of *A. annua* and *M. oleifera* treatment on IgM and IgG antibody responses

The effect of *A. annua* and *M. oleifera* treatment was investigated on the antibody responses using IgM and IgG levels, and IgG avidity index as surrogates. Baseline mean IgM and IgG levels were 1,819 and 16,714 ng/mL, respectively. After 12 months of follow-up, when compared with the baseline time point, we found no significant change or difference in mean levels of IgM in both the *A. annua* and *M. oleifera* and control groups (Figure 2A and B). The control group equally did not show significant changes in IgG levels at the 12 months of follow-up when compared with baseline (Figure 2C).

However, there was a significant reduction in mean IgG levels in the *A. annua* and *M. oleifera*, treatment group when the 12-month time point was compared to the baseline visit (Figure 2D).

Data were expressed as Mean \pm SD. The level of statistical significance was calculated by comparing the mean p24-IgM levels of the control (A) and intervention (B) and mean p24-IgG levels of the control (C) and intervention (D) groups at baseline and exit. P values less than 0.05 were taken to be statistically significant.

Effect of *A. annua* and *M. oleifera* treatment on avidity of anti-HIV p24-IgG antibodies

Subsequently, we investigated whether supplementation

Table 2. Effect of *A. annua* and *M. oleifera* on viral load among PLWH on HAART.

Variable	Median (IQR)		P value (Mann-Whitney test)
	Treatment	Control	
Baseline viral load	73 (0 – 235)	157 (0 – 892)	0.701
Viral Load at 6 months	20 (0 – 280)	120 (0 – 328)	0.239
Viral Load at 12 months	0 (0 – 54)	71 (0 – 349)	0.035*

IQR is inter quartile range. The median viral load readings in the treatment group at the baseline, six, and twelve months were compared with median readings at similar time points in the control.

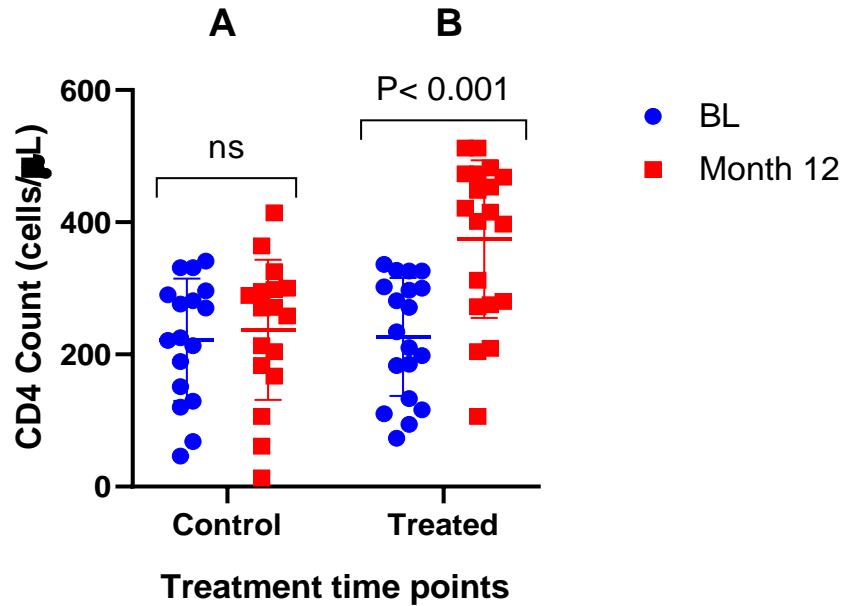


Figure 1. Effect of *A. annua* and *M. oleifera* on CD4 count.

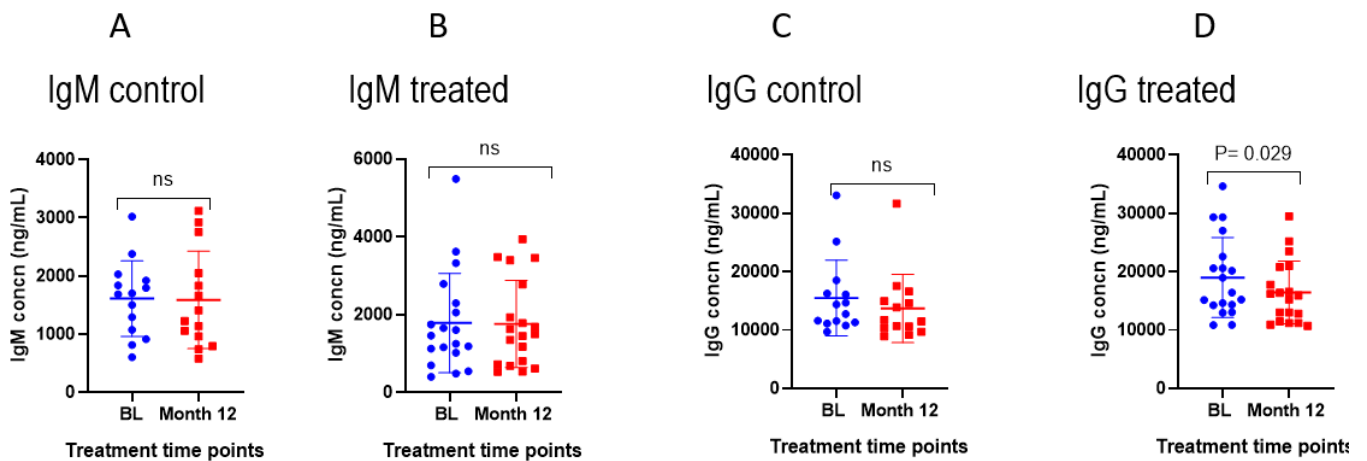


Figure 2. Effect of *A. annua* and *M. oleifera* on HIV anti-p24-IgM and IgG levels.

with *A. annua* and *M. oleifera* alongside HAART had any impact on the binding affinity of HIV-specific antibodies, utilizing the avidity index of anti-HIV-1 p24 IgG as a

surrogate measure. The selection of p24 was primarily influenced by its status as an abundant HIV-1 antigen *in vivo*, eliciting high level of IgG targeting the p24 antigen.

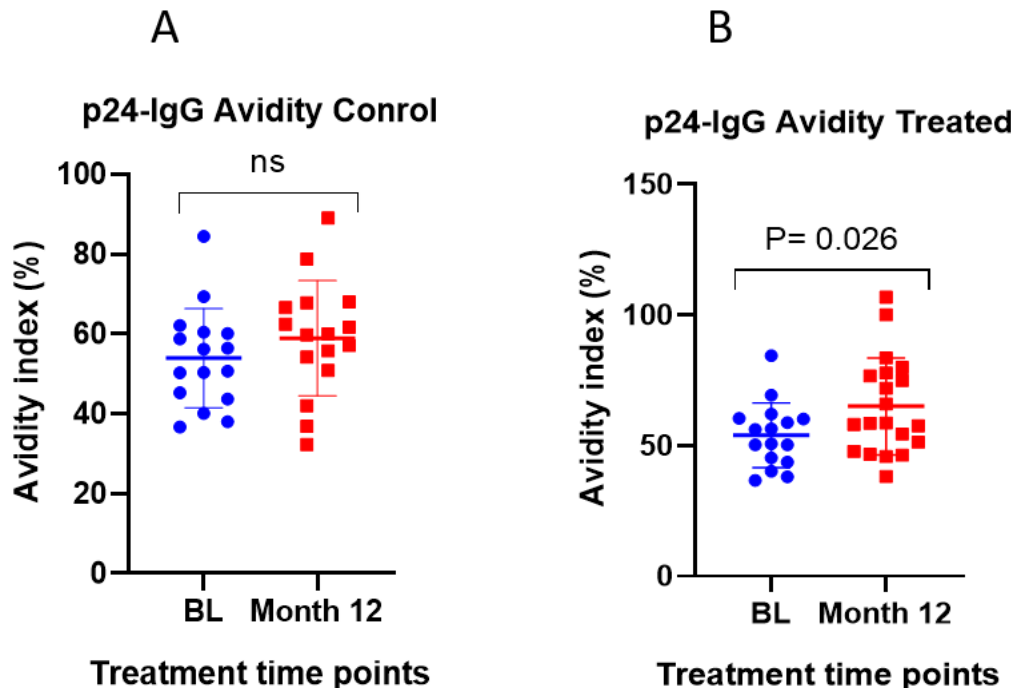


Figure 3. Effect of *A. annua* and *M. oleifera* on the strength of binding between p-24 and IgG antibody among PLWH on ART.

An increase was observed in the mean anti-p24 IgG avidity indices between baseline and 12 months. This increase was not statistically significant in the control group (Figure 3A) but demonstrated statistical significance in the *A. annua* and *M. oleifera* treatment group (Figure 3B).

Data were expressed as Mean \pm SD. The level of statistical significance was calculated by comparing the percentage p24-IgG avidity index of the control (A) and intervention (B) groups at baseline and exit. P values less than 0.05 were taken to be statistically significant.

DISCUSSION

In this study, the impact of supplementing HAART with a combination of *A. annua* and *M. oleifera* leaf powder among PLWH, focusing on p24-specific IgG and IgM antibodies, HIV viral load, and CD4 count was investigated. The average age of the study participants was 39 years (Table 1). This aligns with findings from the Uganda Population-based HIV Impact Assessment (UPHIA 2020-2021), where the age groups with the highest HIV prevalence were 35 to 39 (10.7%), 45 to 49 (12.1%), and 50 to 54 (11.8%), as compared to the national average of 5.8%. This emphasizes that HIV prevalence in Uganda is notably higher among individuals aged 35 and older compared to younger age groups. Our findings align with those of Negin et al. (2016) in Eastern Zimbabwe, where they observed an

increasing HIV prevalence among people aged 45 and older, while the prevalence among younger age groups was decreasing. They attributed their findings to the saturation of infection in high-risk groups, behavior change, and a potential impact of widespread HAART on transmission, as well as increased survival of PLWH due to HAART (Negin et al., 2016).

Therefore, the average age observed in this study is supported by various factors, including the context of Mbarara Regional Referral Hospital, the study site, which is surrounded by HIV high-risk groups, including but not limited to commercial sex workers, refugees, and prisoners.

Despite a significant reduction in viral load observed in the intervention group at 12 months compared to the control, this reduction was gradual and not evident at month 6 following the initiation of the intervention (Table 2). This gradual reduction may be attributed to one of the inclusion criteria for this study, which required patients to have been on HAART for at least one year with a CD4 count of 350 cells/ μ L, potentially causing a delayed rebound of viral load to normal levels. This observation is supported by a study conducted by Liu et al. (2020), who suggested that using the normalization of CD4 counts as the primary evaluation parameter for complete immune restoration in HIV-1 patients under HAART might be insufficient. They noted that the median time for viral load suppression ranges from 3.4 to 4.5 months, irrespective of the HAART regimen, indicating that immune system recovery is a gradual process occurring over an extended

period (Liu et al., 2020).

The findings of this study contrast with those of Gambo et al. (2021), who reported that the *M. oleifera* leaf intervention was not effective in decreasing the viral load of HIV-infected individuals accessing HAART at the S.S Wali Virology Center (Gambo et al., 2021). This disparity can be attributed to the fact that, in this study, *M. oleifera* was combined with *A. annua*, which was not the case in their study. Findings of the present study indicate that after 12 months of patient follow-up, there was a statistically significant reduction in viral load combined with an increase in CD4 count in the intervention group compared to the control. This observation aligns with many studies, including the one by Mugo et al. (2022), where most patients experience a reduction in HIV viral load coupled with an increase in CD4 cell count after initiating HAART. In chronic diseases such as HIV and AIDS, adequate micro and macronutrients are vital for normal body functioning, maintaining optimal immunological function, and improving the efficacy of HAART in people living with HIV (PLWH) (Gambo and Gqaleni, 2022). *M. oleifera* leaves, a rich source of both macro and micronutrients, are often taken as a supplement by PLWH, especially in developing countries, to enhance immunity and manage opportunistic infections (Monera-Penduka et al., 2017). The observed viral load suppression combined with an increase in CD4 cell count in this study can be attributed to the synergistic action of the phytochemicals present in *M. oleifera* and *A. annua* leaf powder that was used to supplement HAART among PLWH.

This study observed a decrease in p24-specific IgM antibody titers, although it was not statistically significant compared to the control (Figure 2A and B). Several studies indicate that untreated PLWH exhibit generalized non-HIV-specific polyclonal B-cell activation, resulting in hypergammaglobulinemia of all immunoglobulin isotypes, as well as increased production of HIV-specific IgG and IgM. Following the initiation of HAART, viremia decreases, along with a reduction in IgM titers. However, findings from this study show a further decrease in IgM titers following the initiation of the intervention, although it was not statistically significant compared to the control. This suggests that p24-specific IgM antibodies may not play a significant role in controlling HIV infection. The findings of the present study align with Tomaras et al. (2008), who demonstrated that IgM antibodies induced by transmitted HIV-1 are capable of binding to HIV virions but have little impact on viremia (Tomaras et al., 2008). In contrast, there was a significant decrease in IgG among the intervention group compared to the control (Figure 2C and D). HIV-1 infection results in an increased concentration of circulating IgG, a phenomenon known as hypergammaglobulinemia and dysregulation of B-cell populations. The increased production of capsid protein-specific IgG antibodies plays an important role in the host immune response to HIV infection (Kardava and Moir,

2019). After HAART, most polyclonal B-cell activation and elevated IgG levels can be restored to levels similar to those in healthy controls, at least in some patients (Song et al., 2020). A similar observation was made in this study in which supplementation of HAART with *M. Oleifera* and *A. annua* leaf powder reduced the HIV viremia leading to a reduction in p24-specific IgG antibody concentration, leading to immunological recovery, hence improving the quality of life among PLWH. As noted by Dang et al. (2018), an elevated p24-specific IgG antibody concentration coupled with elevated viral load in the control group was observed, indicating poor immune response and therefore treatment failure compared to the intervention group (Dang et al., 2018). In the intervention group, the authors did not only observed a reduced concentration of p24-specific IgG antibodies, but these antibodies also exhibited a higher strength of binding to p24, the most abundant HIV protein essential for the assembly of the capsid that encases HIV genetic material (Anywar et al., 2020), compared to those in the control group (Figure 3). Their findings align with those of Bauer (2021), who stated that antibody avidity increases throughout infection and remains elevated. Upon antigen recognition, immunoglobulin heavy and light chains of B-cell receptor (BCR) are further diversified through rounds of somatic hypermutation, leading to affinity maturation. This process selects B cells in the germinal center with improved antigen-binding properties (Ghraichy et al., 2020). Findings of the current study demonstrate the link between B-cell and T-cell responses, as CD4 T cells are necessary for generating high-affinity and long-lived antibody responses. T follicular helper (T_{fh}) CD4 cells drive affinity maturation, a complex dynamical process enabling the immune system to produce antibodies capable of recognizing antigens (Molari et al., 2020; Bauer, 2021) and directing class switch recombination in germinal center B cells (Kamphorst and Ahmed, 2013; French et al., 2017). This process gives rise to IgG antibodies of high avidity (Kimuda et al., 2018), playing a crucial role in antibody-mediated protection against HIV infection among PLWH. The main limitation of this study was compliance, which was monitored through self-reporting by study participants to the investigators.

Conclusion

This study revealed that supplementing HAART with *M. oleifera* and *A. annua* leaf powder diminishes virus-specific immune responses, leading to the suppression of viral replication and an increase in CD4 count. This ensures optimal treatment outcomes in PLWH.

RECOMMENDATION

The authors recommend conducting a similar study in an

environment where strict adherence to HAART is monitored.

Furthermore, they also suggest a study to determine the potential interaction between antiretroviral drugs (ARVs) and a combination of *M. oleifera* and *A. annua*.

ABBREVIATIONS

AIDS, Acquired immune deficiency syndrome; **ARVs**, antiretrovirals; **BCR**, B cell receptor; **HAART**, highly active antiretroviral therapy; **BL**, baseline; **CD4**, cluster of differentiation; **ELISA**, enzyme-linked immunosorbent assay; **GC**, Germinal Center; **HIV**, human immunodeficiency virus; **IgG**, immunoglobulin G; **IgM**, immunoglobulin M; **MUST**, Mbarara University of Science and Technology; **OD**, optical density; **PBS**, phosphate buffered saline; **PLWH**, people living with HIV; **SD**, standard deviation; **UNCST**, Uganda National Council for Science and Technology; **WHO**, World Health Organization.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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