Evaluation of the effect of Artemisia Annua L. and Moringa Oleifera Lam. on CD4 count and viral load among PLWH on HAART at Mbarara Regional Referral Hospital: A Double-Blind Randomized Controlled Clinical Trial

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Abstract

Background

Initiation of HAART among people living with HIV (PLWH) having a CD4 count ≤ 350cells/μl, produces poor immunological recovery, putting them at a high risk of opportunistic infections. *Artemisia annua* and *Moringa oleifera* are among the herbs commonly consumed by PLWH on HAART to boost their immunity, but their clinical benefits and potential interactions with ARVs remain unknown. This study investigated the effect of *A.annua* and *M.oleifera* on CD4 count, viral load, and other clinical and haematological indices among PLWH on HAART at an HIV clinic in Uganda.

Methods

282 HIV-positive participants on HAART with a CD4 count ≤ 350cells/μl were randomized in a double-blind clinical trial to receive daily, in addition to their routine standard of care; 1) *A.annua* leaf powder, 2) *A.annua* plus *M.oleifera*, and 3) routine standard of care only. Our primary outcome was change in the CD4 count at 12 months. Secondary outcomes included change in viral load, complete blood count, renal function tests, liver function tests, ARV plasma levels, and quality of life (QoL). Participants were followed up for a year and outcomes were measured at baseline, 6 and 12 months.

Results

At 12 months of patient follow-up, administration of *A.annua + M.orifera* plus routine standard of care produced an absolute mean CD4 increment of 105.06 cells/μl, ($P < 0.001$), while administration of *A.annua* plus routine standard of care registered an absolute mean CD4 increment of 60.84 cells/μl, ($P = 0.001$) compared to the control group. The viral load reduced significantly ($P = 0.022$) for participants on the *A.annua + M.orifera* compared to those receiving standard of care only. There were significant differences in White blood cell count ($P = 0.03$), platelet count ($P = 0.025$), perceived QoL ($P < 001$) among participants who received *A.annua + M.oleifera* compared to those who received standard of care only. There were no significant differences in the other secondary outcomes.

Conclusion

A combination of *A.annua* and *M.oleifera* leaf powders taken once a day together with the routine standard of care produced significant improvement in CD4 count, viral load suppression, WBCs, platelets, and quality of life among individuals on HAART.

Introduction

HIV's Acquired Immune Deficiency Syndrome (AIDS) is one of the leading causes of morbidity and mortality worldwide [1, 2]. It is estimated that about 36.7 million persons are infected worldwide, with sub-Saharan Africa being the most heavily affected [2]. HIV is known to infect CD4 cells and its replication in these immune cells causes progressive lysis and reduction of the number and quality of functional immune cells. With time, the body fails to contain the viral replication, and immune deficiency sets in, being marked by low CD4 counts, and increased morbidity and mortality from opportunistic infections [3]. A low CD4 count, therefore, represents severe immune destruction, and participants who start ART at a lower baseline CD4 count have been demonstrated to have a poor immune recovery, and a high rate of transmitting HIV [3]. Data from the Comprehensive International Program of Research on AIDS also showed that initiating ART at CD4 cell counts below 200 cells/ml was associated with a four-fold increased risk of mortality and a two-fold increased risk of incident TB.

Although there is improved access to testing, and timely diagnosis for HIV, with increased rollout of HAART, many participants in resource-limited settings still initiate HAART when the HIV infection is in an advanced stage, with sub-optimal immunological recovery [4].

In Uganda, many HIV participants are reported to use herbal medicines before HAART enrolment, or in addition to HAART, including Aloe vera, Vernonia amygdalina and *M. oleifera* [5]. Some herbal therapies have been documented to have potent HIV-1 attachment inhibitors with clinical benefits [6]. Several medicinal plants have also been reported to have anti-HIV effects and immune enhancement effects; for example, *Artemisia annua*, also known as "African Wormwood" [7], has been demonstrated to have immunological effects in laboratory studies as well as anti-HIV effects in vitro [8]. *A. annua* powder which was shown to increase monocytes and lymphocytes levels in pre-clinical studies [8, 9] has been used by some HIV patients in Uganda.

On the other hand, *Moringa oleifera* Lam., is commonly consumed by HIV-infected people on antiretroviral therapy [10], with up to 80% of HIV participants in Africa, and Uganda consuming it as a main nutritional supplement. Moringa leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium, and potassium and act as good sources of natural antioxidants like ascorbic acid, flavonoids, phenolics, and carotenoids that work mutually to strengthen immunity [11, 12]. Individuals reported improved quality of life while using these powders [5]. However, the immunologic effect, clinical benefits, and potential interactions of *A. annua* powder and *Moringa oleifera* use with HAART among HIV-positive patients remained unknown, and have not been demonstrated in any controlled clinical study. This study investigated the effect of *A. annua* powder and *M. oleifera* plus routine standard of care on CD4 count, viral load, ART plasma levels, quality of life, and other clinical and hematological indices in PLWH on HAART for at least one year but with a persistently low CD4 count of ≤ 350cells/µl.

Methods

Study design and setting

We conducted a double-blind randomized controlled trial at the HIV clinic of Mbarara Regional Referral Hospital (MRRH), a publicly funded teaching hospital in southwestern Uganda serving ten districts, with a population of over 5 million people. The study was conducted between December 2017 and August 2020.

Recruitment Of The Study Participants

With the introduction of test and treat protocols in Uganda, our study population consisted of PLWH and on HAART for at least one year. A trained Research assistant screened all HIV-positive participants on HAART attending the MRRH HIV Clinic to identify those that were; 1) 18 years or older, 2) had a CD4 count ≤ 350cells/μl, 3) were living within a 60 km radius from the clinic for ease of transport, 4) had normal haematological, liver and renal function tests, and 5) were of sound mind and able to sign the informed consent. We excluded individuals who had existing opportunistic infections, used other herbal/complementary medicines, or were pregnant. The research assistants obtained informed consent from all participants. A study nurse trained in human participant research obtained informed consent in the local language in a private area of the hospital. All consenting participants gave written informed consent to read and sign in the presence of their witnesses, and for those who could not write, a thumbprint was made on the consent form.

Randomization, Blinding, And Preparation Of Treatments For The Study Participants

Participants were first stratified according to baseline CD4 levels: 350–250, 249–150, and 149 and below before randomisation to the three groups. Computer-generated numbers, delivered by an independent biostatistician were used to assign eligible participants to the three study groups using a simple randomization method in blocks of 10 to either the control group with HAART only, HAART with *A. annua* group, or HAART with *A. annua + M. oleifera* group using a ratio of 1:1:1.

Enrollment of the participants to the study arms/groups was carried out by the research assistants led by the principal investigator according to the computer-generated numbers. The two treatments; *A. annua* and *A. annua + M. oleifera* were separately prepared, packaged, and clearly labeled by the study pharmacist from a pharmacy before dispatch to the study clinic. Participants and research assistants were blinded to the specific name of the treatment they were getting as these were labeled as A (for *A. annua + M. oleifera)* or B (for *A. annua)*. Two research assistants were recruited and trained as observers and data collectors to increase accuracy and consistency in documenting the needed data. They were assisted by the HIV clinic staff and these research assistants were blinded to the hypotheses of this study to minimize observer bias. A senior clinician at the HIV clinic supervised the trial and administration of treatments for HAART participants attending the MRRH clinic. Both herbal treatments were procured from Jenna Herbals Ltd (Kampala, Uganda). Before the use of the medications, we performed quality tests for both intervention herbal materials at MUST Pharmaceutical Laboratory. To blind data collectors, treatments were packaged, labeled, and delivered in opaque parcels from the pharmacist at Devine

Hospital in Mbarara City. Trained research assistants were responsible for distributing *A. annua* and *M. oleifera* powders. Healthcare providers, including nurses, pharmacists, and doctors, as well as phlebotomists, were blinded to the allocation of participants to the study groups.

Study Procedures

Research assistants received opaque parcels already fixed with study codes. These parcels contained either 4g of *A. annua* and 10g of *M. oleifera* or its placebo (starch powder) which were to be taken at least 8 hours apart from each of the daily ARVs dosing. All participants were encouraged to take note and report all side effects.

Preparation of study materials

A. *annua* and *M. oleifera* leaf powders were derived from *A.annua* and *M. oleifera* plants grown in Uganda. They were authenticated by a botanist and specimens were stored in a herbarium at MUST. The leaf powders of *A. annua* and *M. oleifera* were processed and packaged in packets of 4g and 10g respectively by a pharmacist in a setting of good manufacturing practices at Devine Hospital. The concentration of artemisinin in our plant was 0.5 to 0.6% of the dry weight sourced from farmers in Kabale District in South Western Uganda.

Moringa oleifera Lam. is a herb commonly consumed by HIV-infected people on antiretroviral therapy mainly as a nutritional supplement [10]. *M. oleifera* has also been reported to be used in the management of HIV infections in up to 80% of HIV patients in Africa. According to Asare and colleagues [12], the intake of *M. Oleifera* is very safe at levels ≤ 1000 mg/kg body weight. Monera and colleagues also found out that co-administration of *M.oleifera* Lam. leaf powder at the traditional dose did not alter the steady-state pharmacokinetics of nevirapine in HIV-infected adults [10].

Administration Of Treatments

Participants in the *A. annua* study arm self-administered 4g of *A. annua* leaf powder per day pre-packaged in a single dose packet that was emptied in the routinely consumed porridge of choice (cassava, millet, or posho porridges) or water, and taken every morning at 8 am for 12 months. These participants did not receive *M. oleifera* but instead, they were given 10 g of starch powder to take with any porridge of choice at 8 am, or at least 8 hours apart from the routine ARVs dosing.

Participants in the *A. annua* + *M. oleifera* study arm were given 10 g and 4g of *M. oleifera* and *A. annua* powders respectively to take in their drink of choice (cassava, millet, or posho porridges) or water every morning at 8 am for 12 months. The porridge was preferred for uniformity, convenience, and masking the bitter taste of *A.annua* when taken in plain water.

Each parcel supplied to the participant contained herbal treatments to last one month and the parcels had participants' study numbers and dosing instructions in addition to other relevant information on storage and safety. To ensure adherence to the treatments, every participant received a reminder SMS every morning between 7 and 8 am. Participants were requested to respond with the message 'Taken" or a call prompt as a proxy to confirm adherence/ compliance to herbal medicine use. 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 consenting process, we emphasized to all participants that these herbal medicines are 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. All participants had their files with study numbers for easy identification. The study participants were reviewed once a month by the study clinician, except during emergencies where the participant could call on the study clinician as the need arose. Physical examination of the participants was done by the study clinician and documented in addition to self-report by the study participants. Performance status and quality of life were also evaluated and documented. Case report forms were used to capture participants' vital outcome data in addition to laboratory report forms.

Study Measures

A blood sample for the measurement of CD4 count, viral load, complete blood count, and liver and renal function tests was drawn immediately after enrolment but before initiation of herbal treatments, and at six and twelve months following initiation of treatment. An additional blood sample was drawn at 1- and 2 weeks following treatment initiation to assess ARV plasma levels. Research assistants interviewed all participants at baseline, 6 and 12 months to assess the quality of life (QoL) using the WHOQOL-BREF questionnaire [13]. Research assistants also recorded all reported side effects using a standardized data collection form.

Study Outcomes

Our primary outcome measure was a change in absolute and relative CD4 counts after twelve months of follow-up. The CD4 count difference was calculated by comparing the mean CD4 count at the exit with the mean CD4 count at baseline comparing intervention groups with control. Our secondary outcomes included; a change in viral load, complete blood count (CBC), liver function tests (ALT & AST), kidney function tests (creatinine and urea), QoL, antiretroviral plasma levels and reported side effects. Change in viral load, CBC, LFTs, RFTs, QoL, and side effects were determined after twelve months of follow-up. Changes in efavirenz and nevirapine plasma levels were determined after one and two weeks after herbal treatment initiation. The laboratory technicians were blinded by coding the different samples with

participants' unique identification numbers before delivery to the lab. The samples did not indicate any study arm allocated.

Quantitative questionnaire data on socio-demographics, prior herbal usage, depression, health [14] food insecurity[15], alcohol use [16], HIV stigma [17] and social support [18] and quality of life [13] were collected from study participants at enrolment.

Study Sample Size And Statistical Analysis

To compare the mean outcomes between each intervention group with the control group (using the t-test statistic) of a superiority trial, we considered our threshold probability for rejecting the null hypothesis α (two-tailed) to be 0.05. With power at 80% and a 1:1:1 ratio of subjects in each group. The mean CD4 among active participants attending the HIV clinic at MRRH, who have been on ART for more than 1 year and are 18 years or more, with a CD4 ≤ 350 after at least 1 year of initiation, was found to be at 160 (SD = 110). With that mean, we would require a sample size of 282 participants to detect a 30% CD4 count increase, at 80% power, 0.05 alpha, while assuming a 10% loss to follow-up. We would require a total of 94 participants in each of the 3 arms.

All data were cross-checked for completeness before entry. Data were collected using an online questionnaire developed in CommCare application by Dimagi. Data were downloaded in excel and exported into STATA Version 12 for statistical analysis. Data analysis was both by intention-to-treat (ITT) consisting of all participants randomized and thus supposed to be treated [19] and per protocol. Descriptive statistics were used to describe key characteristics of study participants. Different baseline variables were explored for normal distribution and comparison. Independent t-tests were used to compare the distributions and the means for each intervention group and the control. The means and mean differences were presented with their standard deviations. In the per-protocol analysis, the follow-up time for each participant was considered in the analysis, and the mean changes in CD4 count by follow-up time of measurements were compared. In both analyses, a difference was considered statistically different if p < 0.05. Secondary analysis compared the distribution of side effects such as nausea, dizziness, abnormal CBC, and kidney or liver function tests.

The WHOQOL-BREF questionnaire was used to collect data [13]. This questionnaire contains 26 questions, and 4 domains; physical, psychological, environmental, and social questions. Questions were presented on a linkert scale of 1–5, with 1 as poor and 5 as excellent. We transformed the raw linket scores of 1–5 into 1-100 using STATA V12 to enable us make uniform comparisons, especially for the domains that have unequal number of items. We compared the mean QoL scores across all domains using independent t-test.

Ethical approval

The study was approved by the MUST Research Ethics Committee with registration number 27/05-17 and Uganda National Council for Science and Technology (UNCST). The study was also registered with ClinicalTrials.gov NCT03366922. Written informed consent was obtained from each participant and all the procedures used were per the ethical standards of the responsible committee on human experimentation according to the Helsinki Declaration. Participants' identities were kept anonymous throughout the study process.

All treatments administered to participants have previously been documented to be safe when used for prophylaxis or as a supplement [8, 20, 21]. Monthly reviews by a senior clinician were provided in this study, samples and results of ART plasma levels, CD4 cell count, viral loads, liver function, and kidney function tests were provided to the HIV clinic physicians for monitoring. Although severe side effects were not expected, proper management and routine care protocols were adhered to following any such reports. All adverse events were brought to the attention of the Data and Safety Monitoring Committee and Institutional Review Committee in case of safety issues.

Data safety and monitoring

The data and safety monitoring committee was constituted and included senior medical scientists to ensure the safety of participating individuals. Three safety checks were done at 1 month, at 50%, and 75% of recruitment. The data safety management board members were routinely given laboratory and clinical data of all the participants to review and advise the MUST Ethics Committee and the UNCST in Kampala, Uganda where this study was also registered.

Results

Out of the 1844 HIV-positive participants screened for eligibility from December 2017 at Mbarara regional referral hospital HIV clinic in rural Southwestern Uganda, 319 were eligible. A total of 37(11.6%) declined participation in the study (Figure 1), and 282 were randomized and enrolled to receive different treatments. A total of 248 (87.9%) completed all study procedures, 26 (9.2%) participants were lost-to-follow-up, and 8 (2.8%) participants became pregnant and were discontinued from the study. Eighty-three (33%) of the enrolled participants were female and the median age was 39 years (IQR=32,47). Other clinical and demographic characteristics for the three treatment groups at baseline were similar (Table 1). In terms of outcomes, we observed a statistically significant difference when comparing CD4 counts at baseline with CD4 counts at month 12 for each group. At 12 months of patient follow-up, administration of *A.annua* + *M.orifera* produced an absolute mean CD4 increment of 105.06 (SD=17.25) cells/µl, (P <0.001), while administration of *A.annua* only registered an absolute mean CD4 increment of 60.84 (SD=17.20) cells/µl, (P = 0.001) compared to the control group (Table 2). We also calculated the relative difference in CD4 count. At 12 months of patient follow-up, administration of *A.annua* + *M.orifera* showed a relative mean CD4 increment of 30.45 (6.14) cells/µl (P <0.001) while administration of *A.annua* only presented an absolute mean CD4 increment of 26.04 (6.12) cells/µl (P = 0.001) compared

to the control group. There was no statistically significant difference in the absolute and relative CD4 counts nor viral load at 6 months in both treatment intervention groups compared to the control group (Table 2 and Figure 2).

Our data observed that there were more participants with a viral load less than 50 copies/ml in the Artemisia plus Moringa (67, 77.9%), and Artemisia only (61, 69.3%) compared to the control group (P=0.038) (Table 3). Although the mean viral loads decreased steadily for all intervention participants throughout the follow-up period, the absolute differences were not statistically significant (Figure 2). However, when we logarithmically transformed the viral load data, participants who were treated with a combination of *A. annua* and *M. oleifera*, had the potential to suppress viral load by 2.5 times more (mean reduction -2.56 copies /ml; SD = 3.17; P = 0.022) compared to participants receiving standard of care. Participants receiving *A. annua* alone did not show a statistically significant difference in viral load when compared to the control group (mean reduction -1.17 copies /ml; SD = 2.32; P = 0.546) (Table 3). The White blood cell count (Mean difference =2.08 copies /ml, SD = 4.6, P = 0.03), and platelet count (Mean difference =11.04; SD = 3.29; P = 0.025) of participants that received *A. Annua* plus *M. Oleifera* were higher than those individuals who were enrolled in the standard of care (control) group. We also did not observe a statistically significant difference in plasma levels among participants on the efavirenz regimen and those on the nevirapine regimen at one and two weeks following treatment intervention initiation, indicating no interaction between *A. annua* and *M.oleifera* with the referent ARV drugs (Table 3).The Liver function and Kidney function tests, as well as the reported side effects, were generally similar at 12 months follow-up across the 3 groups. We recorded one death in the control group after 5 months into the study. There was generally a significant improvement in the reported QoL, with significant mean differences in the baseline and 12-month scores observed in the physical, social and psychological domains of the WHOQOL-BREF tool for both treatment arms; the highest mean difference scores were reported in the psychological domain for *A. annua* arm (15.7, p = 0.003) and 29.02, p < 001) for the *A.annua* $_+$ *M.oleifera* (Table 4).

Table1. Baseline demographic and clinical characteristics of participants by treatment group

Characteristic	Control Mean (SD) or n (%); (n=74)	Artemisia Mean (SD) or n (%); (n=88)	Artemisia +Mor. Mean (SD) or n (%) (n=86)	P value
Mean age (years) (SD)	39.2 (8.7)	40.9 (9.8)	39.6 (9.9)	0.73
Female gender (%)	25 (33.7)	32 (36.4)	26 (30.2)	0.69
BMI (kg/m^2) ≥25 (%)	11 (14.9)	17 (19.3)	12 (14.0)	0.90
Regular income	10 (13.5)	8 (9.1)	5 (5.8)	0.23
Education level > primary	64 (86.6)	76(86.4)	75(87.2)	0.42
Use of other herbal supplements	26 (35.1)	31 (35.2)	30 (34.8)	-
H/O hospitalization in the last 2 years	22 (29.7)	31 (35.2)	30 (34.8)	0.40
H/O medication defaulting	2(2.7)	0(0)	0(0)	0.80
Severe Food insecurity[a]	13 (17.6)	13 (14.8)	14 (16.3)	0.97
Mean no of adults in household (SD)	2.2 (1.2)	2.3 (1.3)	2.3 (1.3)	0.52
Depression score 0[b]	73 (98.6)	87 (98.9)	86 (100)	0.58
Stigma[c] (moderate to high)	69 (93.2)	80 (90.9)	81 (94.2)	0.84
Mean social support score (SD)	2.7 (1.2)	2.8 (1.3)	2.8 (1.41)	0.273
Overall mean QoL Raw Item Score (SD)	1.82 (0.63)	1.54 (0.72)	1.77 (0.33)	0.77
Disclosed HIV serostatus to a sexual partner	68 (91.9)	82 (93.2)	77 (89.5)	0.52
Mean duration on HAART (months) (SD)	47 (8.5)	45 (4.6)	48 (6.9)	0.76
MeanWBC (10^3 cells/µl) (SD)	3.4 (2.7)	3.5 (2.3)	3.2 (2.3)	0.23
Mean RBC (10^6 cells/µl) (SD)	4.5 (0.8)	4.4 (0.6)	4.5 (0.6)	0.33
Mean Hb level (g/dl) (SD)	14.3 (4.6)	13.7 (2.8)	14.0 (2.9)	0.76

Mean CD4 (cells/µl) (SD)	221.8 (88.9)	214.9 (86.6)	227.5 (75.7)	0.89
Mean VL (copies /ml) SD	7243.4 (27443.9)	9012.9 (45626.0)	27186.3(138408.9)	0.95
ART regimen				
AZT/3TC/EFV	20 (27.0)	24 (27.3)	23 (26.7)	0.31
AZT/3TC/NVP	13 (17.6)	15 (17.0)	16 (18.6)	
3TC/EFV/TDF	41 (55.4)	49 (55.6)	47 (54.7)	

[a]HFIAS>8 means severe food insecurity, [b] this score ranges from1-48 indicating 0 as no depression, [c] Score for stigma ranging from 1-8, with 8 indicating high levels of HIV stigma. [d] this score ranges from 1-4, with 4 indicating high levels of social support. ART antiretroviral therapy; AZT Zidovudine; 3TC Lamivudine; EFV Efavirenz; H/O History of; NVP Nevirapine; TDF Tenofovir; USD US Dollar.

Table 2. Effect of *A.annua, and A.annua + M.orifera* on CD4 count after 1 year of treatment

Categories	Mean difference	Standard deviation	p-value	95%CI
CD4 absolute diff at 6 months				
Control				
Artemisia	8.18	±14.85	0.582	-21.07 - 37.43
Artemisia + Moringa	12.82	±14.97	0.860	-16.677 - 42.30
CD4 absolute diff at 12 months				
Control				
Artemisia	60.84	±17.20	0.001*	26.96 - 94.72
Artemisia + Moringa	105.06	±17.25	<0.001*	71.09 - 139.02
CD4 relative diff at 6 months				
Control				
Artemisia	2.06	±6.21	0.741	-10.18 - 14.29
Artemisia + Moringa	2.70	±6.26	0.667	-9.63 - 15.03
CD4 relative diff at 12 months				
Control				
Artemisia	26.04	±6.12	0.001*	13.98 - 38.10
Artemisia + Moringa	30.45	±6.14	<0.001*	18.36 - 42.54

Statistically significant result ($p<0.05$).

Table 3. Effect of *A.annua, and A.annua + M.orifera* on secondary outcomes after 12 months of treatment

Other secondary outcomes	Mean difference (SD) or frequency (%)	p-value
Viral load<50 copies, n (%)		
Control	44 (59.5)	0.038*
Artemisia	61(69.3)	
Artemisia + Moringa	67 (77.9)	
Mean reduction in viral load per ml		
Control	-418.25 (21840.98)	
Artemisia	-7192.21 (42719.10)	0.467
Artemisia + Moringa	-27286.18 (139215.80)	0.060
Mean LVL		
Control	-0.75 (2.47)	
Artemisia	-1.17 (2.32)	0.564
Artemisia + Moringa	-2.56 (3.17)	0.022*
Mean increment in white blood cell count		
Control	1.21 (2.67)	
Artemisia	1.14 (2.29)	0.080
Artemisia + Moringa	2.08 (4.60)	0.003*
Mean increment in red blood cell count		
Control	0.08 (0.80)	
Artemisia	0.03 (0.70)	0.793
Artemisia + Moringa	0.11 (0.54)	0.133
Mean increment in hematocrit levels		
Control	0.43 (6.45)	
Artemisia	1.28 (3.97)	0.734
Artemisia + Moringa	1.48 (3.92)	0.067
Mean MCV increment		
Control	1. 91 (9.77)	
Artemisia	5.22 (8.45)	0.205
Artemisia + Moringa	16.23 (5.59)	0.141

Mean increment in platelets levels

Control	-7.67 (7.73)	
Artemisia	-15.68 (10.06)	0.952
Artemisia + Moringa	11.04 (3.29)	0.025*

Mean plasma levels of efavirenz and nevirapine (mg/l) (SD)

EFV (n=67)	Baseline	4.66 (0.76)	0.987
	Week 1	4.66 (0.76)	
	Week 2	4.51 (0.68)	
NVP (n=38)	Baseline	7.86 (1.21)	0.991
	Week 1	7.81 (1.05)	
	Week 2	7.67 (0.94)	

Kidney Function tests (Creatinine and or Urea) out of normal range at exit, n (%)

Control	3 (4.1)	0.437
Artemisia	4 (4.5)	
Artemisia + Moringa	3 (3.5)	

Liver Function tests (ALP and or AST) out of normal range at exit, n (%)

Control	14 (18.9)	0.228
Artemisia	17 (19.3)	
Artemisia + Moringa	15 (17.4)	

Other reported side effects, n (%)

Nausea

Artemisia	13 (14.8)	0.324
Artemisia + Moringa	17 (19.8)	

Dizziness

Artemisia	7 (8.0)	0.941
Artemisia + Moringa	7 (8.1)	
Deaths, n (%)		
Control	1 (1.4)	

Statistically significant result (p<0.05).

EFV: efavirenz; mcv: mean corpuscular volume; mch: mean corpuscular hemoglobin; mchc: mean corpuscular hemoglobin concentration; NEV: nevirapine; rbw: red blood cell width; VL: Viral load; LVL: log transformed viral load.

Table 4. Mean differences in Quality-of-life scores (SD) across the 4 domains of the WHOQOL-BREF questionnaire

Domain	Artemisia Arm	Artemisia + Moringa arm	Control	P value	
				Artemisia & Control arms	Moringa arm & control arms
Physical health	7.30 (2.72)	11.23 (3.01)	3.62 (2.53)	0.292	0.001*
Social relations	5.62 (1.03)	13.04 (2.92)	5.01(1.76)	0.714	0.012*
Psychological health	15.47 (4.19)	29.02 (4.02)	6.18 (3.89)	0.003*	0.001*
Environmental	4.20 (0.77)	9.03 (2.14)	4.44 (1.29)	0.319	0.067

Discussion

Among PLWH who had been on HAART for at least one year with CD4 count ≤350cells/µl, administration of *A.annua + M.orifera* for 12 months alongside routine standard of care increased their mean CD4 count by 105.06 (SD=17.25) cells/µl, *(P* <0.001), while administration of *A.annua* alone increased mean CD4 count by 60.84 (SD=17.20) cells/µl, (P = 0.001) when compared to individuals that received standard of care only. The viral load also progressively reduced throughout the follow-up period for both intervention groups, and significantly for participants that received both *A.annua* and *M*.oleifera. The viral load suppression to <50 copies/ml was significantly better, with a 2.5 times more suppression rate observed among participants that received *A.annua* and *M*.oleifera than those that received the standard of care alone. White blood cell and platelet counts increased by 2.08 and 11.04 points respectively among participants who received *A.annua + M.oleifera*. We also observed generally improved QoL scores among participants enrolled in both intervention groups. There were no significant differences between groups in any other secondary outcomes, including the LFTs, RFTs, and ARV plasma levels.

We observed an increment in the CD4 cell count in the *A.annua + M. oleifera* group at 12 months. Our data contribute to an important finding and demonstrate the potential of *A.annua* and *M.orifera* to improve the health and treatment outcomes among PLWH on HAART. This finding is in agreement with a study carried out by[22] which revealed that *Moringa oleifera* leaf supplementation was associated with increased CD4 cell counts of PLHIV on ART in a resource-limited setting in Nigeria. This study recommended *M. oleifera* supplementation as part of a comprehensive approach to ensure optimal treatment outcomes in PLWH. [23],

We observed that a combination of *A.annua* and *M. oleifera* produced a superior increase in absolute CD4 count (105.06±17.25 cells/μl) compared to the treatment with Artemisia alone (60.84±17.20 cells/μl). This may be because combinations of two or more phytochemicals bring about changes in the ultimate biological effects and/or the bioavailability of each component. Several mixtures of pure bioactive compounds or phytochemical-containing plant extracts are reported to provide synergy with regard to antioxidant status, anti-inflammation, anti-cancer, and chemoprevention of several oxidative stress and metabolic disorders *in vitro* [24]. These effects of phytochemical interactions can be classified as addition, synergy, or antagonism. In our study, *M. oleifera* produced a synergistic effect since the CD4 count was higher in the combination group than in the *A.annua* only group. This is a very useful finding because these two plants can be used as adjuvants to ART to improve health outcomes among PLWH since the plants can be cultivated in homes. Both these plants have previously been documented to be safe when used for prophylaxis or as a supplement, [20], and so this combination may provide a readily available and affordable source of supplement that can improve suppression of HIV viral replication and enabling CD4 regain to restore the body's ability to fight against opportunistic infections [3].

We also observed that the CD4 count increased gradually in the intervention groups but the increment did not become statistically significant until month 12. This finding indicates that both *A.annua* and *M.oleifera* if combined with HAART gradually increase CD4 count among PLWH. Our findings are in agreement with a study carried out by in Nigeria which showed a gradual increase in CD4 count and a decrease in viral load but with no statistical difference among treated HIV-positive participants on HAART with *M.oleifera* powder after six months when compared to the control. A study by Ogwang et al 2012 also indicated that Artemisia gradually increases white cell counts with monocytes and lymphocytes increasing becoming significant by 6 and 12 months respectively.

In this study, we observed a statistically significant increment in the white blood cells and platelets counts in the *A.annua + M. oleifera* group compared to participants that were on the standard of care alone. This observation is similar to the study of [25], which was carried out in Nigeria, in which platelet count increased significantly at the end of the study although the white cell count decreased. This could be probably because our study investigated a combination of *A.annua* and *M.oleifera* while Adegbite's investigated the effect of M.oleifera alone. Platelets are important in the formation of platelet plugs during normal haemostasis, clot retraction, and coagulation factor activation. Our findings show that a combination of A.*annua* and *M. oleifera* may provide an alternative method for treating bleeding disorders. White blood cells are involved in protecting the body from infections and among other functions, they kill virus-infected cells, enhance the production of antibodies and engulf foreign materials that enter the body. Our findings are in agreement with findings of which show that consumption of *A.annua* tea increased white blood cell count [26].

Treatment with *A.annua* and *M.oleifera* in addition to HAART did not affect efavirenz and nevirapine plasma levels as a secondary outcome (Table 3). This means that these two plants may not significantly induce or inhibit the production cytochrome P450 2B6 enzyme which primarily metabolizes these two ARVs in the liver [27]. Our findings are in agreement with the study done by [10] who reported no clinically

significant interaction between *M.oleifera* and nevirapine. Our finding is in contrast with the findings of [28] who found that extracts and teas of both *A. annua* and *A. afra* inhibited both CYP2B6 and CYP3A4 activity, the key enzymes responsible for the metabolism of efavirenz and nevirapine. This is probably because their study was done using plant extracts in a rodent model while we used dry plant leaf powders in humans and followed them up to one year after treatment initiation.

Our findings also indicate that *M.oleifera* and or *A.annua* may be safe for use in people on HAART since we did not find differences in the liver and kidney function tests and other reported side effects (nausea and dizziness) among the participants in the control group and the intervention groups. However clinical trials with larger sample sizes are recommended. One death was reported in the control group. Some scholars have indicated that *A.annua* was associated with a form of 'natural healing' as it represented a notion of 'harmlessness' and 'free from side effects' compared to 'modern' pharmaceuticals among HIV patients in Tanzania [29]. Monera-Penduka and colleagues [10] also observed that *M.oleifera* has a good safety profile consistent with its long history of use as food and medicine among HIV-positive participants in Zimbabwe.

Health-related QoL is increasingly recognized as an important outcome in randomized trials [30] because it provides valuable information to assess the health status and well-being of PLWH [31, 32]. We observed a significant improvement in the reported QoL, with significant mean differences between the baseline and 12-month scores observed in the physical, social and psychological domains of the WHOQOL-BREF tool for both individuals using *A. annua* and *M. oleifera* (Table 4). Our findings also indicate that the highest mean difference scores were reported in the psychological domain for both *A. annua*arm (15.7, p = 0.003) and *A.annua* $_+$ *M.oleifera* (29.02, p < 001). These findings are in line with previous studies [31, 33, 34], and the findings provide a useful assessment of how PLWH participants' lives are affected by treatment interventions. The high psychological score may also generally play into [35] improved mental health, adherence to treatment, higher immune response to HIV infection, and well-being of individuals.

Our study had several strengths. All study investigators and clinical staff were blinded to treatment allocation. Although blinding might have been unmasked, particularly by the widely peculiar bitterness of *A. Annua*, we mimicked this with porridge and starch which were taken together at the time of dozing. We also used dried plant powders prepared, packaged, and delivered by an independent study pharmacist, and consumed at least 8 hours apart from the routine HAART dosing to avoid interaction. We also used readily available *A. annua* and *M. oleifera* which may provide an affordable alternative remedy that can induce suppression of HIV viral replication allowing CD4 to regain and restore the body's ability to fight against opportunistic infections, especially in low-resource communities which do not have easy and regular access to HAART. We carried out our clinical trial in a prototypical, publicly funded, and operated hospital in a rural setting with an active HIV clinic managing over 10,000 PLWH, and subject to the standard limitations of public sector health care facilities in a low-resource setting. As such, the study has great potential for generalizability to similar settings. We observed a small rate of eligible participants declining participation (n =37, 11.6%) in our study, mainly because they were disinterested in participating in a non-conventional medicine clinical trial, which was perhaps not unexpected given the stigma on

herbal medicine use in a clinical setting. Despite the strengths pointed out, our study had some important limitations. We observed no mortality in the treatment groups and a good pickup rate for CD4 count and or viral load by the end of the 12-month study period, which may suggest the presence of strict exclusion criteria or the adherence-related possibility of a Hawthorne effect by study participants. We also noted a higher dropout rate, especially in the control arm mainly due to challenges of COVID-19 lockdown such as; transportation, and food insecurity as these were not followed up with SMS reminders to aid compliance/adherence. We also did not specifically maintain objective measures of ART adherence (ART plasma levels, electronic devices, etc) throughout the study period. Although we suspect this may affect the overall outcome, the overall effect may have been diminished by maintaining routine HIV clinical care, refills, and reviews by our study clinician based at the HIV clinic. We also recruited relatively healthy PLWH and therefore our results may not be generalizable for individuals with renal, and liver function problems, existing opportunistic infections, or pregnant.

Conclusion And Recommendation

We found that daily consumption of 10g of *M.oleifera and or 4g of A.annua* leaf powders only given alongside routine standard of care in MRRH improved CD4 count among PLWH who had been on HAART for at least one year and remained with a CD4 count of <350 copies/ml. The addition of *A.annua* and *M.oleifera* leaf powders also improved viral load suppression, WBC count, platelet count, and overall quality of life among PLWH on HAART. Our data also shows that daily consumption of 10g of *M.oleifera and or 4g of A.annua* leaf powders did not affect the ARV blood levels, liver or kidney functionality over one year. These data demonstrate that *A.annua* and *M.oleifera* appear to maintain an important role in effecting HIV-1 activity and with a relatively good safety profile. Based on their HIV-1 activity and good safety profile, further research could be done to rigorously measure and evaluate HAART adherence to rule out differences and its impact across groups. Some clinical studies have shown that these herbal medicines might have the potential to alleviate symptoms, reduce viral load, and increase CD4+ cells for PLWH especially if consumed for a longer period. Our findings show that the participants' viral loads in both intervention arms decreased gradually throughout the 12 months of the study period. Although our findings indicated a potential synergistic effect of *M. oleifera* on *A. annua,* more research is needed to establish the sustained effect of this combination on CD4 count, viral load, CBC, LFT, RFT, and ARV plasma levels over long periods or 5 or more years.

Abbreviations

AIDS: Acquired immune deficiency syndrome; AMAMED: Action for Natural Medicine in the Tropics ART: Antiretroviral therapy; BMI: Body mass index; CD4: Cluster de differentiation 4; CI: Confidence interval; HAART: Highly active antiretroviral therapy; HIV: Human immunodeficiency virus; MUST: Mbarara university of science and technology; PLWH: People living with HIV; QoL: Quality of Life; SD: Standard deviation; SEM: Standard error of mean; UNCST: Uganda national council for science and technology; WHO: World health organization; WHOQOL: World Health Organization Quality of Life

Declarations

Authors' contribution

STS, analyzed the data and drafted the manuscript.

ECA, OPE, and FR designed the study and reviewed the manuscript. JKM conducted the HPLC analysis of the drug levels. All authors read and approved the final manuscript.

MN analyzed the data.

Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request

Consent for publication

Participants' consent was obtained for the study and publication of these data.

Competing Interests

The authors declare that they have no competing interests.

References

1. El-Magd, M., *The effect of Moringa oleifera cookies on CD4+ T cells number and hemoglobin level in HIV patients: A randomized controlled trial.* 2020.
2. Vithalani, J. and M. Herreros-Villanueva, *HIV Epidemiology in Uganda: Survey based on age, gender, number of sexual partners and frequency of testing.* African health sciences, 2018. **18**(3): p. 523-530.
3. Gunda, D.W., et al., *Prevalence and risk factors of poor immune recovery among adult HIV patients attending care and treatment centre in northwestern Tanzania following the use of highly active*

antiretroviral therapy: a retrospective study. BMC research notes, 2017. **10**(1): p. 1-6.

4. Reda, A.A. and S. Biadgilign, *Determinants of adherence to antiretroviral therapy among HIV-infected patients in Africa.* AIDS Research and treatment, 2012. **2012**.

5. Lubinga, S., et al., *Concomitant herbal medicine and Antiretroviral Therapy (ART) use among HIV patients in Western Uganda: a cross-sectional analysis of magnitude and patterns of use, associated factors and impact on ART adherence.* AIDS care, 2012. **24**(11): p. 1375-1383.

6. Helfer, M., et al., *The root extract of the medicinal plant Pelargonium sidoides is a potent HIV-1 attachment inhibitor.* PloS one, 2014. **9**(1): p. e87487.

7. Rath, K., et al., *Pharmacokinetic study of artemisinin after oral intake of a traditional preparation of Artemisia annua L.(annual wormwood).* American Journal of Tropical Medicine and Hygiene, 2004. **70**(2): p. 128-132.

8. Lubbe, A., et al., *Ethnopharmacology in overdrive: the remarkable anti-HIV activity of Artemisia annua.* Journal of Ethnopharmacology, 2012. **141**(3): p. 854-859.

9. Ogwang, P.E., et al., *Use of Artemisia annua L. infusion for malaria prevention: mode of action and benefits in a Ugandan community.* British Journal of Pharmaceutical Research, 2011. **1**(4): p. 124.

10. Monera-Penduka, T.G., et al., *Effect of Moringa oleifera Lam. leaf powder on the pharmacokinetics of nevirapine in HIV-infected adults: a one sequence cross-over study.* AIDS research and therapy, 2017. **14**(1): p. 1-7.

11. Deshmukh, P., et al., *Immunomodulatory activity of Moringa oleifera in albino rats.* Journal of Animal Research, 2015. **5**(2): p. 277.

12. Asare, G.A., et al., *Toxicity potentials of the nutraceutical Moringa oleifera at supra-supplementation levels.* Journal of ethnopharmacology, 2012. **139**(1): p. 265-272.

13. Organization, W.H., *The world health organization quality of life (WHOQOL)-BREF.* 2004, World Health Organization.

14. Bolton, W.K., et al., *Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy.* American journal of nephrology, 2004. **24**(1): p. 32-40.

15. Tsai, A.C., et al., *Food insecurity, depression and the modifying role of social support among people living with HIV/AIDS in rural Uganda.* 2012. **74**(12).

16. Bush, K., et al., *The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking.* Archives of internal medicine, 1998. **158**(16): p. 1789-1795.

17. Kalichman, S.C., et al., *Measuring AIDS stigmas in people living with HIV/AIDS: the Internalized AIDS-Related Stigma Scale.* AIDS care, 2009. **21**(1): p. 87-93.

18. Broadhead, W., et al., *The Duke-UNC Functional Social Support Questionnaire: Measurement of social support in family medicine patients.* Medical care, 1988: p. 709-723.

19. Lesaffre, E., *Superiority, equivalence, and non-inferiority trials.* Bulletin of the NYU hospital for joint diseases, 2008. **66**(2).

20. Andrade, R.J., et al., *Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period.* Gastroenterology, 2005. **129**(2): p. 512-521.

21. Lubbe, A., et al., *Ethnopharmacology in overdrive: the remarkable anti-HIV activity of Artemisia annua.* 2012. **141**(3): p. 854-859.

22. Gambo, A., et al., *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.* PloS one, 2021. **16**(12): p. e0261935.

23. Tshingani, K., et al., *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 complementary and alternative medicine, 2017. **17**(1): p. 1-13.

24. Phan, M.A.T., et al., *Interactions between phytochemicals from fruits and vegetables: Effects on bioactivities and bioavailability.* Critical reviews in food science and nutrition, 2018. **58**(8): p. 1310-1329.

25. Adegbite, O., et al., *Effects of Moringa oleifera leaves on hematological indices in humans.* Ann Hematol Oncol, 2016. **3**(8): p. 1107.

26. Ogwang, P.E., et al., *Artemisia annua L. infusion consumed once a week reduces risk of multiple episodes of malaria: a randomised trial in a Ugandan community.* Tropical Journal of Pharmaceutical Research, 2012. **11**(3): p. 445-453.

27. Maseng, M.J., et al., *Association of CYP2B6 Genetic Variation with Efavirenz and Nevirapine Drug Resistance in HIV-1 Patients from Botswana.* Pharmacogenomics and Personalized Medicine, 2021. **14**: p. 335.

28. Desrosiers, M.R., A. Mittleman, and P.J. Weathers, *Dried leaf Artemisia annua improves bioavailability of artemisinin via cytochrome P450 inhibition and enhances artemisinin efficacy downstream.* Biomolecules, 2020. **10**(2): p. 254.

29. zu Biesen, C.M., *Notions of Efficacy around a Chinese Medicinal Plant: Artemisia annua–an Innovative AIDS therapy in Tanzania.* Religion and AIDS Treatment in Africa: Saving Souls, Prolonging Lives, 2016: p. 271.

30. Johnston, B.C., et al., *Improving the interpretation of quality of life evidence in meta-analyses: the application of minimal important difference units.* Health and quality of life outcomes, 2010. **8**(1): p. 1-5.

31. Khademi, N., et al., *Quality of life of HIV-infected individuals: insights from a study of patients in Kermanshah, Iran.* BMC Infectious Diseases, 2021. **21**(1): p. 1-12.

32. Hsiung, P.-C., et al., *Comparison of WHOQOL-BREF and SF-36 in patients with HIV infection.* Quality of Life Research, 2005. **14**(1): p. 141-150.

33. Dhakne, S., S. Dargar, and P.S. Tawri, *Assessment of quality of life and its associated factors among people living with HIV/AIDS.* AIDS, 2018.

34. Nobre, N., et al., *Quality of life of people living with HIV/AIDS: a cross-country comparison study of Finland and Portugal.* 2016. **28**(7): p. 873-877.

35. Nobre, N., et al., *Quality of life of people living with HIV/AIDS: a cross-country comparison study of Finland and Portugal.* AIDS care, 2016. **28**(7): p. 873-877.
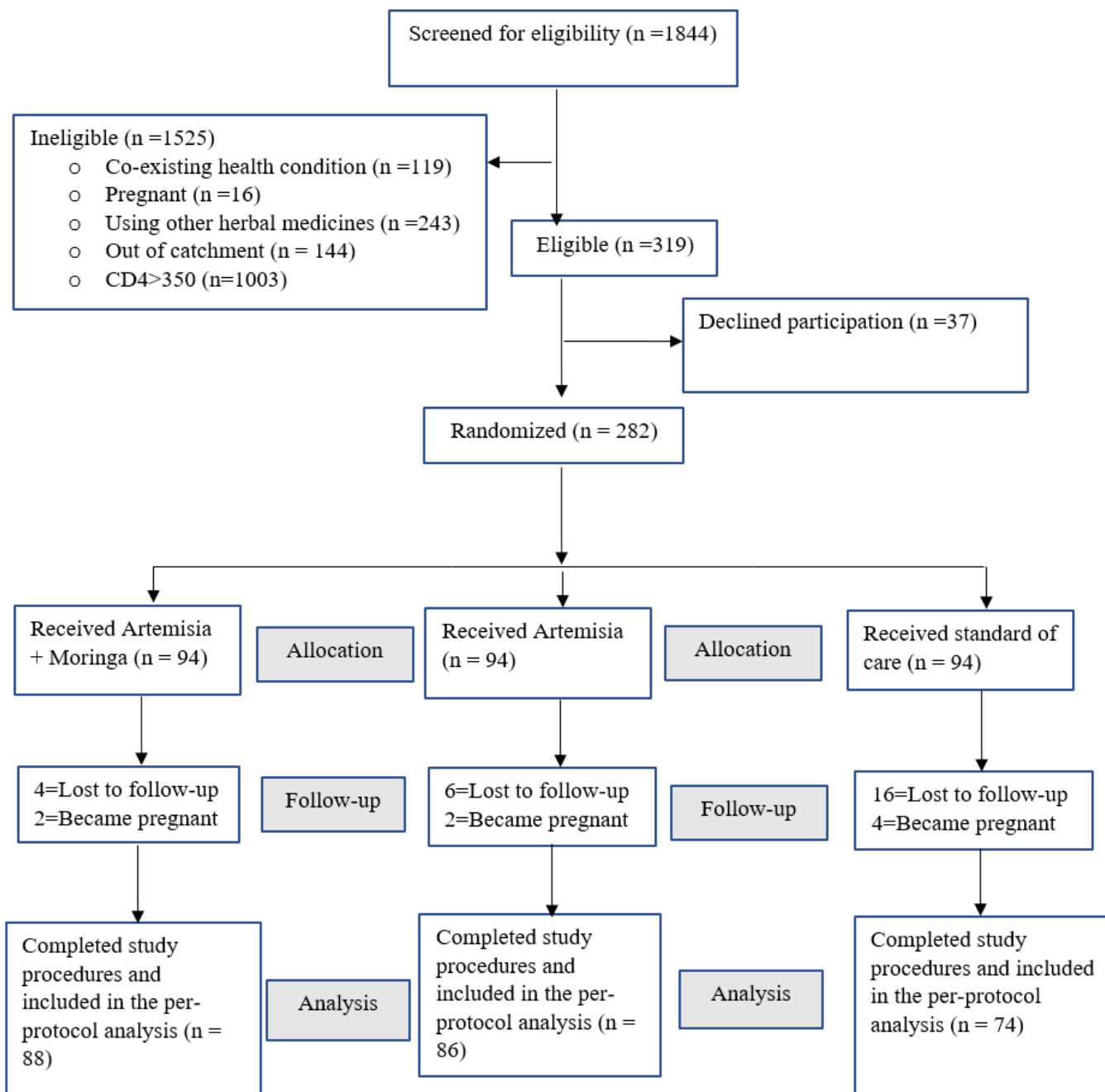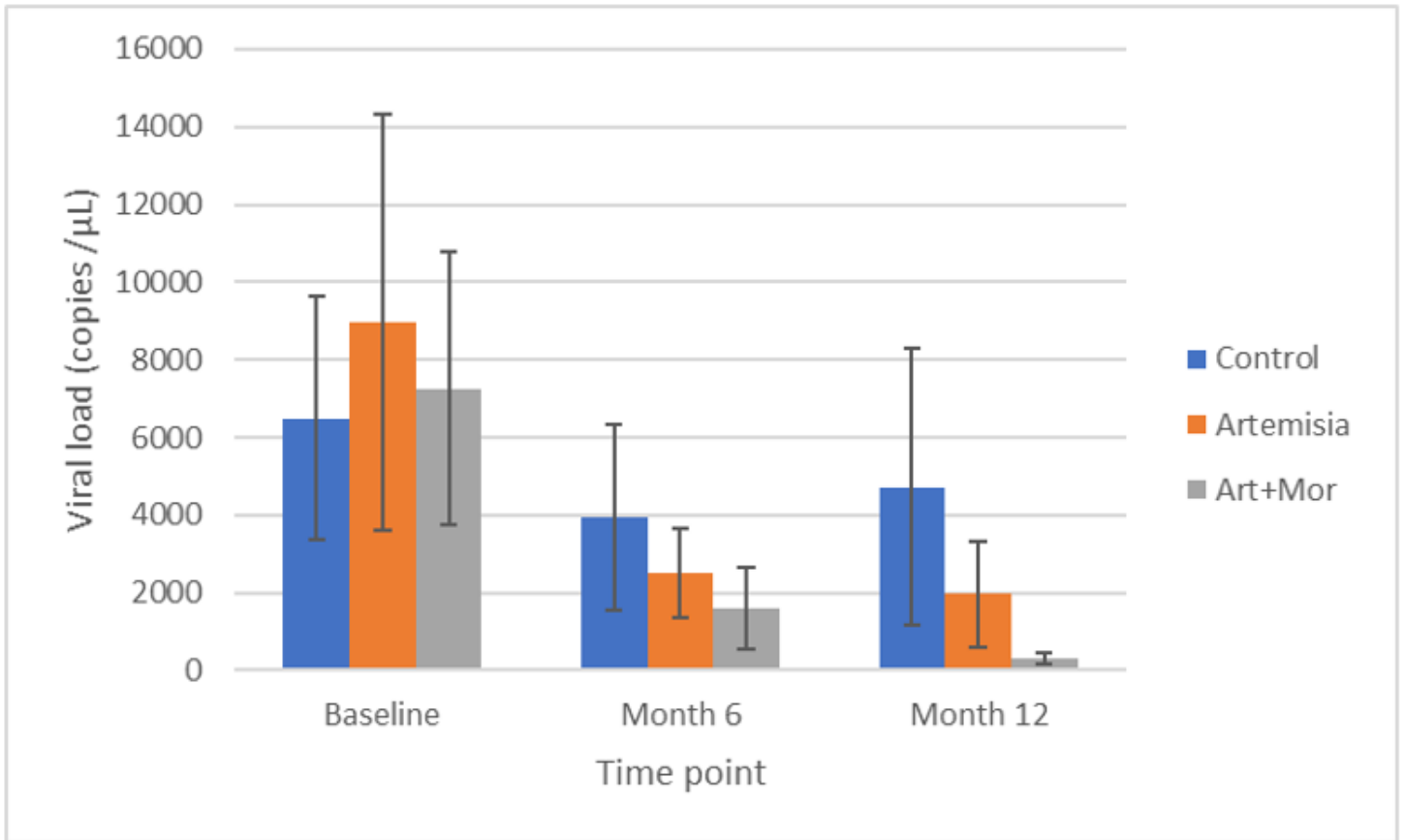
Figures

Figure 1

Trial profile

Figure 2

Effect of *A.annua* and *M. oleifra* on viral load among participants in the three study arms throughout the study period