Original Research Article

Effect of *Moringa oleifera* supplementation on CD4+ and CD8+ T cell patterns and renal functions of HIV/AIDS patients receiving highly active antiretroviral drugs (HAART)

ABSTRACT

Aims:HIV infectionis managed by expensive ARVs that cause side effects and lead to drug resistance and drug failure. The use of nutritional remedies in the treatment of HIV/AIDS is becoming popular. *Moringa oleifera* is a nutrient-dense plant with medicinal properties that results from its wide range of phytochemicals. The main objective of this study was toinvestigate the effect of *Moringa oleifera* supplementation on CD4+ and CD8+ T cell patterns, and also renal functions of HIV/AIDS patients receiving highly active antiretroviral drugs (HAART).

Study Design: A quasi-experimental study of regression discontinuity type was carried out at the Comprehensive Care Center ofMbagathi County Hospital, Nairobi, Kenya.

Methodology: One hundred and seventy-three HIV seropositive participants treated with ARV and attending the regular clinic were assigned to the intervention group (n=99)supplemented with *Moringa oleifera* leaf powder or the control group (n=74)that was not supplemented. The primary endpoints for this study were the safety and influence of *M. oleifera*supplementation on CD4+ and CD8+ T cell patterns. The results were obtained as computer printouts of the BD FACSCalibur analyzer for the CD4 and the CD8 T cell counts and the HumaLyzer Primus Chemistry Analyzer for creatinine levels, and analyzed using the computer software XLSTAT 2021.2.1. Any time-dependent variations within the individual groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests. Variations between the two study groups were analyzed using unpaired *t*-tests. *P* values less than 0.05 were considered statistically significant.

Results: No significant differences in CD4+ (p= 0.523) and CD8+ T cell counts (p= 0.908),werefound between the supplemented and non-supplemented groups. However, *M. oleifera*supplementation did not havean effect on renal function.

Conclusion: The study concluded that*M. oleifera*supplementation does not have an effect onimmune functions in HIV/AIDS patients. Consumption of *M. oleifera* is safe.

Keywords*: Moringa oleifera*, *Supplementation, CD4, CD8, HIV, ARVs, Kenya*.

1. INTRODUCTION

Human immunodeficiency virus (HIV) affects the cells of the immune system such as CD4+ T cells, CD8+ T cells, macrophages, and dendritic cells[1]. CD4+ and CD8+ Tcells are important in controlling HIV infection [2]. Depletion of CD8+ Tcell responses is associated with infection; while regeneration restores control of viral replication [3].

The reduction in CD4+ Tcell counts signifies immune deficiency[4].It may becaused by the death of uninfected bystander cells by apoptosis and the killing of infected CD4+ Tcells by cytolytic lymphocytes [5]. This phenomenon indicatesthe progression of HIV in infected individuals [6]. A count below 200 CD4+ cells/µl leads to the development of various complications in HIV/AIDS. Patients with a CD4+ Tcell count of 500 cells/µl are healthy[7]. The progression of the disease and an adequate response to ART therapy for most patients is associated with an increase in CD4 count in the range of (50-150) cells/mm^3 during the first year of treatment [8]. Any measures that can restore immune functions can help reduce pain, mortality, and morbidity, due to HIV/AIDS infection.

The use of ART results in decreased Tcell death and depletion, as well as a restoration of their numbers [9].Therapy helps prolong the lives of HIV and AIDS patients, but it is accompanied by drawbacks, such as challenges in adhesion, drug resistance, and other health complications [10].In search of an alternative, traditional remedies have become common among patients. These natural products, especially those of plant origin, are an excellent source of new anti-HIV drugs [11,12] that exhibit modulatory effects on HIV-1 replication[13]. Clinical tests of some of these compounds have produced remarkable results[14,15].

Moringaoleifera (Moringaceae) is a multipurpose tree with nutritional and medicinal properties that can be used as an alternative remedy to various health conditions in HIV/AIDS. The plant is packed with nutrients such as proteins, minerals, vitamins, fats, and fiber[16]; and minerals such as magnesium, copper, sulfur, sodium, phosphorus, zinc, and potassium [17]. This study used *Moringa oleifera,* which is a cheap, readily available, nutrient-rich, plant-based supplement that can bridge the gap of malnutrition, especially for patients who cannot afford a proper diet.
The results of this study are useful in understanding the effect of *M. oleifera* consumption onCD4+ T cells and CD8+ T cellpatterns of patients with HIV and AIDS.

2. MATERIALS AND METHODS

2.1 Study Design

The study was a quasi-experimental study of regression discontinuity type involving HIV-positive male and female adults aged 18 to 55 years, all on highly active antiretroviral drugs (HAART) and attending the Comprehensive Care Center (CCC), Mbagathi County Hospital in Nairobi, Kenya.Patients with gastrointestinal infections and liver, cardiovascular, renal, or endocrine disorders thatcould interfere with the observations, as well as expectant, and breastfeeding mothers,were excluded. We screened 261 patients for eligibility,of which 173 participantswere recruited into the study. Then these were placed into two groups: the treatment group receiving *M. oleifera* supplementation (n=99)and the control group not(n=74). CD4+ T cell counts and CD8+ T cell counts were monitored at baseline and in the third and sixth months of the study.

Participants in the intervention group were supplemented with *Moringa oleifera* leaf powder obtained from East Africa Nutraceutical Company, Nairobi. Each study participant in the experimental group received a monthly supply of 300 grams of *Moringa oleifera* leaf powder in teabags weighing 2 grams each. Participants were advised to use four tea bags (about 8 grams) daily [18] by adding them to hot water in a standard 200 ml mug and steeping for ten minutes(2 tea bags in the morning and 2 tea bags in the evening).

2.2 Determination of CD4+and CD8+ Tcell counts

CD4+ and CD8+ Tcell counts were determined by flow cytometry according to the manufacturer's instructions (BD FACSCaliburanalyzer, BD Biosciences, USA) at baseline, in the third and sixth months of the study.Briefly, fifty microliters of whole blood were added to the CD4 and CD8 reagent tubes and incubated. Fifty microliters of 5% formaldehyde were added and processed in the BD FACSCalibur™analyzer. The tests were carried out with washing cycles between the assays, and the results were acquired by computer printing.The results were obtained as computer printouts of the BD FACSCalibur analyzer for the CD4 and the CD8 T cell counts.

2.3Evaluation of toxicity

To determine whether*M. oleifera* had toxic effects on participants,creatinine tests were performed on each study subject at the beginning of the study, in the third and sixth months.The creatinine values were applied on theCock Croft Gault formula (CGF) [19]to calculate the glomerular filtration rate (GFT). The age was in years, the weight in kilograms, and the creatinine (Cr)in mg/Dl.

$$GFR = \frac{(140 - age) \times weight}{Cr\ (Creatinine) \times 85}$$

Low GFRs indicate a decrease in renal functional capacity. Creatinine levels were measured using HumaLyzer Primus Chemistry Analyzer equipmentaccording to the manufacturer's instructions (Wiesbaden, Germany).

2.4 Data analysis

The data input was done in Microsoft Excel and exported tothe computer software XLSTAT 2021.2.1 for analysis.Analysis of variance (ANOVA) followed by Tukey's post hoc tests assessed any time-dependent changes in the means of CD4+ Tcell and CD8+ T cell counts, as well as the creatinine levels of the two study groupsacross the study period(within the groups). Unpaired t-tests assessed any differences in the means of the parameters between the two study groups at baseline, months three and six(between the groups). The results were significantly different if $p < 0.05$.

3. RESULTS

3.1 Social demographic characteristics ofstudy participants

Table 1 shows the demographic characteristics of the study participants. There were 62 males (35.8%) and 111 females (64.2%). The females were more than the males in both study groups. The age subset 46 – 55 years had the most participants (54.3%), while the age subset 18 – 25 years, had the least (2.3%).

Table 1: Social demographic characteristics of study participants

Characteristic	N	(%)
Age Groups		
18-25	4	2.3
26-35	23	13.3
36-45	52	30.1
46-55	94	54.3
Sex		
Male	62	35.8
Female	111	64.2

3.2 Study process

Figure 1 shows the CONSORT flow chart for the study. Of the two hundred and sixty-one (261) patients screened for eligibility, only one hundred and seventy-three participated in the study. Twenty-two (22) participants did not complete the study; three were lost to follow-up, fourteen (14) refused follow-up, three (3) were discontinued due to health problems, and two (2) succumbed to cancer detected during supplementation.

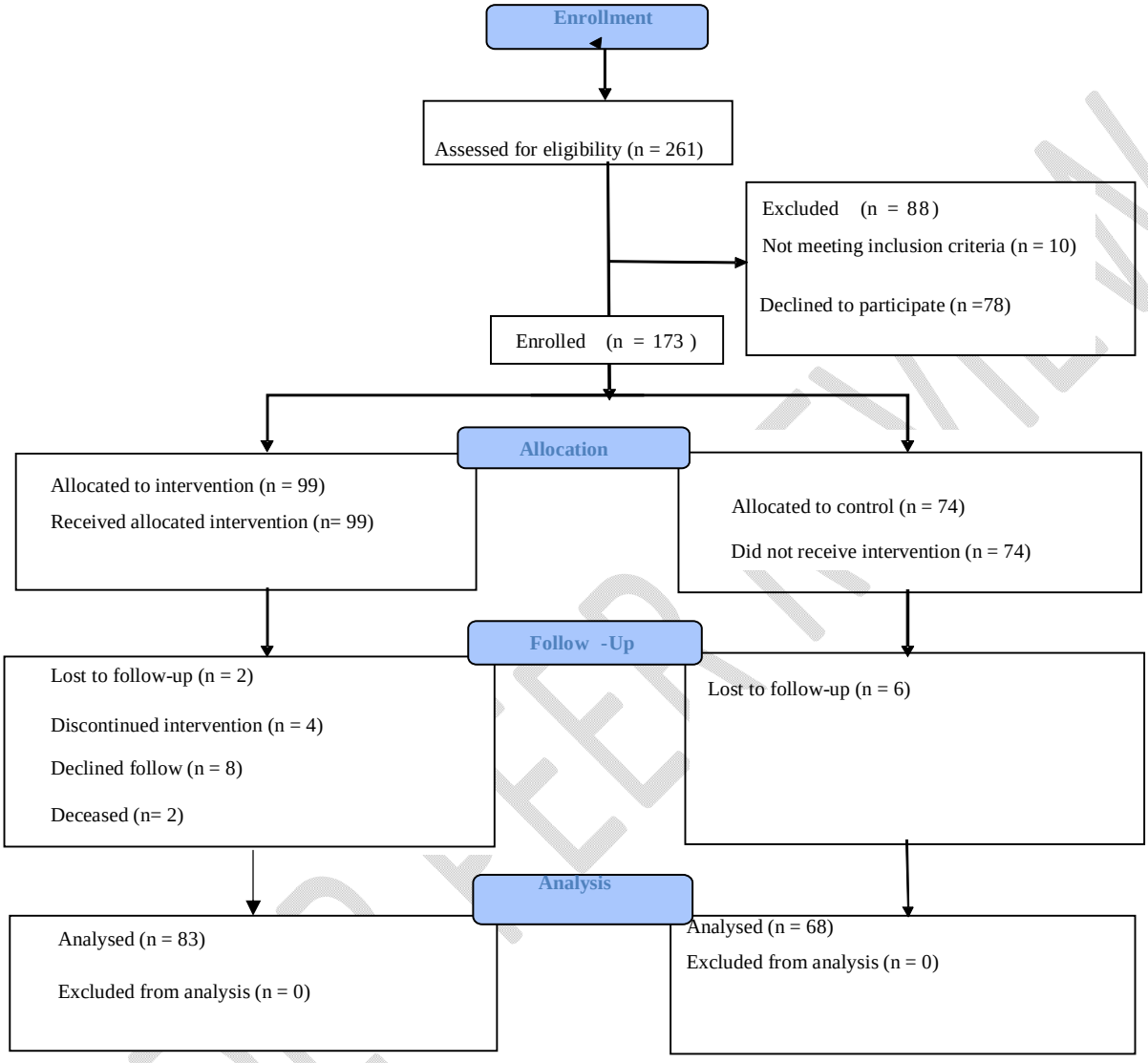

Figure 1: Participant flow diagram (CONSORT FLOW 2010)

3.3 Changes in CD4+and CD8+ Tcell counts

Table 2 shows the analysis of variance (ANOVA) comparingthe meancounts of CD4+ Tcells and CD8+Tcellsof each of the two study groups from baseline, month three, and month six(changes with time within each of the study groups). The CD4+ T cell count of the intervention group increased significantly from a baseline value of (521.60 ±215.40) to (656.23 ± 274.8) at month six after the start of the intervention (p< 0.005). The CD4+ Tcell count of the control group also increased significantly from a baseline value of (449.24 ± 214.82) to (624.53 ± 299.32) at month six after the start of the study (p <0.005). There was no significant change in the baseline CD8+ Tcell counts of the intervention group (F - 0.0065; p = 0.994) and the control group (F = 0.0466; p = 0.954) during the follow–up period.Though there were some quantitative changes in the CD8+ T cell count at month six from the baseline levels, the changes were not statistically significant.

Table 2: Analysis of variance of mean values of CD4+ and cd8+ T cell counts during follow-up

	Parameter	Baseline	Month 3	Month 6	ANOVA F - value	*P* – value
Intervention Group	**CD4 cells/µl**	521.60 ± 215.40	559.52 ± 214.43	656.23 ± 274.8	6.2863	0.002*
	CD8 cells/µl	1075.71 ± 485.55	1071.08 ± 412.1	1079.47 ± 437.69	0.0065	0.994
Control Group	**CD4 cells/µl**	449.24 ± 215.82	581.26 ± 297.92	624.53 ± 299.32	6.8949	0.001*
	CD8 cells/µl	1058.92 ± 442.25	1084.68 ± 513.78	1070.58 ± 451.99	0.0466	0.954

Table 3 shows a comparison of mean CD4+ and CD8+ Tcell counts of month 3 and month 6 of the intervention and control groups(between groups); using unpaired *t*-tests. Although the mean CD4+ T cell count of the intervention group was higher (656.23 ± 274.8) than that of the control group (624.53 ± 299.32) in the sixth month of the study, the rate of change in cell counts between the two groups was not statistically different. There was no significant difference in the rate of change in the cell count as indicated by the P values for the means of CD4+ T cell counts and CD8+ T cell counts in months three, and six between the two groups (p <0.005).

Table 3: Unpaired *t*-tests for the means of months 3 and 6 CD4+ and CD8+ T cell counts of the intervention and control groups

Time point	**Parameter**	**Intervention group (n = 83)**	**Control group (n = 68)**	**Difference (*P* – value)**
Month 3	CD4 cells/µl	559.52 ± 214.43	581.26 ± 297.92	0.633
	CD8 cells/µl	1071.08 ± 412.1	1084.68 ± 513.78	0.867
Month 6	CD4 cells/µl	656.23 ± 274.8	624.53 ± 299.32	0.523
	CD8 cells/µl	1079.47 ± 437.69	1070.58 ± 451.99	0.908

3.4 Toxicity

The toxicity of the kidneys was evaluated by changes in kidney glomerular filtration rates calculated from creatinine levels. At the beginning of the study, the mean GFR of the control group (84.35 ± 22.97) was significantly higher (p = 0.002) than that of the intervention group (72.83 ± 22.57).It remained significantly higher (108.72 ± 32.09) (p = 0.004) thanthat of the intervention group (94.21 ± 27.71) at the end of the study(Figure 2).

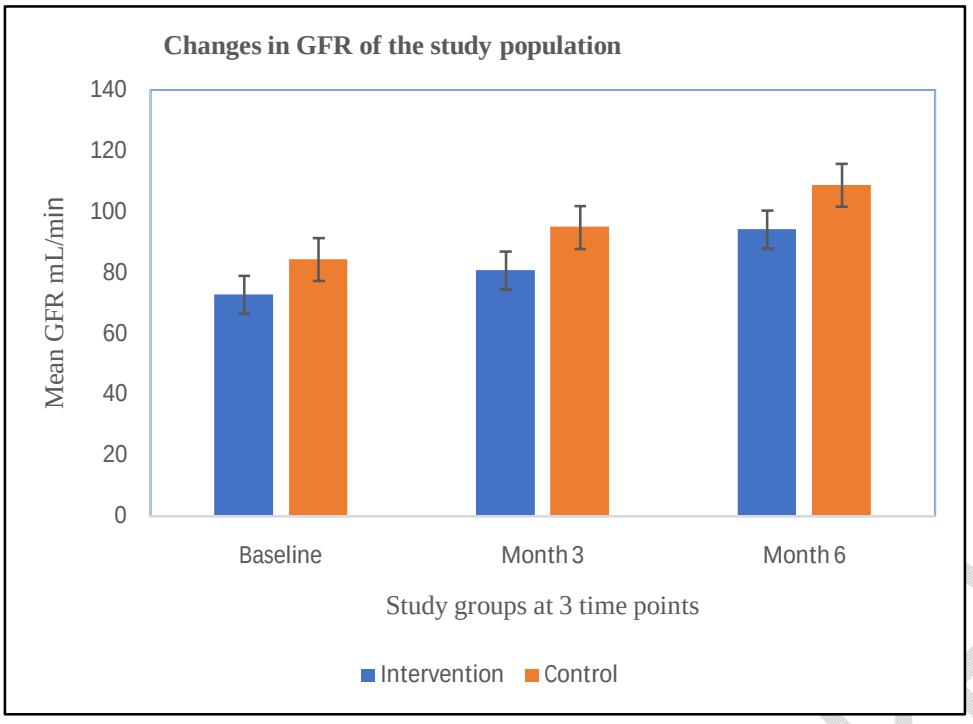

Figure 2: Changes in the glomerular filtration rate (GFR)

4.DISCUSSION

The results of the current study indicate that the mean CD4+ Tcell count of the intervention group was higher than that of the control group at the end of the sixth month, but the difference was not significant. However, an increase in baseline CD4+ Tcell count was observed over time within the two groups, suggesting that the two groups had improved immunity.

The effect of *M. oleifera* on CD4+ Tcell counts has been investigated *in vitro*, in animal models, and inhumans. The results of these studies are varied; some suggest up-regulation, no change, or dose-dependent suppression of CD4+ Tcell counts. A team of scientists observed an increased CD4+ Tcell activity in diabetic mice[20]. An *in vitro* study at the University of Brawijaya, Malang, Indonesia, observed immunomodulatory activity through active *Moringa* compounds such as flavonoids and saponins,which stimulated CD4+ Tcell counts and other immune cells. Low doses were effective and high doses caused immunosuppression [21]. A dose-dependent decrease in CD4+ Tcell counts and their respective pro-inflammatory cytokines was observed in diabetic-induced Balb/C mice[22]. The results obtained from these animal models may not be duplicated in humans.

In a study conducted by Novitra*et al*. (2020)at Dr.RubiniMempawah Hospital, Indonesia, in patients with HIV/AIDS and also using ARV, the study team observed an increase in CD4+ Tcell count that was dose-dependent and duration-dependent [23]. Another study conducted in southeast Nigeria demonstrated that 20g of *M. oleifera*consumed over 2 monthsimproved the CD4 + Tcell count of HIV-positive patients on HAART[24]. These studies used a sample size less than the recommended sample size for thenormal distribution.Waruguru *et al*. (2023), in a systematic reviewof a few studies, also suggested an increase in CD4+ T cell count resulting from*M. oleifera* supplementation [25].Furthermore, the mean increase in CD4+ Tcell count of the intervention group of our study (690.07 ± 292.60) was much higher than those achieved by other studies. The findings of a recent study conducted in Kano, Nigeria, found no differences in mean CD4+ Tcell counts throughout the study period, except for the sixth month, a difference influenced by some sociodemographic characteristics of the participants[26]. Another study conducted in Kinshasa in the Democratic Republic of Congo (DRC) also did not observe anysignificant change in CD4+ Tcell counts[27].

These contradictory findings from the current study could be explained by the differences in the designs, sample sizes, the heterogeneity of the study participants, and the differences in the amounts and formulations of *M. oleifera* used. While the current study was a quasi-experimental study that does not involve randomization of participants, the reviewed studies were double and single-blinded randomized controlled trialsThese are factors that future research can address before concluding. In environments of food insecurity, an intervention using *Moringa* alone may not affectpeople who are malnourished. It is also possible that the doses administered were too low to influence the results(compared to a range of

15g to 30g used in the studies reviewed).Although these differences are small, they are a signalof the possibility that *M. oleifera* consumed by participants in the intervention group may increase CD4+ Tcell counts in HIV/AIDS patients.

CD8+ Tcells are a critical component of the cellular immune response during viral infection, resulting in decreased viremia [5]. Cytolysis due to CD8+ Tcells is high during the asymptomatic phase of HIV infection [28] but decreases as the patient progresses to AIDS [29].This study indicates no changes in CD8+ Tcell counts during the follow-up period. The findings of other authors indicate that *M. oleifera* stimulates CD8+ Tcell activities. In a study conducted in Malang, Indonesia [21],it was observed that low doses of leaf extract stimulated CD8+ Tcells and other immune cellsin mice. Another study conducted in Cairo, Egypt, made similar observations [20]. However, a dose-dependent decrease in CD8+ Tcell activity was observed in diabetic Balb/C mice[22]. A plausible explanation for these contradictory findings is that all the studies were with animal models and may not be duplicated in humans. Little information is available on the effects of *M. oleifera* on CD8+ Tcell counts in humans.

The findings of our study show that the creatinine levels and GFR of both the intervention and control groups were within normal ranges. Several animal studies suggest that various preparations of *M. oleifera* leaves may be very safe at the amounts and doses commonly used [17]. No studies involving*M. oleifera*usein human subjects have recorded side effects. So far, there has been no documented effect of *M. oleifera* on renal function in humans. It can be concluded that the use of *M. oleifera* is not toxic.

Given the observational and non-randomization nature of this study, the results may have been limited and subjected to potential confounding factors such as age, gender, and selection bias, Further investigations using designs such as randomized controlled trials (RTCs) are recommended to confirm the above findings.

5. CONCLUSION

In conclusion, *M. oleifera*consumptiondoes not increase CD4+ T cell counts in HIV/AIDS patients at the dose used in this study but may be effective at higher doses.It is still unclear what dose of *M. oleifera* leaf powder may improve patientoutcomes. The different studies reviewed have used different doses. We recommend thatthe same experiment be repeated using varying doses of *M. oleifera* to establish any dose-dependent impact on the immune system.

Ethical Approval and Consent

The Kenyatta University Ethics Review Board and the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (SERU) approved the study. Before participating in the study, all participants gave their informed consent.

Availability of data

The data used to arrive at the conclusions of this work will be provided upon request.

Artificial intelligence

Author(s) hereby declare that NO generative AI technologies and text-to-image generators have been used during the writing or editing of this manuscript.

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