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EFFECT OF HERBAL SOUP ON IMMUNOLOGICAL PARAMETERS OF PEOPLE LIVING WITH HIV, KENYA - A PARALLEL SINGLE BLIND RANDOMIZED CONTROL TRIAL

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ABSTRACT

Approximately 39 million people living with HIV (PLWH) are at risk of infection with severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) that causes Coronavirus Disease 2019 (COVID 19). COVID 19 can lead to serious negative health outcomes for PLWH with a low CD4+ count and increased viral load (VL). The objective of this study was to determine the effect of herbal soup (formulated by mixing; dried ginger powder, dried garlic powder, roasted butternut squash powder, ground chia seeds, ground sunflower seeds, dried turmeric powder and chili pepper powder) on the immunological parameters of PLWH. This was a parallel single blind randomized control trial (RCT) conducted in Kakamega County Teaching and Referral Hospital (KCTRH) Comprehensive Care Centre (CCC). Block randomization and sequentially numbered opaque envelopes were used to assign respondents to either control group (received standard care including Nutrition counselling and anti-HIV drugs) or treatment group (received herbal soup in addition to the standard care). Participants in the treatment group prepared and consumed 1 serving (16.35g) of the soup daily for 3 months. A total of 60 respondents were selected for the control group (n=30) and treatment group (n=30), of whom, a small sample size of 41 completed the study limiting the study findings from drawing policy conclusions. Statistical Packages for Social Sciences was used for data organization and management. Paired t-test was used to determine the effect of herbal soup on CD4+ cell count and Viral Load (VL). The study findings indicated no significant effect on the mean CD4+ cell count (p=0.838), (±277) and mean VL (p=0.051), (±42) in the control group. There was a significant effect on mean CD4+ cell count (p=0.012), (±199), however, no significant effect on the mean VL (p=0.102), (±74) in the treatment group. The study concluded that the herbal soup had an effect on the CD4+ cell count and can be adopted for use at household and facility level to improve the immune defence of PLWH during emerging virus-related epidemics such as COVID 19.

Key words: HIV/AIDS, SARS-CoV-2, COVID 19, CD4+ cell count, viral load, herbal soup

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INTRODUCTION

The estimated global burden of HIV/AIDS is approximately 39 million [1]. The HIV epidemic is disproportionately concentrated in sub-Saharan Africa (SSA) with an estimated 25.6 million PLWH [2]. Out of the 25.6 million PLWH,20.4 million are in Eastern and Southern Africa [1]. Kenya is among the world's top countries with the highest number of PLWH at an incidence of 1500 PLWH in every 100,000 persons [3]. The country has a population of 1.4 million PLWH and a national prevalence of 3.7% [4]. Since the first case of HIV that was reported in 1984, the Government of Kenya (GoK) established the National AIDS and STI Control Program (NASCOP), formulated policy guidelines and established the National AIDS Control Council (NACC) to fight against the virus [5]. The country is on track to achieving zero new infections, zero HIV related deaths, and zero discrimination through prevention and treatment services [6]. Despite these efforts, Kenya is still the fourth largest HIV epidemic zone globally [5]. The overall cascade performance indicates that Kenya has attained 79:78.9:73.4, and therefore a gap to achieving the 90:90:90 goal by 2030 [6]. A total of 22,000 new infections and more than 18,000 HIV related deaths were reported in 2023 [4]. Notably, HIV incidence differs across counties, with Kisumu and Siaya recording as high as 6.9 per 1000 and Wajir and Mandera recording as low as 0.001 per 1000 [6]. Out of the 47 counties, 25% of them including Homa Bay, Siaya, Kisumu, Migori, Kiambu, Kajiado, Mombasa, Kisii, Nairobi, Nakuru, Uasin Gishu and Kakamega are rated as high incidence counties [6].

COVID 19 an infectious disease caused by SARS-CoV-2 was declared a pandemic on 13th March 2020 by the World Health Organization (WHO) [7]. More than 55,928,327 cases and 1,344,003 fatalities have been reported in 220 countries globally [7]. Kenya suffered five waves of COVID 19, with a total of 331,324 confirmed cases and 5,488 deaths as at 30th January 2022 [7]. Sub-Saharan Africa is at risk of having a burden of COVID 19 driven by the proportion of HIV patients with superimposed bacterial pneumonia [8, 12]. Prioritizing COVID 19 over HIV in Africa can lead to a 10-year reversal of HIV treatment progress [9]. A study conducted in Kenya reported that PLWH are afraid of contracting COVID 19 causing an impediment to accessing health care services [10]. This could lead to non-adherence of Highly Active Antiviral Treatment (HAART) posing a risk of treatment failure which may mean no cure for HIV/AIDS [11]. Poorly controlled HIV infection with increased VL and inadequate CD4 cell count poses a risk to severe COVID 19 [12]. Hence, this study aims to test and find out whether this herbal soup is able to improve the CD4 cell count and suppress viral replication among PLWH.

Medicinal plants have been used for a long time to boost immunity of PLWH [13]. Hence, they may serve as a source of new and effective drugs [14]. In Kenya, a combination of nine plants; *Opuntia ficus-indica, Aloe vera, Carissa edulis,*

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Triumfetta macrophylla, Clerodndrum myricoides, Leonotis nepetifolia, Maesa lanceolata, Prunus Africans, and *Azadirachta indica* in liquid or powder formulation have been used by herbalists to treat HIV/AIDS [15]. *Combretummolle, Hypoxis hemerocallidea, Pelargonium sidoides, Rheum species, Vernoniaamygdalina, Sutherlandi frutescens,* and *Hypericum perforatum* are some of the plant species that have shown remarkable anti-HIV activity and are therefore worthy of further research for the development of new anti-HIV chemotherapeutic drugs [16]. Kenya is yet to fully exploit the potential that herbal medicine holds [17]. Despite the many studies done on plant remedies, there is a need to assess their validity, phytochemistry and bio efficacy in clinical trials [18].

The herbal soup is a new innovation formulated by a research team at Masinde Muliro University of Science and Technology (MMUST). It is made from seven herbal ingredients: Ginger (*Zingiber officinalis)*, Garlic (*Allium sativum)*, Chilli pepper (*Capsaicin*), Turmeric (*Curcuma longa*), Chia seeds (*Salvia hispanica)*, Butternut squash (*Cucurbita moschata),* and Sunflower seeds. Gingerols in ginger have anti-inflammatory, antibacterial, anticancer, antivirus and antioxidant health benefits [19]. *Allium sativum* stimulates immune cells leukocytes and γ globulins and strengthens the CD4 T lymphocytes [18, 20]. Ajoene, an organosulfur compound in garlic exhibits antiviral action on HIV-1 [21]. The immune enhancing capabilities of *Capsaicin* include increased spleen lymphocyte proliferation [22]. *Curcuma longa* boosts the CD4+, CD8+ T cells, Th1 type cytokines, reduces Treg cells and decreases T cell apoptosis [22]. Curcumin shows activity against HIV by inhibiting HIV protease and integrase and prevents transcription of HIV-1 [23]. Chia seeds (*Salvia hispanica)* have omega-3 and omega-6 known for boosting immunity [24]. Butternut squash known as *Cucurbita moschata* is beneficial for boosting immunity and shows anti-inflammatory activity [25]. Sunflower seeds are a rich source of phosphorus, magnesium, selenium, vitamin B-6 and E nutrients needed for good immunity [26, 27, 28]. This study seeks to validate the bio efficacy of combining these herbal plants formulated into herbal soup on immunological parameters of PLWH.

MATERIALS AND METHODS

Study Design and Area

This was a parallel single blind randomized control trial conducted from January to April 2022 in Kakamega County Teaching and Referral Hospital (KCTRH) at the Comprehensive Care Clinic (CCC) in Kenya. There were two study groups; control group (n=30) and treatment group (n=30). The control group was exclusively administered standard care while the treatment group was administered herbal soup alongside the standard care.

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Sample size and sampling techniques used

The study adopted the study power, standard deviation and effect size from a previous similar study [30].

$$n = 2 (Z \alpha + Z [1-\beta])^2 \times SD^2 / d^2$$
$$n = 2 (1.96 + 1.28)^2 \times 3.3^2/1.95^2 = 60$$

Where n = sample size required (60), Z_α = level of significance ($P < 0.05$), 1-β = study power (80% or 0.8), SD = standard deviation (3.3) and d = effect size (1.95). In this study, 60 participants were included, assigning control group (n=30) and treatment group (n=30).

Inclusion - Exclusion criteria

The study included PLWH attending the KCTRH CCC aged 15-65 years with adherence to anti-HIV treatment for at least three years, with a CD4+ cell count >200cells/µL of blood and those who gave a written consent to take part in the study. People living with HIV on other medications besides the anti-HIV drugs, on ARVSaquinavir and Ritonavir, and pregnant and lactating women living with HIV were excluded from the study.

Randomization

This was a single blind RCT. Only the researcher was aware of the intervention allocation. Participants in both the treatment group and control group did not have information on the intervention allocation. Systematic random sampling was used to recruit the participants. The participants were organized into blocks based on their age. Simple random sampling using sequentially numbered opaque envelopes was used to assign the participants to either the control group or treatment group within each block. The control group was exclusively allocated standard care (Nutrition counselling and anti-HIV drugs) while the treatment group was allocated herbal soup alongside standard care.

Formulation of herbal soup

Herbal soup was formulated by a research team of Masinde Muliro University of Science and Technology (MMUST). The formulation consisted of dried ginger powder (0.75 g), dried garlic powder (0.5 g), roasted butternut squash powder (3.75 g), grounded chia seeds (0.5 g), grounded sunflower seeds (0.5 g), dried turmeric powder (0.25 g) and chili pepper powder (0.1 g). The ingredients were collected in their natural form. Processing involved washing, sorting, peeling, dicing and drying on open aluminium trays in an oven at 50^0C until the ingredients were felt to be non-sticky. This was preceded by milling each ingredient separately using a milling machine to produce fine powder. The ingredients were then mixed in proportions that made the soup palatable. The mixed powder was passed through 0.01mm sieve to obtain a fine powder. The powder was filled in small transparent polythene sachets

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each weighing 16.35g that were then sealed using an electric manual sealer; then packed into larger packets containing 7 sachets each. Table 1 and Table 2 show the ingredients and nutrient composition of herbal soup. Microbial analysis indicated 70 CFU/100µL meeting the WHO food safety standards. Nutrient analysis report by the Kenya Bureau of Standards (KEBS) indicated that it is rich in vitamins, minerals and bioactive compounds.

Administration and Compliance of Intervention

Control group (n=30) was exclusively administered standard care while the treatment group (n=30) was given herbal soup alongside standard care. Standard care entailed nutrition counselling and adherence to anti-HIV drugs. Nutrition counselling was done by the researcher and the research assistant registered by the Kenya Nutritionists and Dietitians Institute (KNDI). The counselling session was done once after enrollment to the study. Food models, flip charts, food pyramids and tables were some of the materials that were used for counselling. The participants were counselled on strategies to meet the nutrients and energy needs [30]. The counselling session took a period of 30 to 40 minutes. The study participants were reminded to adhere to their prescribed anti-HIV medication.

One serving (16.35g) of the soup was prepared and consumed by mixing the formulation with 250 ml of cold water, brought to boil for 5 minutes and served while hot. The soup was consumed plain or accompanying other meals at any time of the day. Compliance to the herbal soup prescription was achieved through strict instructions not to share the prescribed herbal soup with other family members. Follow up to ensure adherence was made through daily phone calls and short messages. Home visits were done after every 3 days to monitor how the participants prepared and consumed the soup. The study participants were put in their respective care for 3 months to allow enough time to evaluate the effectiveness of the herbal soup.

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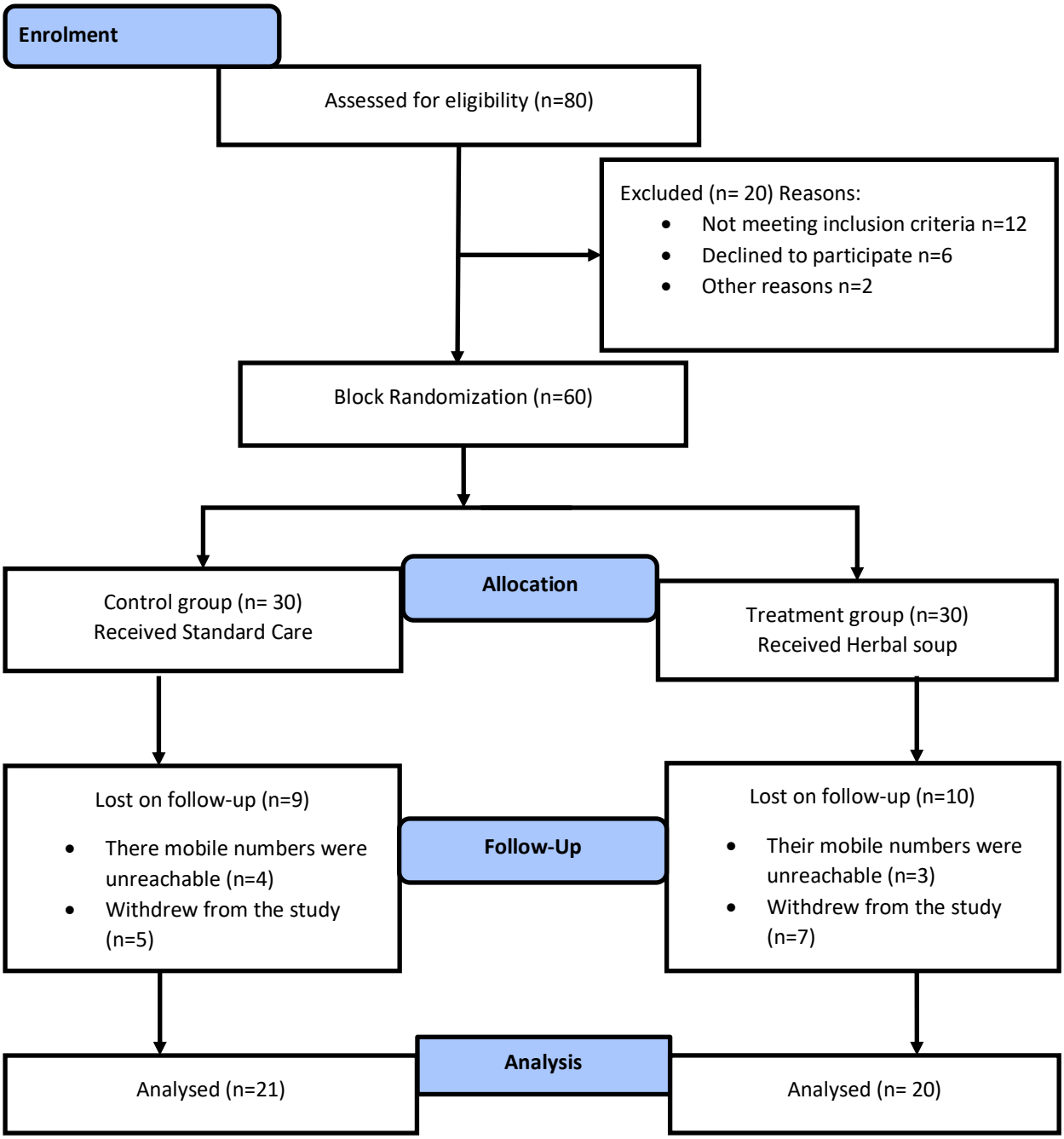

Figure 1: Study design flow diagram

Figure 1 is a consort 2010 flow diagram reporting on the overall summary of the study design. A total of 80 PLWH were assessed for eligibility, of which 20 did not participate: not meeting the inclusion criteria (n=12), declined to participate (n=6), and students attending boarding schools (n=2). The remaining 60 participants were randomized into either the control group (n=30) or treatment group (n=30). In the control group (n=9) and in the treatment group (n=10) participants were lost on follow-up due to communication challenges while some dropped out of the study. A

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total of 41 responses, control group (n=21), and treatment group (n=20) were included in the analysis.

Data analysis

Data was analyzed using Statistical Packages for Social Sciences (SPSS) version 21 at 95% level of confidence. Descriptive statistics was used to collect and analyze numerical facts. Continuous variables were computed with standard methods and presented as mean and SD. Categorical variables were reported as frequencies and percentage of each category. Independent sample t-test was used to analyze the significant difference in the study parameters at baseline, and post intervention between the control group and treatment groups. Paired t-test was used to analyze the effect of herbal soup on the CD4+ cell count and VL.

Ethical Considerations

The study upheld the Declaration of Helsinki guidelines. All procedures involving human subjects were approved by the Masinde Muliro University of Science and Technology Institutional Ethics Research Committee with reference number (MMUST/IERC/130/2023) and the National Commission for Science, Technology and Innovation (NACOSTI) with license number (NACOSTI/P/23/28083) to conduct the study. The study was registered with Pan African Clinical Trial Registry (PACTR) with reference number (PACTR202307740330512). Written informed consent was obtained from all study participants. Participants below the age of majority <18+ years consented to participate through their caregivers. Confidentiality, autonomy, beneficence and justice was upheld in the study.

RESULTS AND DISCUSSION

Table 3 shows that both the control group and treatment group had a high number of the study respondents within the age bracket of 45-54 years - 90.5% (n=19) and - 45% (n=9), respectively. This is similar to a study done in China that found the middle-aged HIV/AIDS populations have the highest incidence [31]. Higher percentage of females with HIV was reported in both the control group - 66.7% (n=14) and the treatment group - 90% (n=18). This is in agreement with another study that was looking at gender disparity in epidemiological trend of HIV/AIDS infection and treatment in Ethiopia that found out that new infection and AIDs related deaths is by far higher among adult women than men [32]. Majority of the current study respondents in the control group 47.6% (n=10) and treatment group 55% (n=11) had acquired primary education level and tertiary education level was the least. This contrasts to findings of a study in Bangladesh that had 29.9% participants with primary education and 35.9% with secondary education, however, tertiary education was the least at 8.2% [33]. This difference could be due to the previous study population that included only women. The current findings indicate that the

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majority of the respondents in control group 52.4% (n=11) and treatment group 85% (n=17) did not receive any form of financial or social support, a factor that reduces the level of depression [34]. Table 3 also indicates that the majority of the respondents in both the control group 100% (n=21) and treatment group 55% (n=11) had small family sizes consisting of 2-5 members.

Table 4 shows changes in the mean CD4+ cell count. A statistically significant difference (+120.6, ± 199.3 change at p=0.012) was observed in the treatment group. The results suggested the herbal soup may have had a positive effect on the CD4+ cell count of PLWH. This could be attributed to the several mixtures of micronutrients, bioactive, and phytochemicals present in the soup that provide synergy with regard to antioxidant, anti-inflammation and prevention of oxidative stress [35]. *Moringa oleifera* Lam. Leaves were also found to improve immune response of HIV positive adults [36]. These could be attributed to the high content of vitamin C, potassium, calcium, iron, vitamin A and proteins found in *Moringa oleifera* [37]. Similarly, the beneficial effect of carrot-ginger blend on the immune status of PLWH is due to vitamin A, C and E which have an antioxidant role [19]. However, given the small sample size and the high dropout rate, further research is required to confirm these findings.

As shown in Table 5, the intra group baseline and post study comparison show that the treatment group had -25, ±73.5 change in VL at p=0.102 indicating that this change was not statistically significant. Hence, the herbal soup had no significant effect on the VL of PLWH. This was also true in the study of the effect of *Moringa oleifera* leaf powder supplementation on the VL of adult HIV patients [39]. However, *Artemisia annua* and *Moringa oleifera* Lam, were found to have a significant improvement on VL suppression. This could be due to the combined antioxidant and anti-inflammatory effect of *Artemisia annua* and *Moringa oleifera* Lam [35].

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The study suggests that the herbal soup has no significant effect on the VL of PLWH in KCTRH. However, the herbal soup has a significant effect on the CD4+ cell count of PLWH. Therefore, the study recommends undertaking a larger study to confirm these findings, prior to considering the use of the herbal soup among the immune-suppressed population of PLWH to boost their immunity in terms of CD4+ cell count as a mitigating strategy against emergence of virulent diseases such as COVID 19. Future research should consider conducting a crossover RCT to ascertain the effect of herbal soup on CD4+ cell count, and other immunological parameters involving a larger study population and extended study period. In order to understand the effect of herbal soup on CD4+ cell count, there is need for further research on the nutrient

composition and compounds of the soup, their phytochemistry, bio-efficacy, toxicity and safe intake levels.

Limitations of the study

The relatively small sample size and a high dropout rate limits the study findings from being inferred to the whole population of PLWH. Moreover, the treatment and control groups had significant differences in characteristics. Adherence to nutrition counselling and herbal soup depended on the self-efficacy of the patients to prepare and consume as prescribed. Barriers such as financial status, sharing with other family members among others could interfere with the study findings and therefore not provide a true picture.

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CONFLICT OF INTEREST

None of the authors declared conflict of interest

CONTRIBUTORS

AK, SC and JS conceived the idea and designed the study. AK and SC developed the herbal soup. AK and SC performed data collection. JS and LM supervised the data collection. AK performed data analysis and drafted the manuscript. JS and LM offered overall supervisory oversight.

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Table 1: Nutrient composition of one serving of herbal soup

Ingredients of one (16.35g) serving of herbal soup		
Common name	**Scientific name**	**Quantity (g)**
Butternut squash	*Cucurbita moschata*	3.75
Ginger	*Zingiber officinalis*	0.75
Garlic	*Allium sativum*	0.5
Chilli pepper	*Capsaicin*	0.1
Sunflower seeds	*Helianthus annuus*	0.5
Chia seeds	*Salvia hispanica*	0.5
Turmeric	*Curcuma longa*	0.25
Wheat flour (thickening agent)	*Triticum aestivum*	10
Total		16.35

Table 2: Kenya Bureau of Standards (KEBS) nutrient analysis

Nutrient	In 16.35g (One serving)	In 100g
Calorie (Kj)	62.29	383.42
Fat (g)	0.66	4.032
Sodium (Mg)	5.46	33.43
Total CHO (g)	13.31	81.40
Dietary Fibre (g)	1.40	8.62
Sugar (g)	0.91	5.57
Protein (g)	2.09	12.79
Calcium (Mg/dl)	25.19	154.11
Iron (Mg)	1.08	6.60
Potassium (Mg)	166.39	1017.71
Choline (Mg)	1.87	11.46
Folate (Mcg)	30.04	183.72
Pantothenic (Mg)	0.21	1.23
Riboflavin (Mcg/l)	0.14	0.80
Thiamine (Ug/l)	0.12	0.74
Lutein & Zeaxanthin (Mg)	10.46	63.99
Vitamin C (Mg)	0.37	2.32
Vitamin B6 (Mg)	0.07	0.47
Copper (Mg)	0.059	0.36
Magnesium (Mg)	6.80	41.62
Manganese (Mg)	0.46	2.84
Phosphorus (Mg)	36.06	220.55
Selenium (Ug/dl)	4.89	29.93
Zinc (Mg/l)	0.23	1.38

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Table 3: Socio-demographic characteristics of PLWH in KCTRH CCC

Demographic characteristics		Control group n=21 (%)	Treatment group n=20 (%)	*P* value
Age	15-24 years	1 (4.8)	0 (0%)	0.001
	25-34 years	0 (0%)	2 (10%)	
	35-44 years	1 (4.8)	4 (20%)	
	45-54 years	19 (90.5%)	9 (45%)	
	55-64 years	0 (0%)	5 (25%)	
Gender	Male	7 (33.3%)	2 (10%)	0.071
	Female	14 (66.7%)	18 (90%)	
Education Level	Primary	10 (47.6)	11 (55)	0.541
	Secondary	6 (28.6)	7 (35)	
	Tertiary	5 (23.8)	2 (10)	
Number of household members	2-3 members	21 (100)	5 (25)	<0.0001
	4-5 members	0 (0)	11 (55)	
	6-7 members	0 (0)	4 (20)	
Duration of Infection	1-5 years	21 (100)	19 (95)	0.300
	6-10 years	0 (0)	1 (5)	
Type of occupation	Employed	4 (19)	2 (10)	0.464
	Owns Business	3 (14.3)	4 (20)	
	Farmer	8 (38.1)	9 (45)	
	Casual Worker	1 (4.8)	4 (20)	
	Retired	1 (4.8)	0 (0)	
	Student	1 (4.8)	0 (0)	
	Others	3 14.3)	1 (5)	
Receipt of financial/social Support	Yes	10 (47.6)	3 (15)	0.025
	No	11 (52.4)	17 (85)	

Data is presented as frequency and percentage as indicated. All statistical analysis was performed using chi-square except for age and education level where Fisher's exact test was used. Data on age, gender, education level, number of household members, duration of infection, type of occupation, and receipt of support are presented by categories under each variable. Age and duration of infection are presented in years. The significant P values are in bold

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Table 4: Effect of herbal soup on CD4+ cell count of PLWH in KCTRH

Control Group n=21			Paired T test	Treatment Group n=20		Paired T test	T test *P*	
BL		PS	*P*	BL	PS	*P*	BL	PS
CD4 + cell count (cells/µl)	440.4±199.7	449.4±276.7	0.838	417.6 ± 165.3	538.2±199.3	**0.012**	0.694	0.248
Change	+9.0			+120.6				

Data is presented as means and standard deviation as indicated. Statistical analysis was performed using an independent sample t test and paired t test. This presents data on CD4+ cell count for both the control and treatment group at baseline (BL) and post study (PS). The significant P value at the 5% level is in bold

Table 5: Effect of herbal soup on VL of PLWH in KCTRH CCC

Control Group n=21			Paired T test	Treatment Group n=20		Paired T test	T test *P*	
BL		PS	*P*	BL	PS	*P*	BL	PS
VL cp/ml	86.3±48.2	76.8±41.8	0.051	97.3±76.7	95.0±73.5	0.102	0.587	0.332
Change	-9.5			-25.1				

Data is presented as means and standard deviation as indicated. Statistical analysis was performed using an independent sample t test and paired t test. This presents data on VL for both the control and treatment group at baseline (BL) and post study (PS)

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