TRIAL PROTOCOL

Effect of a Locally Formulated Carrot–Ginger Micronutrient Supplement on Immunological and Liver Function Biomarkers among HIV Patients Receiving Antiretroviral Therapy in Kaduna State, Nigeria.

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1.0 Background and Rationale

Human Immunodeficiency Virus (HIV) infection remains a major public health challenge worldwide, particularly in Sub-Saharan Africa, which accounts for approximately two-thirds of the global HIV burden. Despite remarkable advances in antiretroviral therapy (ART), HIV continues to contribute substantially to morbidity and mortality, especially in resource-limited settings where nutritional deficiencies and co-morbidities complicate treatment outcomes (UNAIDS, 2023; Deeks *et al.*, 2021).

Although ART has significantly improved survival, reduced viral replication, and enhanced quality of life among people living with HIV, micronutrient deficiencies remain common and may impair immune recovery, increase oxidative stress, and predispose patients to opportunistic infections and metabolic complications (Okonkwo *et al.*, 2024). Vitamins A, C, and E, as well as trace elements such as selenium and zinc, play essential roles in maintaining immune competence, antioxidant defense mechanisms, and cellular function. Deficiencies in these micronutrients have been associated with reduced $CD4^+$ T-cell recovery, increased inflammation, and poorer clinical outcomes among HIV-infected individuals receiving ART (Adebayo & Olanrewaju, 2023; Okonkwo *et al.*, 2024).

Commercial micronutrient supplements have demonstrated benefits in improving nutritional status and immune function; however, their cost and limited accessibility often restrict their use among patients in low-resource settings (Jibril *et al.*, 2024). Consequently, there is increasing interest in affordable, food-based nutritional interventions derived from locally available plant materials that can complement ART and improve long-term health outcomes.

Carrot (*Daucus carota*) is an excellent source of β-carotene, vitamin A precursors, and antioxidant compounds that support immune function and protect against oxidative damage. Ginger (*Zingiber officinale*) contains biologically active compounds including gingerols and shogaols, which possess antioxidant, anti-inflammatory, and hepatoprotective properties (Kumar & Singh, 2022). Emerging evidence suggests that plant-derived bioactive compounds may enhance antioxidant capacity, support immune cell function, and reduce ART-associated oxidative stress and hepatotoxicity (Kumar & Singh, 2022; Jibril *et al.*, 2024).

Therefore, combining carrot and ginger into a locally formulated micronutrient supplement may provide a sustainable, affordable, and culturally acceptable nutritional intervention capable of enhancing immune recovery and reducing liver-related complications among HIV-positive individuals receiving ART. This randomized controlled trial was designed to evaluate the effectiveness of a locally formulated carrot–ginger micronutrient supplement compared with a commercial micronutrient supplement and standard ART care among HIV patients attending Kafanchan General Hospital, Kaduna State, Nigeria

2.0 Aim of the Study

To evaluate the effects of a locally formulated carrot–ginger micronutrient supplement on immunological status, nutritional outcomes, and liver function biomarkers among HIV-positive adults receiving antiretroviral therapy.

3.0 Objectives

Primary Objectives

1. To determine the effect of carrot–ginger supplementation on $CD4^+$ T-cell counts among HIV-positive adults receiving ART.
2. To evaluate the effect of carrot–ginger supplementation on liver function biomarkers (ALT and AST).

Secondary Objectives

1. To assess changes in serum vitamin A, vitamin C, vitamin E, selenium, and zinc concentrations.
2. To determine the effect of supplementation on body mass index (BMI).
3. To examine correlations between micronutrient status and $CD4^+$ T-cell recovery.

4.0 Hypotheses

Null Hypothesis (H0)

Carrot–ginger micronutrient supplementation has no significant effect on $CD4^+$ T-cell counts, nutritional status, or liver function biomarkers among HIV-positive adults receiving ART.

Alternative Hypothesis (H1)

Carrot–ginger micronutrient supplementation significantly improves $CD4^+$ T-cell counts, nutritional status, and liver function biomarkers among HIV-positive adults receiving ART.

5.0 Study Design

This study was a randomized controlled, assessor-blinded, parallel-group intervention trial with three treatment arms.

Participants were randomly assigned in a 1:1:1 ratio to:

- Group I: ART only (Control)
- Group II: ART plus SelACE® micronutrient supplement
- Group III: ART plus carrot–ginger micronutrient supplement

The intervention period lasted 90 days.

6.0 Study Setting

The study was conducted at the HIV/AIDS Special Treatment Clinic, General Hospital Kafanchan, Jama'a Local Government Area, and Kaduna State, Nigeria.

7.0 Study Population

HIV-positive adults attending the HIV/AIDS Special Treatment Clinic and initiating antiretroviral therapy.

8.0 Eligibility Criteria

Inclusion Criteria

Participants were eligible if they:

- Were confirmed HIV-positive adults.
- Were between 18 and 49 years of age.
- Had baseline $CD4^+$ counts between 350 and 400 cells/μL.
- Were newly initiating ART.
- Provided written informed consent.

Exclusion Criteria

Participants were excluded if they:

- Were pregnant.
- Had active tuberculosis infection.
- Had chronic liver disease.
- Had previously commenced ART.
- Declined informed consent.

9.0 Sample Size

A total of 90 participants were enrolled, with 30 participants allocated to each study arm.

The sample size was based on participant availability and operational feasibility within the study setting.

10.0 Recruitment Procedures

Potential participants attending the HIV/AIDS Special Treatment Clinic were screened for eligibility.

Eligible individuals were informed about the study objectives, procedures, risks, and benefits. Written informed consent was obtained before enrollment.

Recruitment commenced in April 2017 and continued until the required sample size was achieved.

11.0 Randomization and Allocation Concealment

A computer-generated randomization sequence was prepared by an independent researcher.

Allocation concealment was achieved using sequentially numbered sealed opaque envelopes containing treatment assignments.

The envelopes were opened only after participant enrollment and completion of baseline assessments.

12.0 Blinding

Because of the nature of the intervention, participant blinding was not feasible.

However:

- Laboratory personnel were blinded to treatment allocation.
- Outcome assessors were blinded.
- Statistical analysts were blinded.

The study therefore employed assessor blinding.

13.0 Intervention

Group I (Control)

Participants received standard antiretroviral therapy only.

Group II (Commercial Supplement)

Participants received ART plus SelACE® micronutrient supplementation according to manufacturer recommendations.

Group III (Carrot–Ginger Supplement)

Fresh carrot and ginger were washed, sliced, and dried at 40–45°C for approximately 10 hours.

Dried materials were milled and blended in a 75:25 ratio (carrot).

The formulation was packaged into sterile 10 g sachets.

Participants consumed one sachet twice daily for 90 days in addition to ART.

14. Outcome Measures

Primary Outcomes

- $CD4^+$ T-cell count (cells/μL)
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)

Secondary Outcomes

- Serum vitamin A
- Serum vitamin C
- Serum vitamin E
- Serum selenium
- Serum zinc
- Body mass index (BMI)

15.0 Data Collection Schedule

Measurements were performed at:

- Baseline (Day 0)
- Day 30
- Day 60
- Day 90

16.0 Laboratory Procedures

$CD4^+$ T-cell Count

Determined using flow cytometry.

Vitamin A

Determined using spectrophotometric methods.

Vitamin C

Determined by titrimetric analysis.

Vitamin E

Determined by spectrophotometric methods.

Selenium and Zinc

Measured using atomic absorption spectrophotometry.

ALT and AST

Determined using the Reitman–Frankel method.

17.0 Anthropometric Assessment

Body weight and height were measured using standardized procedures.

Body Mass Index was calculated as:

$BMI = \text{Weight (kg)} / \text{Height}^2\ (m^2)$

18.0 Safety Monitoring

Participants were monitored at each follow-up visit for adverse events and potential supplement-related side effects.

No serious adverse events attributable to the interventions were reported.

19.0 Statistical Analysis Plan

Data were analyzed using SPSS version 20.0.

Analyses included:

- Descriptive statistics
- Two-way Analysis of Variance (ANOVA)
- Tukey post hoc multiple comparison tests
- Pearson correlation analysis

Statistical significance was accepted at $p \leq 0.05$.

20.0 Ethical Considerations

Ethical approval was obtained from the Kaduna State Ministry of Health Ethics Committee.

Approval Number:

MOH/ADM/744/VOL.1/520

Written informed consent was obtained from all participants prior to enrollment.

The study adhered to the principles of the Declaration of Helsinki.

21.0 Data Management and Confidentiality

Participant information was coded and stored confidentially.

Only authorized study investigators had access to identifiable participant information.

Data were analyzed and reported in aggregate form.

22.0 Dissemination of Results

Study findings will be disseminated through:

- Peer-reviewed journal publications
- Scientific conferences
- Academic presentations
- Policy and public health stakeholder engagements

23.0 Trial Registration

This study was retrospectively registered on **17/7/2026** with the Pan African Centre for clinical trials **(PACTR**) and with unique identification number for the registration as **PACTR202607531692452.**

24.0 Funding

No external funding was received for this study.

25.0 Protocol Version

Version 1.0

Prepared for submission to Scientific Reports in accordance with WHO Recommended Format for Research Protocols and CONSORT 2010 guidelines.