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Hepatoprotective and immune-reconstitution potentials of carrot-ginger blend among HIV-infected patients taking antiretroviral therapy in Kaduna, Nigeria

[1§]Joshua Zamani Pius, [2]Abarshi Muawiyam Musa, [2]Sani Ibrahim, [2]Olumuyiwa Owolabi Adeyemi, [2]Mada Sanusi Bello, [2]Dallhatu Rhoda Yakubu

[1]Department of Science Laboratory Technology, Nuhu Bamalli Polytechnic, Zaria
[2]Department of Biochemistry, Ahmadu Bello University, Zaria.

[§]**Corresponding author:** Joshua Zamani Pius. zamanijo2013@gmail.com, Phone: +234(0)8035777866

Abstract

Hepatotoxicity, micronutrients insufficiency and cost of micronutrient supplements are challenges faced by HIV infected patients on antiretroviral therapy (ART). This study investigated the effect of natural plant micronutrients (vitamins A, C, and E, selenium and Zinc supplements from carrot-ginger (75:25) blend on liver enzymes: Alanine transaminase (ALT) and Aspartate transaminase (AST), CD4 + T lymphocytes and body mass index (BMI) of HIV-infected-patients taking ART. Ninety HIV-infected-patients attending Special Treatment Clinic, Kafanchan General Hospital, Kaduna State, Nigeria, were randomized into three groups of thirty patients each: Group 1 is control group and received ART alone, Group 2 is standard group and received ART with ready to use commercial micronutrient supplement (SelACE^R supplement) while Group 3 is supplement group and received ART + Carrot-Ginger blend for 90 days. Serum Alanine, Aspartate transaminase, CD4 + T lymphocytes and BMI were assessed using standard methods at baseline (day 0), 30 days, 60 days and 90 days. The results indicated that patients on CarrotGinger blend and SelACE^R micronutrients supplements show significant ($p<0.05$) reduction in ALT and AST level. However, there was no significant ($p>0.05$) difference in patients treated with ART alone when compared to their baseline values. The results indicated that patients on carrot-ginger blend and SelACE® supplements had significant ($p<0.05$) increase in BMI, CD4+ T-cell counts, serum vitamins A, C, E, selenium and zinc from day zero. There was no significant ($p>0.05$) difference in patients treated with ART alone compared to their baseline values. In addition, patients on SelACE® supplement revealed significant ($p<0.05$) difference in their mean BMI, CD4+ T-cell counts, serum vitamins A, C, E, Selenium and Zinc compared to patients on carrot-ginger blend after 90 days. The results also indicated a strong positive association **(r=0.97)** between serum ALT and AST activity and between CD4+ T cell counts and body mass index **(r=0.77)** after 90 days. Therefore, micronutrients supplementation of HIV patients during ART treatment with Carrot-Ginger blend could also be a beneficial adjunct to ART due to its potentials to reconstitute the immune system and protect the liver in HIV individuals on ART.

Keywords: Human Immunodeficiency Virus, Antiretroviral therapy, Carrot-Ginger, Antiretroviral therapy, Hepatoprotective

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INTRODUCTION

HIV/AIDS has remained a major focus of public health discussion since the last two decades. By the end of 2015, about 40 million people globally were living with HIV/AIDS (WHO/UNAIDS, 2016) and out of this figure 30million live in low and middle income countries. Thus, 87.2% of the world population living with HIV/AIDS lives in low and middle income countries. Sub-Saharan Africa accounts for the largest population, (67%), of the world's HIV/AIDS patients (UNAIDS, 2017).Nigeria is classified as a low income country by the World Bank. With a population of over 150 million people Nigeria has 1.9 million people living with HIV/AIDS (NACA, 2020). Infection with human immunodeficiency virus (HIV) is associated with a decline in immunity and the inability to fight infection and progress to acquired immunodeficiency syndrome (AIDS).Thus a vicious cycle has been envisaged in which undernourished HIV-infected persons have micronutrient deficiencies, leading to further immune-suppression and oxidative stress and subsequent acceleration of HIV replication and CD4+ T-cell depletion (Semba and Mehta *et al*; 2007).

Micronutrient insufficiencies and HIV disease progression are thought to interrelate synergistically progressively aggravating each other (Yeldu *et al*., 2016)].Human Immunodeficiency Virus (HIV) infection induces a wide range of immunological alterations causing the progressive development of opportunistic infections and malignancy, which results in acquired immunodeficiency syndrome (AIDS). Contributing to this progression, is oxidative stress induced by the production of reactive oxygen species (ROS) which may play a critical role in the stimulation of HIV replication and the development of immunodeficiency(Allard *et al*; 2006).Moreover, enhanced oxidative stress may be involved in the pathogenesis of impaired T-Cell responsiveness and enhanced T-cell apoptosis during HIV infection, and it may also play a role in the development of certain HIV-related clinical disorders, including malignancies and HIV related encephalopathy (Raphael *et al*., 2016)).Studies have demonstrated that HIV-infected individuals, particularly those with advanced disease have greater oxidative DNA damage in CD4+ T-cells, as assessed by increased 7,8–dihydro-8-oxoguanine (8-oxo-G) accumulation with a marked decline in DNA glycosylase activity, an enzyme necessary for the repair of oxidative base lesion in CD4+T-cells (Beisel, 2000; Fawzi, *et al;* 2005). The increased activity of inflammatory cytokine, tumour necrosis factor-α (TNF-α) and altered intracellular glutathione redox status found in HIV-infected patients may be responsible for stimulating oxidative DNA damage in CD4+T-cells.

Several prospective, randomized studies suggest that, micronutrients help to reinforce the immune system, improve clinical outcomes and significantly increase CD4+cell count and reduce the severity and impact of opportunistic infections in people living with HIV/AIDS (Fawzi *et al*, 1998; Baum *et al*, 2011). Sufficient nutritional status helps immunity and physical performance, while malnutrition especially through its negative effects on the immune system, further worsens HIV infection by increasing the risk of opportunistic infection and death (Olaniyi and Arinola, 2007, Bilbis *et al*., 2010). In turn, HIV-infected persons are at advanced risk for malnutrition, and certain conditions can magnify the risk such as anorexia, difficulty in swallowing, malabsorption and diarrhoea, altered metabolism of nutrients, increased utilization of nutrients, and greater loss of nutrients (Semba and Tang, 1999).Because of the crucial role of micronutrients in supporting the body's functions, HIV- infected people very much need to have adequate micronutrient status (Depee and Semba, 2011). Timely nutritional support for people living with HIV (PLHIV) may help extend the asymptomatic period of relative health or where severe immune deterioration has already occurred, it may reduce the risk of death (UNAIDS, 2017).

Micronutrient supplements have been used as part of a regular care package obtainable in the medical management of HIV/AIDS patients. However the results were either not well defined or conflicting, as some but not all studies show immunological and clinical benefits (Mashhadi *et al*., Mao *et al*., 2019). Micronutrient supplementation could be a relatively low cost strategy to defer the initiation of expensive, potentially toxic and lifelong

antiretroviral therapy. Supplementation of natural antioxidants is always desirable to modulate the process of free radical production .Spices are important components of human diet and used as condiments in nearly all recipes, they are usually plants based materials added to food to give some specific flavor, aroma to enable the acceptability commonly used spices include ,Carrot,Ginger,Garlic. They are also important in improving the health of individuals due to the presence of bioactive components, and they are widely use in traditional and modern medicines (Okay *et al*; 2003).Even with these advantages of micronutrients supplementation during ART treatment, the cost of micronutrients supplements continue to be a problem in developing countries most especially in Sub-Saharan Africa due to scarce resources (Joshua *et al.,* 2013). These have continued to affect treatment outcome even in the ART era. Presently, there are no data on the use of natural plant micronutrients supplements to support treatment outcome among HIV-Infected-patients taking ART. The present study was designed to investigate the hepatoprotective and immune reconstitution potentials of Carrot-Ginger blend among HIV positive adults taking ART in Nigeria.

MATERIALS AND METHODS:

MATERIALS

The study area was the HIV/AIDS Special Treatment Clinic (STC) in Kafanchan General Hospital, Jama'a Local Government area (LGA) of Kaduna State North-Western Nigeria. Kafanchan being the headquarter of Jama`a LGA is one of the oldest LGA in Southern senatorial District of Kaduna State with a population of 375,500 according to the National Population Commission 2016 population projection. Jama'a Local Government Area is located between latitude 9 o 11' and 9 o 30' N and longitude 8 o 00' and 8 o 30' E. The Local Government is bounded in the East by Kagoro in Kaura LGA, in the North by Zonkwa and Agwan Rimi District of Zango Kataf LGA, to the West by Jaba LGA and in the South by Nasarawa State and in the South-East by Sanga LGA respectively. Jama`a LGA has 11 political wards, the people are mostly commercial farmers and their cash crops include Ginger, sorghum, millet, maize and finger millet.

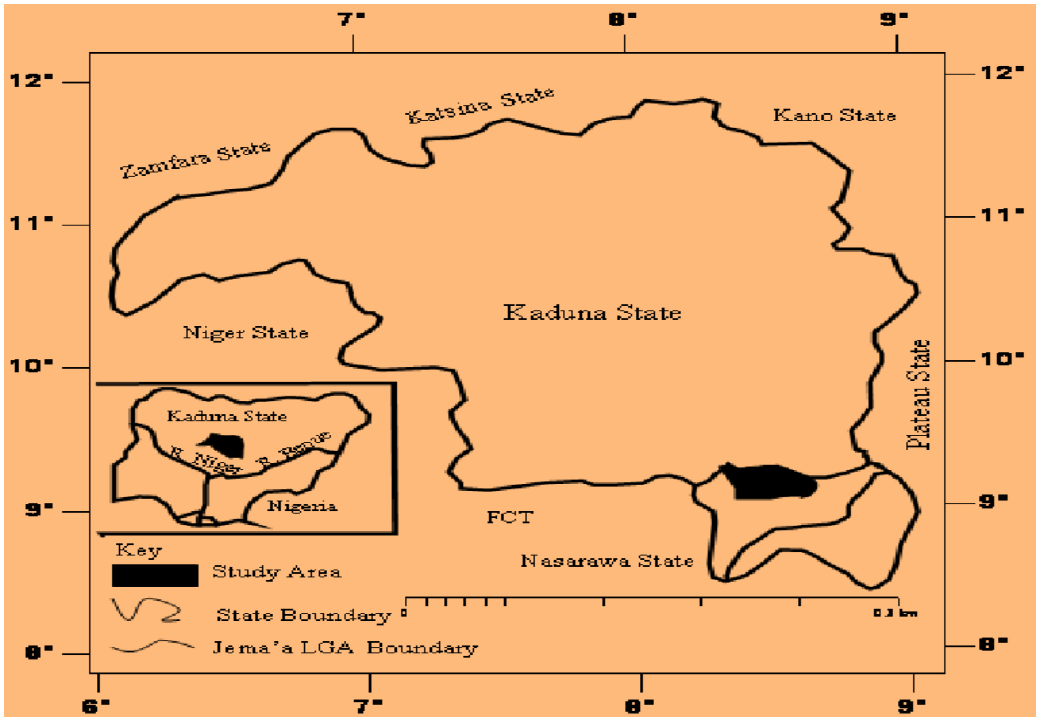

Figure 1: Map of Nigeria and Kaduna State showing the study area

Source: Kaduna Geographical Information System, 2017

Study Design

Enrollment took place from August to September 2017 at the HIV/AIDS Antiretroviral therapy special treatment clinic (STC) in Kafanchan General Hospital, Jama,a Local Government area of Kaduna State North-Western Nigeria. The study was a prospective cohort study involving consenting eligible HIV Positive Male and Female adults attending Kafanchan General Hospital in Kaduna State who were enrolled to either receive commercial micronutrients supplement or micronutrients supplement from 75:25 Carrot-Ginger blends for 90 days. Those who have CD4+ T-cell counts were between 350-400 cells/µl and who were about to commence ART therapy and were between 18-49years were eligible, while pregnant women and those with any metabolic syndrome were excluded. After their baseline assessment the effects of micronutrients supplementation was assessed after 90 days on liver enzymes(ALT and AST), also their CD4+ T-cell counts for 90days were done. On enrollment a semi structured questionnaire was administered and information on patient's socio demographic status which included age, sex, level of education, marital status and income level was evaluated.

Ethical Approval

The study design and protocol were approved by the Ethics and Research Committee of the Kaduna State Ministry of Health **(MOH/ADM244/VOL.1/520**). The research was carried out in accordance with the 1964 affirmation of Helsinki concerning the ethical principles for medical research involving human subjects. Written informed consent was obtained from all study participants before enrolment.

Study Regimen

The study regimen used was a commercially formulated micronutrient supplement that included 5 ingredients: Pro Vitamin A (β-Carotene-150µg), Vitamin C (Ascorbic acid-500mg),Vitamin E(α-Tocopherol-12.06µg),Selenium-250µg and Zinc-50mg and with trade name SelACE® procured from Meyer PVC organics India and distributed in Nigeria by Meyer Vitabiotics Ikeja, Lagos while the micronutrients supplements local formulation contains, Vitamin A (β-Carotene-748.44µg), Vitamin C (Ascorbic acid-3.87mg),Vitamin E(α-Tocopherol-6.07µg),Selenium-3.56µg and Zinc-11.58mg . The subjects were randomly assigned to receive micronutrient supplement SelACE^{R} tablet 1 daily or 2 sachets of 10,000mg/sachet of 75:25 Carrot-Ginger blend after meal for 90 days. The participants were not allowed to use another micronutrient or natural health product. Compliance with the study regimens was assessed according to the methods of Kupka and co-researchers (2008), Kawai and coworkers (2010) and adopted by Yeldu *et al* ;(2016). The HIV-positive ART patients were asked to bring the unused SelACE®tablets back during the next Clinic visit. Participants exchanged a used bottle with a new bottle that contained SelACE®tablets. Compliance with the SelACE®supplement was calculated as the number of SelACE®tablets absent from the returned bottles. This was used as the indicator of the subject's compliance to the study medication.

Collection of Blood Samples.

About 10 mls of blood sample were collected by a trained phlebotomist using venipuncture; immediately 1 ml was transferred into an Ethylene Diamine Tetra acetic Acid (EDTA) tube and about 9 ml into a plain tube. The blood samples were collected at different phases of the study (day 0, day 30, day 60 and day 90). After collection, the samples were delinked from donor's information except that concerning age and sex. Blood samples in EDTA tubes were used for the CD4+ T-cell counts while samples in plain tubes were allowed to clot and then centrifuged at1200 g for 5 min to obtain sera which were used to analyze for Vitamins A, C and E, Zinc, and Selenium using standard methods.

Formulation of Carrot-Ginger Blend.

Fresh Carrot and Ginger were obtained from the market in Zaria and Kafanchan in Jema,a Local Government Area Of Kaduna State, Nigeria in January,2017. The plant was authenticated in the Department of Biological Sciences Ahmadu Bello University, Zaria in Nigeria 2017. The method described by Singh and Kulshrestha (2008) was used. The Carrot and Ginger were sought and washed with distilled water to remove dirt and other contaminants and sliced into smaller sizes of 0.3mm.The samples were sun dried for 2 hours in a stainless tray to reduce moisture contents followed by oven drying at 40 - 45^{o}C for 10 hours. This was followed by grinding of the dried samples and passing through a 0.01mm sieve to obtain a fine powder. 75:25 of the Carrot-Ginger powder were mixed together in an electric stainless blender to obtain

consistency. 10,000mg of the formulation were sealed in a polythene sachet using electric manual sealer and taken for micronutrients analysis.

Determination of Aminotransferases.

The determination of Aspartate Transaminase (AST) was carried out as described by Reitman and Frankel (1957).

Principle and Procedure

Aspartate + α-Ketoglutarrate → Glutamate +Oxaloacetate + 2,4 ditrophenylhydrazine→2, 4 ditrophenylhydrazone

Aspartate substrate react with Ketoglutarrate in the presence of Aspartate Transaminase to give Glutamate and oxaloacetate and under an alkaline pH the oxaloacetate react with 2,4 dinitrophenylhydrazine to form 2,4 dinitrophenylhydrazone which is read spectrophotometrically. 0.1cm^3 of the patients serum was measured into a clean test tube and labeled Test another 0.1cm^3 of distilled water was also measured into a separate tube and labeled blank.0.5cm^3 of Aspartate substrate was introduced into each of the tubes and swirled to mix the tubes were incubated at 37oc for 30mins after which the tubes were removed from the incubator and 0.5cm^3 of 2,4dinitrophenylhydrazine was added to both tubes and swirl to mix and allowed to stand for another 20mins. 5cm^3 of 0.4NNaOH was further added to both tubes the tubes were allowed to stand for another 5mins,the blank was used to zero the spectrophotometer and the absorbance was measured at 505nm.

The determination of Alanine Transaminase (ALT) was carried out as described by Reitman and Frankel (1957).

Principle and Procedure

Alanine + α-Ketoglutarate → pyruvate + L-glutarate + 2,4DNPH → pyruvate dinitrophenylhydrazone.

Alanine substrate react with Ketoglutarrate in the presence of Alanine Transaminase to give Pyruvate and L-glutamate under an alkaline pH the Pyruvate react with 2,4 dinitrophenylhydrazine to form pyruvate 2,4 dinitrophenylhydrazone which is read spectrophotometrically. 0.1cm^3 of the patients serum was measured into a clean test tube and labeled Test another 0.1cm^3 of distilled water was also measured into a separate tube and labeled blank.0.5cm^3 of Alanine substrate was introduced into each of the tubes and swirled to mix the tubes were incubated at 37oc for 30mins after which the tubes were removed from the incubator and 0.5cm^3 of 2,4dinitrophenylhydrazine was added to both tubes and swirl to mix and allowed to stand for another 20mins. 5cm^3 of 0.4NNaOH was further added to both tubes the tubes were allowed to stand for another 5mins, the blank was used to zero the spectrophotometer and the absorbance was measured at 505nm

Determination of CD4+ T-Cell Counts

Principle and Procedure

A beam of Laser passes through the cells labeled with fluorescence in a cuvette. The cells are individually illuminated by the Laser beam. Due to excitation, the molecule emits fluorescence of characteristics colour (emission wavelength spectrum). This fluorescence light is separated into colour ranges by means of optical filters. The intensity of each colour range is analyzed. This was determined by the flow cytometry technique, using Cyflow Counter machine (PARTEC GmbH, Germany). Cyflow counter flow cytometer was used to determine CD4+Tcellcount. Sheaths fluid bottle was filled to 800 ml mark and air was expelled from filter before corked tightly. The fluid was discarded in waste bottle and rinsed with 10% hydrochloride solution and corked tightly. The sample was prepared as follows: into a Rohren test tube, 20 µl of CD4+ T-cell count PE mAb was added and 20 µl of well mixed EDTA with whole blood that was collected within 6 hours was added, mixed and incubated in the dark for 15 min at 25^{o}C.Exactly 800 µl of CD4+ T-cell count buffer was added, mixed and read on the cyflow. The prepared samples was then plugged to the sample port of the cyflow and wait for acquisition and displayed of data.

Measurement of Anthropometric Parameters

Anthropometric parameters were measured using the method described by (Mustapha *et al.,* 2011). Measurement was taking at day 0, 30, 60 and 90 days. Subjects were weighted with minimum clothing to the nearest 0.1 kg by using a regularly calibrated weighing health scale; model ZT 120(Seca Gmbh and Co. Germany), while the heights were measured by using a calibrated Stadiometer, model 220 (Seca Gmbh and Co. Germany). Body mass

index (BMI) was calculated as: BMI = Weight (kg)/Height (m^2).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 20.0 and Microsoft Excel were used for data analysis. Two Way Analysis of variance (ANOVA) and Turkey multiple range Post Hoc test were used to analyze variations from between and within the groups. Pearson's moment correlation was also used to determine relationships between duration of treatments and concentrations of nutrients, CD4+ T-cell counts, and BMI and liver enzymes. Result from the analysis was considered statistically significant when P-Value was less than 5% ($P<0.05$).

RESULTS

Sociodemographic Characteristics of Study the Population

Table 1 displays the general Sociodemographic characteristics of the study population. The data reveal that 62 (68.9%) of the study population were females and 28 (31.1%) were males. Thirty nine (43.3%) were within the age range of 18-28 years while 5(5.6%) were > 50years of age. 43(47.8%) were married while 8(8.9%) were widows. Their levels of education shows that 34(37.8%) have tertiary education while 16(17.8%) have primary education. Their monthly income earnings in Naira reveal that majority (44%) of the study population are poor, earning a meager N10, 000-19,000 monthly and only an insignificant few earn up to or higher thanN40, 000

Table 2 presents the micronutrient concentrations of vitamin A, C, E, Selenium and Zinc for Carrots, Ginger, Carrot-Ginger formulation and standard micronutrient supplement (SelACE^R) and their corresponding percentage daily value intakes (%DVI). The result reveal the amount of Vitamin A in 75:25 carrot-ginger formulations as 748.44±0.43 µg/100g which contributes 249.48% of the RDA required for Vitamin A almost five times than that labeled on the standard micronutrient supplement SelACE^{R,} 150.00µg. The result indicates, the vitamins C, E, selenium and zinc for 75:25 Carrot-Ginger formulation as 3.87±0.04mg/100g, 6.07±0.06µg/100g,3.56±0.09µg/100g, and 11.58±0.74mg/100g which are less when compared to that reported on the SelACE^{R,} micronutrients supplements as, 500.00 mg, 12.06 µg, 250.00 µg and 50.00 mg. Vitamin E and Zinc contributes 30.20 and 77.20 percentage of the RDA required for vitamin E and Zinc respectively

Table 3 presents the mean BMI of the study population. The result show no significant difference ($P >0.05$) between Carrot-Ginger, SelACE^R and the control (22.52±1.83, 22.43±1.92 and 22.03±1.87 Kg/m^2) at day 0. The results reveal that HIV-Infected-patients on Carrot-Ginger formulation and SelACE^R micronutrients supplement recorded the highest BMI (25.17±0.64, 26.65± 0.68 kg/m^2) after 90 days, which were significant ($P<0.05$) when compare with HIV-Infected-patients on ART alone (22.96± 0.95kg/m^2).Table 3 also indicate a significant increase ($P<0.050$) in BMI of HIV positive patient taking Carrot-Ginger formulation and SeLACE^R micronutrients supplement between day 0 to day 90 (22.52±0.52 -25.17±0.64 and 22.43±26.65±0.68 kg/m^2) ,while no significant difference was observed for HIV positive patient on ART alone (22.33±1.87 -22.96±0.95) respectively.

Figure I shows serum alanine transaminase (ALT) activities among HIV-Infected-patients on ART and micronutrient supplement after 90 days. Serum ALT activity among patients on ART alone were significantly higher ($P>0.05$) (12.35 ± 0.10 IU/l) when compared with patients on Carrot-Ginger formulation and SeLACE^R micronutrient supplement (10.00± 0.04 and 9.42 ± 0.14 IU/l), who recorded lesser ALT activity.

Table 4 shows Aspartate transaminase (AST) activity among HIV-Infected- ART and micronutrient supplements after 90 days. HIV-Infected-patients on Carrot-Ginger formulation and SeLACE^R micronutrient supplement show a significant reduction ($P<0.05$) in their AST activity (26.66 ± 0.38, 25.46 ± 0.36 IU/l) when compared with patients on ART alone (28.05 ± 0.35 IU/l) who show a significant increase ($P<0.05$) in their AST activity after 90 days respectively.

Table 5 presents the CD4+ T-cell counts of HIV-Infected-patients on ART and micronutrients supplement after 90 days. There was significant increase ($P<0.05$) in $CD4^+$ cell count among all the patients after 90 days, while patients on Carrot-Ginger formulation and SeLACE^R micronutrients supplement recorded the highest Count ($P<0.05$) (401.86±9.03 , 477.23±8.29 Cells/µl) when compared to HIV-Infected-patients on ART alone (380.47±11.02 Cells/µl).

Figure II shows the Pearson Moment Correlation analysis result for ALT and AST values. The result suggests that there is a very strong positive significant correlation between ALT and AST parameters (R = 0.978, P = 0.000). This implies that there is a 97.8% positive relationship between ALT and AST values. In other words, as ALT values increase/decrease, AST values also increase/decrease. Figure 1 also shows this relationship.

Figure III: demonstrates the relationship between BMI and serum CD4+ T-cell counts of HIV-Infected-patients on antiretroviral therapy. There is a linear positive and significant relationship between patients serum CD4+ T-cell counts and BMI (r = 0.771). This implies that there is a 77.1% positive relationship between CD4+ and BMI parameters. In other words, as CD4+ values increase/decrease, BMI values also increase/decrease

Table 1: Socio-Demographic Characteristics of HIV-positive patients attending Kafanchan General Hospital, Kaduna State

Characteristics of Respondents	**N=90**	**Percentage (%)**
Gender		
Female	62	68.9
Male	28	31.1
Age Category(years)		
18-28	39	43.3
29-39	17	18.9
40-50	29	32.2
>50	5	5.6
Marital Status		
Single	18	20
Married	43	47.8
Widowed	8	8.9
Divorced	21	23.3
Level of Education		
No formal Education	16	17.8
Primary	18	20
Secondary	22	24.4
Tertiary Education	34	37.8
Monthly income(Naira)		
<5000=00	29	32.2
10,000=00-19,999	40	44.4
20,000=00-29,000=00	7	7.8
30,000=00-39,000=00	10	11.1
>40,000=00	4	4.4

Table 2: Micronutrients Compositions of 75:25 Carrot-Ginger Formulation and their Percentage Daily Value Intakes (DVI)

Micronutrients	Carrot	DVI	Ginger	DVI	Carrot-Ginger	DVI	SelACE^{R}	DVI	RDA
Vitamin A (µg/100g)	1013.15 ±0.49	337.66	2.29 ±0.42	0.76	748.44 ±0.43	249.48	150.00	50.00	300.00
Vitamin C (mg/100g)	4.19 ±0.03	6.98	2.07 ±0.02	3.45	3.87 ±0.04	6.45	500.00	833.30	60.00
Vitamin E (µg/100g)	6.66 ±0.02	33.13	4.12 ±0.01	20.50	6.07 ±0.06	30.20	12.06	60.00	20.10
Selenium (µg/100g)	1.42 ±0.13	2.03	10.02 ±0.06	14.31	3.56 ±0.09	5.09	250.00	357.34	70.00
Zinc (mg/100g)	15.32 ±0.05	102.13	1.84 ±0.01	12.27	11.58 ±0.74	77.20	50.00	333.13	15.00

Results are expressed as mean ±S.D of triplicate determinations

DVI=Daily Value Intake RDA=Recommended Daily Allowance SeLACE^{R} = Standard Micronutrient supplement

Table 3: Effects of Micronutrients Supplementation from Carrot-Ginger Formulation on Body Mass Index (Kg/m^2) of HIV-Infected-patients on ART at Kafanchan General Hospital, Kaduna State

Groups	Day 0	Day 30	Day 60	Day 90
GROUP 1:	22.33±1.87^a	22.24±2.02^a	22.87±1.89^a	22.96±0.95^a
GROUP 2:	22.43±1.92^a	24.75±1.76^b	25.23±1.77^c	26.65±0.68^c
GROUP 3:	22.52±1.83^a	22.22±1.69^a	24.77±1.88^b	25.17±0.64^b

Results are expressed as Mean ± S.D of 30 patients per group and values with different superscript alphabets (a-c) along respective horizontal axis are statistically different. (Turkey's multiple range post-hoc test, $p<0.05$). Group 1: HIV-Infected-Patients on ART alone; Group 2: HIV-Infected-Patients on ART and supplemented with Standard (SelACE®) supplement; Group 3: HIV-Infected-Patients on ART and supplemented with Carrot/Ginger Formulation.

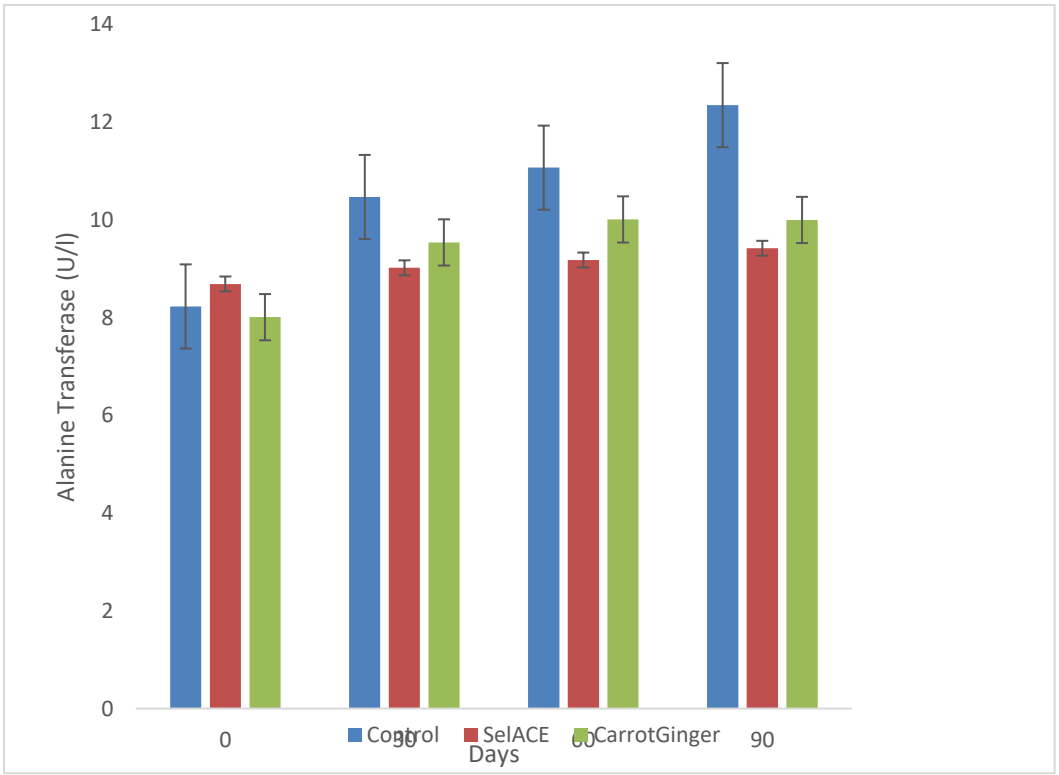

Figure I: Mean ALT activity of HIV-Infected-patients before and after micronutrients supplementation

Table 4: Effects of Micronutrients supplementation on serum Aspartate Transaminase (AST) activity (IU/l) of HIV Positive patients on ART at General Hospital, Kafanchan, Kaduna State

Groups n=30	**Day 0**	**Day 30**	**Day 60**	**Day 90**
Group 1:	27.00 ± 0.34^{b}	27.43 ± 0.51^{b}	28.15 ± 0.29^{c}	28.05 ± 0.35^{c}
Group 2:	27.02 ± 0.38^{a}	26.92 ± 0.36^{a}	26.44 ± 0.22^{a}	25.46 ± 0.36^{a}
Group 3:	27.01 ± 0.41^{b}	27.22 ± 0.42b	27.82 ± 0.36^{b}	26.66 ± 0.38^{b}

Results are expressed as Mean ± S.D of 30 patients per group and values with different superscript alphabets (a-c) along respective horizontal axis are statistically different. (Turkey's multiple range post-hoc test, $p<0.05$). Group 1: HIV-Infected-Patients on ART alone; Group 2: HIV-Infected-Patients on ART and supplemented with Standard (SelACE®) supplement; Group 3: HIV-Infected-Patients on ART and supplemented with Carrot/Ginger Formulation.

Table 5: Effects of Carrot-Ginger Supplementation on CD4+ T-cell counts (Cells/µl) of HIV-Infected-patients at General Hospital, Kafanchan, Kaduna State

Groups	0 Day	30 Days	60 Days	90 Days
Group 1:	375.97**±**16.04^{b}	378.30**±**9.59^{a}	381.23**±**9.92^{a}	380.47**±**11.02^{a}
Group 2:	373.23**±**16.34^{a}	419.87**±**6.52^{c}	455.57**±**8.53^{c}	477.23**±**8.291^{c}
Group 3:	372.74**±**16.42^{a}	389.27**±**6.56^{b}	391.85**±**8.45^{b}	401.86**±**9.03^{b}

Results are expressed as Mean ± S.D of 30 patients per group and values with different superscript alphabets (a-c) along respective horizontal axis are statistically different. (Turkey's multiple range post-hoc test, $p<0.05$). Group 1: HIV-Infected-Patients on ART alone; Group 2: HIV-Infected-Patients on ART and supplemented with Standard (SelACE®) supplement; Group 3: HIV-Infected-Patients on ART and supplemented with Carrot/Ginger Formulation.

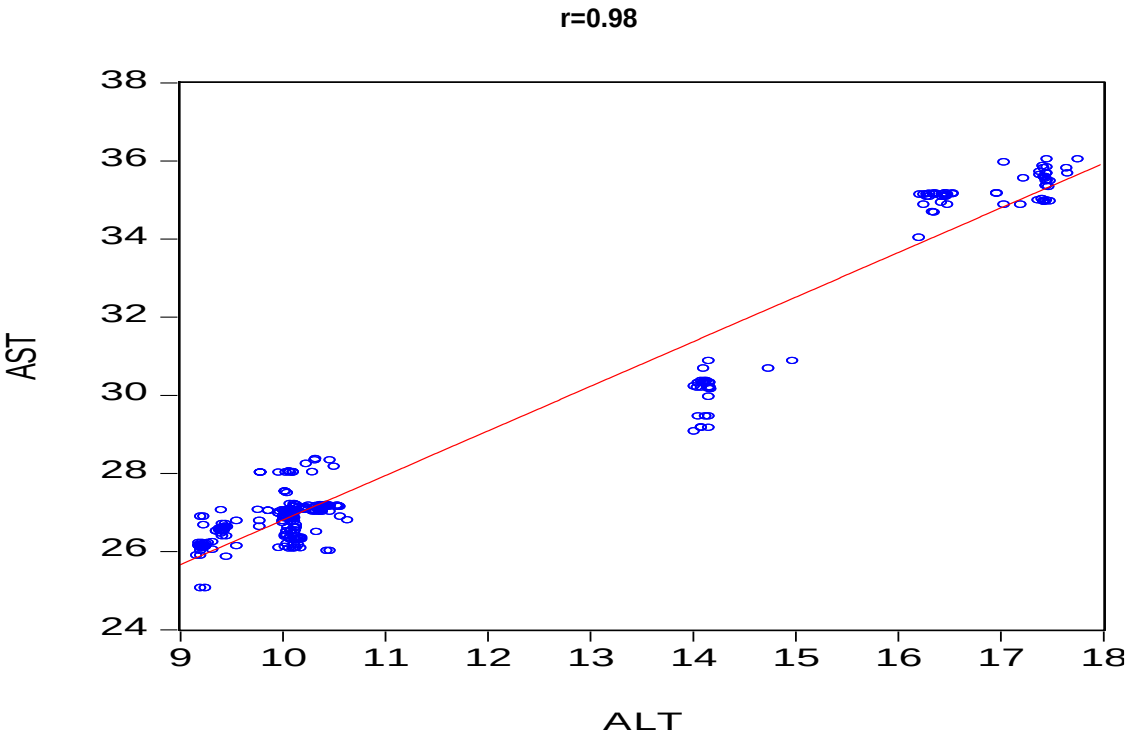

Figure II: Association between ALT and AST

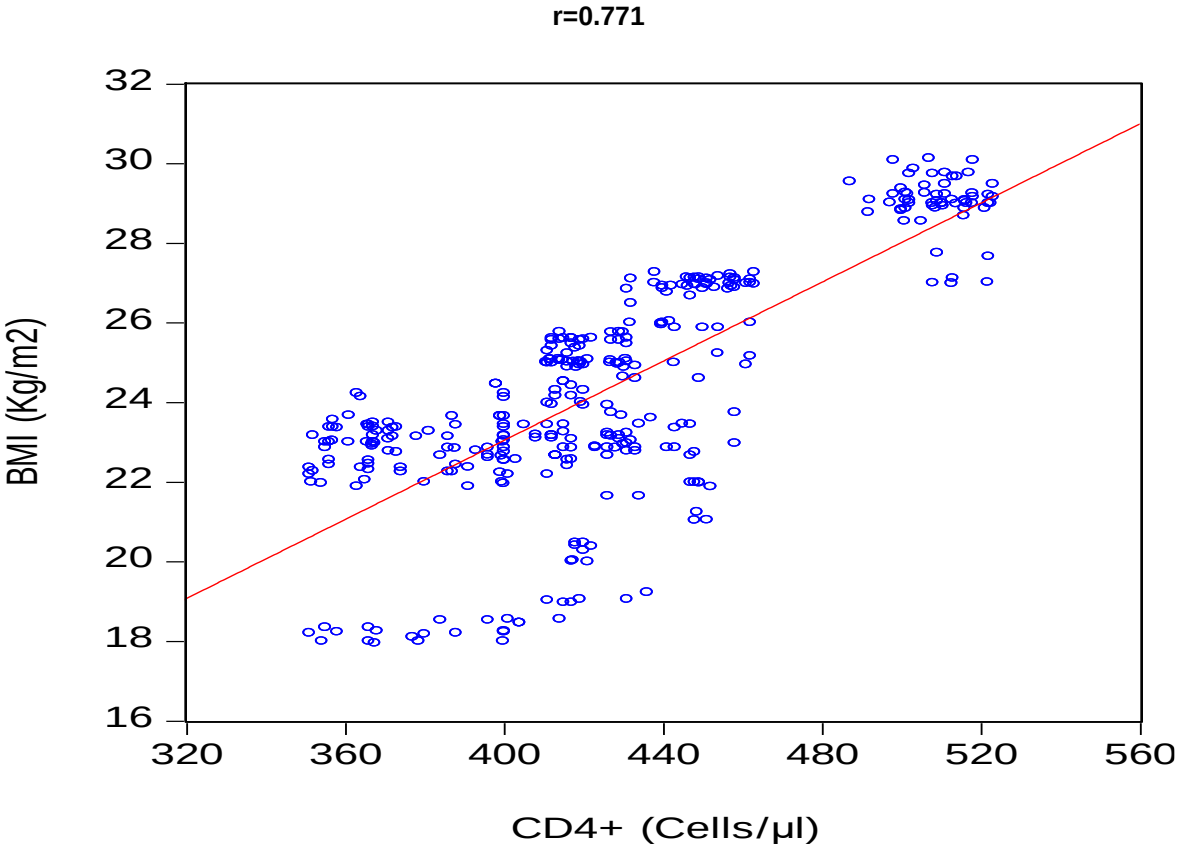

Figure III: Association between serum CD4+ T-cell counts and Body Mass Index

DISCUSSION

The current study revealed that socio-economic factors such as level of education, income, gender, number of partners and sexual habit are some of the major predisposing factors that are likely to enhance the prevalence of HIV/AIDS in low income communities. Findings from the study indicate that HIV/AIDS prevalence was higher in female than in their male counterparts. This could likely be due to the facts that more female's presents themselves for medical checkup and access HIV services while the males for fear of stigmatization and consequents do not visit the hospital to check their health status until they are sure (NACA (2017). These findings are in agreements with the report of Yeldu *et al* (2016) and NACA (2018). The same studies also reported that low level of education and low income were high among females than the male counterparts; this could likely be one of the reasons while women are more affected than men. These findings also agreed with the report of UNAIDS, 2017.

This study showed that carrot-Ginger blend is a good source of micronutrients A, C, E, Selenium and Zinc compared to carrot or Ginger alone, 75:25carrot-Ginger blend is able to give close amounts of value to meet the Recommended Dietary Allowance (RDA)) as the costly synthetic micronutrient supplements SelACE^{R}. The compositions of vitamins A, C, E, Selenium and Zinc displayed on table 1 is in agreement with the findings of Krishan *et al;* (2012) who reported that carrot powder provide good amounts of vitamins A, C, E, Selenium and Zinc. In a chemical analysis to determine the bioactive components presents in Ginger roots Latona *et al*, (2012) also reported the presence of vitamins A, C, Selenium and Zinc in Ginger roots respectively. The present study also showed that Carrot-Ginger blend can provide a better DVI for vitamins A, C, E, Selenium and Zinc that can match the one provided by the synthetic expensive SelACE^{R} micronutrients supplements as such it has the ability to improve the antioxidant pool and rebuild the immune system (Jibril *et al.,* 2016), thereby reducing oxidative stress experienced by HIV-Infected-patients taking ART.

Asymptomatic Liver injury (ALI) is also a contributing factor increasing morbidity and mortality among HIV-Infected-patients on ART.Mild liver toxicity was observed among all the HIV infected patients enrolled for this study, this could possibly be because of the absence of risk factors that can contribute to the development of severe liver injury in all the patients. This finding agrees with that of (Sulkowaski *et al*., 2000). This study demonstrates the beneficial role of micronutrients supplementation in reducing aminotransferases elevation during ART therapy. these findings are in line with that reported by Sha *et al*; 2015 who reported that natural antioxidant poses protective role against hepatocytes, they also demonstrated that Natural antioxidants contained in edible or medicinal plants often possess strong antioxidant and free radical scavenging abilities as well as anti-inflammatory action, which are also supposed to be the basis of other bioactivities and health benefits. These findings are also in agreements with the findings of Raphael *et al*., (2016) who also reported the antioxidant protective roles against markers of Kidney disease in 54 HIV-Infected-patients on ART. This was also reported by Joshua *et al.,* (2013). The current studies have shown that highly active antiretroviral therapy (ART) was found to be associated with low level hepatotoxicity at initiation, regardless of drug class or combination. Elevated transaminases due to antiretroviral therapy was independent of patient's age and increased levels of transaminases showed a significant positive linear relationship with increase in the duration of treatment. This is also in line with the findings of Lucien *et al., (*2010) who in a prospective cross sectional study involving 150 HIV positive Cameroonians on ART reported similar findings.

Antiretroviral therapy (ART) was found to be associated with low level hepatotoxicity at initiation, regardless of drug class or combination. This study shows a positive correlation between the mean serum ALT and AST levels in all the patients, this is in line with that documented by Lucien et al (2009) The studies of Soriano *et al*.., (2001) found that certain co morbidities, such as chronic hepatitis B (HBV) or hepatitis C (HCV) infection, may predispose patients to ARLI. Also, all the patients who presented with elevated levels of transaminases were found to present with first degree hepatotoxicity which correspond to low level liver toxicity Spengler *et al.,* (2002).The mechanism by which the study regimen protect the liver cells during ART medication has not been documented, but it may be linked to the antioxidant defense properties of vitamin A, vitamin C, vitamin E, selenium and zinc by suppression and elimination of free radicals generated due to the activities of the virus and ART medication.

The present study further justifies other studies on the role of micronutrients supplementation in HIV positive Patients. There would be immune

reconstitution, reduced oxidative stress, and increased CD4+ count in HIV diseases conditions (Allard *et al*., 1998); Burbano *et al*., 2002).The increase in CD4+ count of HIV-Infected-patients on SelACE^{R} and Carrot-Ginger supplements with increase in supplementation periods from their baseline, in the present study agree with that reported by Raphael *et al*..,(2016).The present study also shows that the increase in the mean serum CD4+ cell counts and immune reconstitution observed for the SelACE^{R} and Carrot-Ginger group could could be due to increase appetite caused by the intake of micronutrients from the carrot-ginger formulation which could be responsible for improving their BMI, immune reconstitution and recovery and also as a results of the antioxidant defense properties of Vitamins A, C, Selenium and Zinc to suppress and eliminate free radicals that are generated from the activities of the virus itself and from the ART medication also, the findings from these study is in line with so many scientific findings, Increase in the mean serum CD4+ counts in the body of HIV positive individuals have been reported as indices for improvement in their health status, especially HIV positive pregnant women and neonates, through reduction in clinical HIV disease progression and HIV related mortality (Fawzi *et al.,* 1998; Mocchegiani *et al.,* 1999; Jiamton et, *al*., 2003; Chaisilwattana *et al.,* 2003).

The effect of the carrot-ginger formulation observed in this study could be linked to the antioxidant defense properties of the micronutrients such as Vitamins A, C and E, Selenium and Zinc through suppression and elimination of free radicals that are generated due to the activities of the virus itself and from ART medication. Carrot-ginger blend has beneficial role in boosting and recovering the immune system and reducing liver toxicity usually observed among HIV-positive patients on ART medication. Carrot-ginger is available, affordable, and sustainable which could substitute the commercial micronutrients supplements which are expensive. HIV-positive patients who are yet to be enrolled for ART medication and those in low-income communities may be giving Carrot-ginger blend, this will not only save cost it may delay disease progression and reduce morbidity and mortality in HIV positive patients.

CONCLUSION

Based on the results from the current study it can be concluded that natural plants micronutrients supplementation from Carrot-Ginger blend which is cheaper and readily available and play a beneficial role to support HIV-infected-patients on ART, due to their ability to increase CD4+ T cell counts and protect the liver, eliminate waisting and reconstitute the immune system. The study found a strong positive correlation between ALT and AST and also between CD4+ T-cell counts and Body mass index (BMI). Also this data further demonstrated the antioxidant defense properties of micronutrients supplement during ART therapy. The present findings have shown the beneficial role of carrot-ginger formulation in the recovery of the immune system and protection of the liver. However other study can focus on giving the Carrot-ginger blend for a longer duration and its bioavailability, toxicity and effects on other biochemical profile can be investigated.

Conflicts of Interest

The authors have no known competing financial interest or personal relationship that could have influenced the work reported in this paper

REFRENCES

Allard, J.P., Aghdassi, E. and Chau, J. (1998). Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV**-**infected subjects. *AIDS,* **12:**1653–1659.

Baum MK, Lai S, Sales S, Page JB Campa. (2010). A. Randomized controlled clinical trial of zinc supplementation to prevent immunological failure in HIV-positive adults. *Clinical Infectious Diseases.* 2010;**50**(12):1653-1660.

Beisel WR. AIDS. In: Gershwin ME, German JB, Keen CL, eds. (2000). Nutrition and Immunology: Principles and Practices. Totowa, NJ: Humana Press, **2**:389-403.

Bilbis LS, Idowu DB, Saidu Y, Lawal M, Njoku CH. (2010). Serum levels of antioxidant vitamins and mineral elements of human immunodeficiency virus positive subjects in Sokoto, Nigeria. *Annals Africa Medicine***, 9**:235-239.

Fawzi, W.W., Msamanga, G. I. and Spiegelman, D. (2004). A randomized trial of multivitam**in** supplements and HIV disease progression and mortality. *New England Journal of Medicine,* **351**:23–32

Fawzi, W.W., Msamanga, G.I.and Spiegelman, D. (1998). Randomized trial of effects of vitam**in** supplements on pregnancy outcomes and T cell counts in HIV**-**1-

infected women in Tanzania. *Lancet;* **351:**1477–1482.

Jiamton, S., Pepin**,** J. and Suttent, R. (2003). A randomized trial of the impact of multiple micronutrient supplementations on mortality among HIV**-**infected individuals li**v**ing in Bangkok. *AIDS,*17:2461–2469

Joshua, Z. P, Aminu, A.M, Mariam, I. S, Sidi, M.T. (2013). Effects of micronutrients supplementation and highly active antiretroviral therapy on the CD4+ counts, vitamins, minerals and liver enzymes among HIV-Infected-patients attending Barau Dikko General Hospital, Kaduna State. *Nigerian Journal of Nutrition Sciences,* **34**(1): 43-48.

Kenako,J. J. (1999). Clinic Biochem of Animal 4th edition. Academic press inc. New York, pp.932.

Krishan D. S, Nayan S. T, Surekha A. (2012). Chemical compositions and functional properties of Carrots. *Journal of Food Science Technology*, **49**(1**):**22-32

Kupka R, Mugusi F, Aboud S, Mmsamanga G.I, Finkelstein J.L,Spiegelman A. D. (2008). Randomized, double blind, placebo, controlled trial of selenium supplements among HIV-infected pregnant women in Tanzania: Effects on material and child outcomes. *American Journal of Clinical Nutrition,* **87**:1802-1808.

Latona D. F, Oyeleke G. O, Olayinwola A.O. (2012). Chemical Analysis of Ginger Roots. IOSR I *Journal Applied Chemistry,* **1(**1): 47-49

Lucien, K.F.H, Clement, A.N.J., Welediji, P.,Ndikvu ,C.P.(2010).The effects of Antiretroviral treatment on the liver function enzymes among HIV infected outpatients attending the Central Hospital of Yaoundé Cameroon. *African Journal of Clinical and Experimental Microbiology,* **11**(3)*:*174-178.

Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. B. (2019). Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe). Foods (Basel,Switzerland), **8**(6): 185.

Mashhadi, N. S., Ghiasvand, R., Askari, G., Hariri, M., Darvishi, L., & Mofid, M. R. (2013). Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: Review of current evidence. *International Journal of Preventive Medicine*, **4**(1):36–S42.

Mustapha, K.B., Ehianeta, T.S and Kirim, R.A. (2011). Highly active antiretroviral therapy (HAART) and body mass index (BMI) relationship in people living with HIV/AIDS (PLWHA) in the federal capital territory, Nigeria and the neighboring states. *Journal of AIDS and HIV Research,* **3**(3):57-52.

National Agency for the Control of AIDS. 'Nigeria GARPR 2020 Report. Abuja, Nigeria: NACA; 2020. https://naca.gov.ng/nigeria_garpr_2020_report; 2020 [accessed 13 October 2020].

Olaniyi JA, Arinola OG. (2007). Essential trace elements and antioxidant status in relation to severity of HIV in Nigerian patients. *Medical Principles and Practice*, **16**:420-450.

Semba, R.D.(1999).Vitamin A and immunity to viral, bacterial and protozoan infections. *Proceedings on Nutrition, 4:* 719–727.

Singh P, Kulshrestha K. (2008). Nutritional quality of food supplements based on carrot powder and grits*. Journal of Food Science and Technology;* **45**:99–101

Soriano, V., Puoti, M., Garcia-Gasco, P., Rockstroh, J. K., Benhamou, Y., Barreiro, P., Spengler,U; Lichterfeld, M., Rockstroh, J.K. (2002). Antiretroviral drug toxicity-Therapy. Reuters Health, **22**(14):55-59.

Spengler, U; Lichterfeld, M.; Rockstroh, J.K. (2002). Antiretroviral drug toxicity-Therapy. *Reuters Health*, **22**(14**):**55-59

United Nations Programme on HIV/AIDS.(2017). Overview of the global AIDS epidemic. *Report on the global AIDS epidemic*. *FDC ID:170393 NDB Number:* 11124.

World Health Organization. (2014). World Health Organization. Report on the global AIDS pandemic;*http:www.who/en/ressources/campaign/factsheet/*2014/6/0172236066357.htm

World Health Organization. (2017). World Health Organization. Report on the global AIDS pandemic in Sub-Saharan Africa and Low income Countries. Available from http://www.who.org/globalreport/2017/10/0181036062356.htm

Yeldu, M. H, Das, S.C, Bilbis, L. S, Saidu,Y, Njoku, C.H.(2016).Effects of Micronutrient supplementation on CD4+Cell count and anthropometric parameters in HIV-positive adults on highly active antiretroviral therapy and treatment naïve in Sokoto, Nigeria**.** *British Journal of Medicine & Medical Research,* **12**(1): 1-9.