

Clinical Study on the Effect of *Moringa oleifera* on Body mass index, Serum Level of High density lipoprotein and Triglyceride in Subjects Taken Tenofovir, Lamivudine and Efavirenz Combination Regimen.

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Abstract

Introduction/Aim: Antiretroviral drugs can have adverse effects. Most are manageable, but some can be serious. The aim of this clinical study is to evaluate the potential benefits of taking *Moringa oleifera* on body mass index (BMI), blood triglyceride and High density lipoprotein (HDL) level in patient taking Tenofovir/Lamivudine/efavirenz (TLE) combination.

Materials/Method: The study was designed as a Longitudinal Randomized Comparative Trial (LRCT) involving 140 HIV adult subjects (56 males, 84 females) who have been on Tenofovir/Lamivudine/efavirenz (300/300/600mg) TLE combination for at least 6 months prior to the study. They were recruited from a Teaching Hospital in Nigeria. *Moringa oleifera* capsules (200mg) were administered by the subjects to be used beginning from the first day of visit 0, through visit 1 (after four weeks) and 2 (after 12 weeks). Blood samples of subjects were collected at each visit (visit 0, 1 and 2) and analyzed for triglyceride and HDL level.

Result: There was no significant reduction in serum HDL level ($P < 0.01$) of subjects in visit 1 but there was significant ($P < 0.01$) increase on visit 2 when compared to visit 0. There was also significant improvement in blood triglyceride level ($P < 0.01$) in visit 1 and 2 compared to visit 0 of tenofovir/Lamivudine/Efavirenz (TLE) moringa combination

Conclusion: Results from the study suggests that *Moringa oleifera* may be useful in improving triglyceride and cholesterol level of patients receiving TLE combination.

Keyword: *Moringa oleifera*, Blood, Glucose, Tenofovir, Triglyceride.

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Introduction

HIV is a retrovirus that targets the immune system, which is the system that fights off infection and disease. The virus damages or destroys white blood cells called CD4 cells. This makes it difficult for the body to fight off illness [1-3]. Antiretroviral therapy prevents the virus from multiplying, which reduces the amount of HIV in the body [4]. This gives the immune system a chance to produce more CD4 cells. Although antiretroviral therapy cannot completely remove HIV from the body, it keeps the immune system strong enough to combat infections and some HIV-related cancers. The aim of antiretroviral therapy is to reduce the amount of HIV in the blood to very low levels. Viral suppression occurs when the count reaches fewer than 200 copies of the virus per milliliter of blood [5].

HIV drugs have improved over the years, and serious side effects are less likely than they used to be. However, HIV drugs can still cause side effects [6]. Some are mild, while others are more severe or even life-threatening. A side effect can also get worse the longer a drug is taken. It's possible for other medications to interact with HIV drugs, causing side effects. Other health conditions can also make the side effects from

HIV drugs worse [7]. For these reasons, when starting any new drug, people with HIV should tell their healthcare provider and pharmacist about all the other medications, supplements, or herbs they're taking [8,9].

Antiretroviral formulations blocks HIV at certain stages of the viral "life cycle" [10,11]. Processes such as "binding, fusion and entry, reverse transcription and integration, proviral transcription, cytoplasmic expression" are involved in the viral cycle [12], replication, assembly and budding, release, maturation. *Moringa oleifera* Lam (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics [13]. The *Moringa* leaf is prepared for consumption either fresh, dried, or as extract of an aqueous solution [14-16]. Some populations consume it in their daily diet, whereas others use as a nutritional supplement and for medicinal purposes, mainly for diabetes. Common ailments such as malaria, typhoid fever, swellings, cuts, hypertension and diabetes are treated with the

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leaves [17]. They are also used to bring about milk production in lactating women [18], sediment impurities of water [19], detoxify the system of free radicals [20], improves immunity (to manage HIV/AIDS and treat related symptoms). The aim of this study is to evaluate the clinical effect of taking *Moringa oleifera* with Tenofovir/Lamivudine/efavirenz (300/300/600mg) (TLE) regimen on the blood cholesterol and the triglyceride level.

Materials and method

The study designed was a Longitudinal “Randomized Comparative Trial” (LRCT) as applicable in clinical investigation involving two or more patient treatment groups, over a time frame. This study is designed in line with a part of the FDA (Food and Drug Administration)/WHO Phases during “randomized controlled clinical trials” (RCCT) of drugs. However, details about the application of RCCT have been clarified by FDA/WHO which made the purpose of such investigation explicit; stating that it was designed to affirm and or set aside hypothetical clinical claims [21] of administrable substances. Groups were analyzed in 3 phases as baseline (commencement) 4weeks follow-up and 12 weeks post commencement of supplements (conclusion of administration).

Procedure

Subjects were recruited at the out-patient department of a Teaching Hospital HIV-clinic. Prospective participants were officially and properly informed prior to the exercise, doubts were cleared and benefits explained to the patients. The Longitudinal Randomized Comparative Trial (LRCT) was employed and used.

The study was designed as a Longitudinal Randomized Comparative Trial (LRCT) involving a total of 140 HIV adult subjects (56 males, 84 females) who have been on Tenofovir/ Lamivudine/ efavirenz (300/300/600mg) TLE combination for at least 6 months. Subjects were categorized into groups as underweight, normal weight, over weight and obese. On visit 0, blood samples of the subjects already on TLE regimen (without *Moringa* or any supplements) for at least 6 month were taken for analysis. *Moringa oleifera* capsules (200mg) were given to each subject to be taken from commencement (baseline) to 12 weeks post commencement of study. Blood samples of subjects were collected at each visit (visit 1 and 2) and analyzed for HDL and triglyceride level.

Data collection

Anthropometric parameters (weight and height) and blood samples were determined for eligible patients (participants) distributed into the various categories; after duly signed consent forms were retrieved. Blood samples were analyzed at the UPTH Hematology research lab within the hospital premises.

Blood Sample

Analysis of samples was done at the hematology laboratory of the University of Port Harcourt Teaching Hospital (UPTH), Rivers state, Nigeria. “Computerized clinical chemistry analyzer” (VS10) (Vitro Scient) operating with the principle guided by “Beer-lambert’s law” was used to determine concentration of biochemical parameters under study.

Data analysis

Data was presented in tables using SPSS (IBM® version 23) and MATLAB (version 17). Descriptive statistics was used to express variable characteristics (with continuous data stated as mean (S.D) while categorical data as frequency [%]). Dunnette T3 Post Hoc test of multiple comparisons was used to compare means, while binary logistic regression was used to predict factors contributing to the changes in variables. Variable interactions were tested at 95% confidence level; with $P \leq 0.05$ taken to be significant.

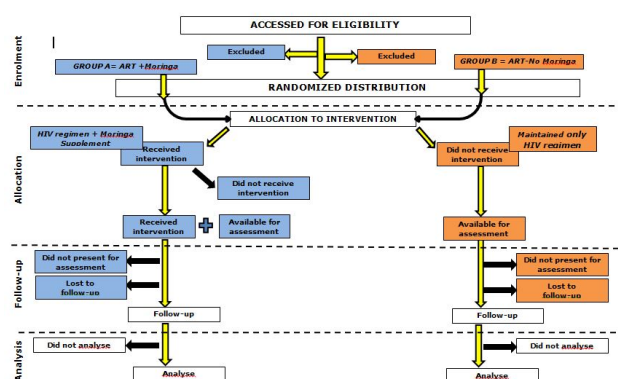


Figure 1. Model for random comparative trail with modification by the researcher.

Results

High density lipoprotein (HDL) and triglyceride level of ART subject taking TLE on visit day 0. Underweight subjects were found to be 6 subjects, normal weight were 76 subjects, overweight were 44 subjects while obese were found to be 14 subjects (table 1).

Effect of *Moringa oleifera* on ART patient taking TLE on visit day 1

There was no significant differences ($P < 0.001$) in HDL level observed in mean values of TLE/*Moringa* subjects between visit 0 and visit 1. There was significant ($P < 0.001$) decrease in the level of triglyceride in visit 1 when compared to visit 0. Also, there was no significant difference between TLE/*Moringa* (visit 1) and TLE/Non *Moringa* (visit 1) in the level of serum triglyceride of the subjects (table 2,3,4,5 and 6).

Effect of *Moringa oleifera* on ART patient taking TLE on visit day 2

There was statistically significant ($P < 0.001$) different in mean values of the TLE/Moringa subjects between visit 0 and visit 2 in HDL and triglyceride levels, while there was no significant differences ($P < 0.001$) between TDF/Non Moringa (visit 2) and TDF/Non Moringa (visit 0) in the level of serum HDL and triglyceride (table 2, 3, 4, 5 and 6).

Table 1. Socio-demographic and anthropometric characteristics of the study population.

	Sex	N	Mean \pm S.D	S.E
Age (yrs.)	Male	54	39.11 \pm 10.46*	1.43
	Female	86	35.63 \pm 8.33	0.89
	Total	140	36.01 \pm 9.41	0.77
Weight (kg)	Male	54	69.00 \pm 9.76	1.3
	Female	86	66.43 \pm 12.1	1.25
	Total	140	67.38 \pm 11.3	0.92
Height (m)	Male	54	1.71 \pm 0.09**	0.01
	Female	86	1.64 \pm 0.08	0.01
	Total	140	1.66 \pm 0.09	0.01
BMI (kgm-2)	Male	54	23.77 \pm 3.26	0.44
	Female	86	24.79 \pm 4.60	0.47
	Total	140	24.41 \pm 4.17	0.35

Table 2. The descriptive characteristics and test of mean differences of metabolic profile of the HIV patients on Tenofovir based ART at Visit 0 (Baseline).

Descriptive statistics					T-test of mean difference		
PARAMETERS	Sex	N	Mean \pm S.D	S.E	t-value	P-value	Inf
TG (mmol/l)	Male	56	1.43 \pm 0.49	0.07	2.305	0.023	S
	Female	84	1.24 \pm 0.47	0.05			
	Total	140	1.32 \pm 0.48				
HDL (mmol/l)	Male	56	1.38 \pm 0.44	0.06	0.526	0.6	NS
	Female	84	1.34 \pm 0.56	0.06			
	Total	140	1.36 \pm 0.51				

Note: TG=Triglyceride, HDL=High density lipoprotein N=Distribution, S.D=Standard deviation, S.E=Standard error of mean, Min=Minimum, Max=Maximum, P-

value=Probability value, t-value=t-test calculated value, Inf=Inference (S=Significant, NS=Not Significant).

Table 3: The descriptive characteristics of metabolic profile of the HIV patients on Tenofovir with Moringa (TDF/M) and Tenofovir alone (TDF/NM) at Visit 1 (4 weeks of administration).

GROUPS		T.G (mmol/l)	HDL (mmol/l)
TDF/M (Male=56)	Mean \pm S.D	1.25 \pm 0.40	1.27 \pm 0.50
	S.E	0.05	0.06
	Range(Min – Max)	0.42 - 2.17	0.39 - 2.27
TDF/NM (Female=84)	Mean \pm S.D	1.27 \pm 0.37	1.32 \pm 0.49
	S.E	0.04	0.05
	Range(Min– Max)	0.24 - 2.21	0.4 - 2.25
Total (N=140)	Mean \pm S.D	1.26 \pm 0.38	1.30 \pm 0.49
	S.E	0.03	0.04
	Range(Min– Max)	0.24 - 2.21	0.39 - 2.27

Table 4. The descriptive characteristics of metabolic profile of the HIV patients on Tenofovir with Moringa (TDF/M) and Tenofovir alone (TDF/NM) at Visit 2 (12 weeks of administration).

GROUPS		T.G (mmol/l)	HDL (mmol/l)
TDF/M(Male=56)	Mean \pm S.D	1.09 \pm 0.55	1.87 \pm 0.57
	S.E	0.07	0.08
	Range(Min –Max)	0.02 – 3.25	1.17 – 4.27
TDF/NM (Female=84)	Mean \pm S.D	1.15 \pm 0.47	1.42 \pm 0.46
	S.E	0.05	0.05
	Range(Min– Max)	0.23 - 3.18	0.17 - 3.35
Total (N=140)	Mean \pm S.D	1.13 \pm 0.50	1.60 \pm 0.55
	S.E	0.04	0.05
	Range(Min– Max)	0.02 - 3.25	0.17 - 4.27

Table 5. Post Hoc (Dunnette T3) multiple comparison of the metabolic profile of HIV patients on TDF not taking Moringa (TDF) supplement across the various visits.

Parameters	Visits	Mean \pm S.D	Min	Max	S.E	95% C.I for Mean	
						Lower Bound	Upper Bound
T.G (mmol/l)	Visit 0	1.24 \pm 0.47	0.3	2.3	0.05	1.14	1.35
	Visit 1	1.27 \pm 0.37	0.24	2.21	0.04	1.19	1.35

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	Visit 2	1.15 ± 0.47	0.23	3.18	0.05	1.05	1.25
	Total	1.22 ± 0.44	0.23	3.18	0.03	1.17	1.28
HDL (mmol/l)	Visit 0	1.34 ± 0.56	0.28	2.31	0.06	1.22	1.46
	Visit 1	1.32 ± 0.49	0.4	2.25	0.05	1.21	1.42
	Visit 2	1.42 ± 0.46	0.17	3.35	0.05	1.32	1.52
	Total	1.36 ± 0.50	0.17	3.35	0.03	1.3	1.42

Table 6. Post Hoc (Dunnette T3) multiple comparison of the metabolic profile of HIV patients on TDF taking Moringa (TDF+M) supplement across the various visits.

Parameters	Visits	Mean ±S.D	Min	Max	S.E	95% C.I for Mean	
						Lower Bound	Upper Bound
T.G (mmol/l)	Visit 0	1.43 ± 0.49 ^{^A}	0.58	2.83	0.07	1.3	1.56
	Visit 1	1.25 ± 0.40 ^I	0.42	2.17	0.05	1.14	1.35
	Visit 2	1.09 ± 0.55	0.02	3.25	0.07	0.94	1.23
	Total	1.26 ± 0.50	0.02	3.25	0.04	1.18	1.33
HDL (mmol/l)	Visit 0	1.38 ± 0.44 ^{^A}	0.24	2.14	0.06	1.26	1.5
	Visit 1	1.27 ± 0.50 ^I	0.39	2.27	0.07	1.14	1.41
	Visit 2	1.87 ± 0.57	1.17	4.27	0.08	1.72	2.02
	Total	1.51 ± 0.57	0.24	4.27	0.04	1.42	1.59

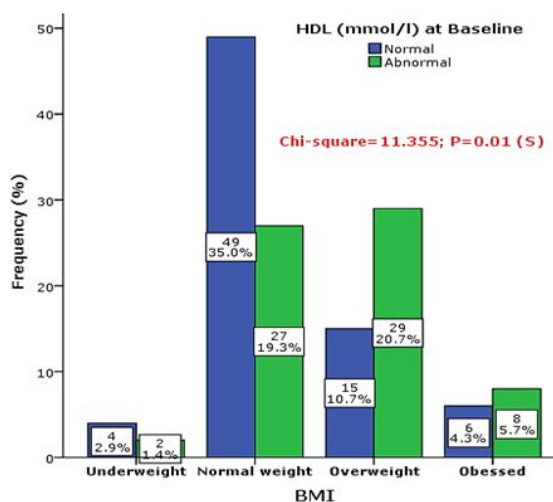


Figure 2. BMI associated high density lipoprotein (HDL) classification and distribution at Visit 0 (Baseline).

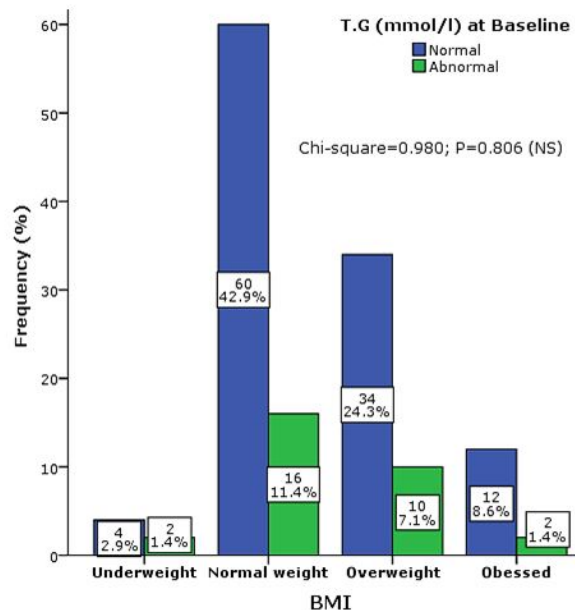


Figure 3. BMI associated triglyceride classification and distribution at Visit 0 (Baseline).

Discussion

The primary goal of antiretroviral therapy for human immunodeficiency virus (HIV) infection is suppression of viral replication [3, 7, 22]. Evidence indicates that the optimal way to achieve this goal is by initiating combination therapy with two or more antiretroviral agents. This therapy helps keep the body healthy and prevent infections [12, 26]. Specifically, successful antiretroviral therapy prevents people from developing advanced HIV and makes it impossible to transmit the virus to others. Although the study was abinitio designed to investigate the effect of *M. oleifera* supplementation on TDF dependent HIV patients; however various reports of ARV therapy associated metabolic abnormalities [22-25] informed this investigation. Also the increased prevalence of these abnormalities necessitated the evaluation of the relationship TDF based regimen has with abnormal presentation of selected metabolic profile. Prevalent abnormal metabolic abnormalities were higher in proportion with abnormal BMI; mostly overweight and obese; as proportion of HIV patients with HDL-C and hypertriglyceridemia was almost 2 times that observed in normal body weight HIV patients. This trend has been observed by Joseph et al. [24] although in normal individual.

Tradomedical practices and other scientific researches (mostly on animal models) have suggested that Moringa significantly (positively) affect abnormal metabolic profile induced by various physiologic factors [24-28]. Therefore it would be worthwhile to determine how effective an already established Moringa supplement is in ameliorating some metabolic abnormalities induced by ART drug such as TDF base regimen.

Despite lower HDL levels, at visit 1 (4 weeks after administration of Moringa supplement, no significant increase

was observed; However, a significant decrease in both the mean values as well as proportion of subjects with high TG and significant increase HDL-C was observed at Visit 2; when compared to the TDF-NM group which had a reverse result, is an indication of positive gradual effect of *M. oleifera* supplement. These observations are in accord with those of [29-31] which they reported the “hypcholesterolemic and hypoglycemic” effect of *M.oleifera*. The “antilipidemic effect of Moringa” in this study is in accord with the findings of Siegfried et al., [32] and Horvath et al., [33] as they mentioned that the presence of a bioactive phyto-constituents, that is β -sitosterol played the significant role. Different parts of the MO tree have been established as being good sources of unique glucosinolates, flavonoids and phenolic acids, carotenoids, tocopherols, polyunsaturated fatty acids (PUFAs), highly bioavailable minerals, folate etc. most of these compound have established to excercised various pharmacological activity [17,21,25,34] observed that *M.oleifera* consumed in dietary form lowered the serum CHOL, PHOSLIPID, TG, VLDL, LDL, cholesterol to “phospholipid ratio and atherogenic index”, but increased the “HDL/HDL-total cholesterol ratio”. The “antilipidemic effect of Moringa” in this study is in accord with the findings of Adedapo et al. [35] and Adias et al., [36]; as they mentioned that the presence of a bioactive phyto-constituents, that is β -sitosterol played the significant role. *M.oleifera* appears not to have much effect on serum level of high density lipopolysaccharide at the early stage (first 4 weeks) but after 12 weeks of administering the drug there was significant improvement in the HDL level when compared to first visit of commencement of therapy. This suggests that for proper clinical improvement in metabolic profile of patient on antiretroviral drug regimen, there may be the need to take MO for a prolong period of time.

Conclusion

Result from this work suggests that consumption of *Moringa oleifera*, may improve the metabolic parameters in patients on antiretroviral regime over a sustained period of time. Further study may be necessary understand molecular and pharmacology activity and mechanism of action of this plant in improving the metabolic profile of patient on HIV drugs.

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