ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Clinical evaluation of the potential benefits of taking Moringa oleifera on blood triglyceride and cholesterol level in patient taking Tenofovir/Lamivudine/Efavirenz (TLE) combination.

Joseph Oyepata Simeon[1] Sabastine Aliyu Zubairu[2] and Joseph Opeyemi Tosin[3]

[1]*Department of Pharmacology, Faculty of Pharmaceutical Sciences, Federal University, Oye - Ekiti State, Nigeria.*

[2]*Department of Pharmacology and Therapeutics, Faculty of Pharmacy,Gombe State University, Gombe State, Nigeria*

[3]*Department of Pharmacy, University College Hospital, Ibadan, Oyo State, Nigeria*

Abstract

The main treatment for HIV is a class of drugs called antiretrovirals. Sticking to a treatment plan isn't always easy. Antiretroviral drugs can cause side effects that can be severe enough to make some people stop taking them. A side effect can also get worse the longer a drug is taken. An example is change in metabolic parameters. The aim of this clinical study is to evaluate the potential benefits of taking Moringa oleifera on blood triglyceride and cholesterol level in patient taking Tenofovir/Lamivudine/efavirenz (TLE) combination.

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 cholesterol level. There was significant reduction in blood triglyceride and cholesterol level ($P<0.01$) of subjects in visit 1 and 2 when compared to visit 0. There was also significant improvement in blood triglyceride and cholesterol level ($P<0.01$) in visit 2 compared to visit 0 of tenofovir/Lamivudine/Efavirenz (TLE) combination, when compared to subjects that did not received Moringa oleifera. There was no significant difference in the blood triglyceride and cholesterol level ($P<0.01$) in the non moringa subjects.

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

Keyword: Moringa oleifera, blood, cholesterol, tenofovir, triglyceride

INTRODUCTION

The human immunodeficiency virus (HIV) targets the immune system and weakens people's defense against many infections and some types of cancer [1]. As the virus destroys and impairs the function of immune cells, infected individuals gradually become immunodeficient. Immune function is typically measured by CD4 cell count. Immunodeficiency results in increased susceptibility to a wide range of infections, cancers and other diseases that people with healthy immune systems can fight off [2]. The most advanced stage of HIV infection is acquired immunodeficiency syndrome (AIDS), which can take many years to develop if not treated, depending on the individual. AIDS is defined by the development of certain cancers, infections or other severe long term clinical manifestations [3,4].

HIV is a virus that damages the immune system. Untreated HIV affects and kills CD4 cells which are a type of immune cell called T cell. Over time, as HIV kills more CD4 cells, the body is more likely to get various types of conditions and cancers. HIV continues to be a major global public health issue, having claimed almost 33 million lives so far [5]. However, with increasing access to effective HIV prevention, diagnosis, treatment and care, including for opportunistic infections, HIV infection has become a manageable chronic health condition, enabling people living with HIV to lead long and healthy lives. HIV is different in structure from other retroviruses [6]. It is roughly spherical with a diameter of about 120 nm, around 60 times smaller than a red blood cell [7]. It is composed of two copies of positive-sense single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle [8].

This is, in turn, surrounded by the viral envelope, that is composed of the lipid bilayer taken from the membrane of a human host cell when the newly formed virus particle buds from the cell. The viral envelope contains proteins from the host cell and relatively few copies of the HIV envelope protein, which consists of a cap made of three molecules known as glycoprotein (gp) 120, and a stem consisting of three gp41 molecules that anchor the structure into the viral envelope [8,9]. The envelope protein, encoded by the HIV env gene, allows the virus to attach to target cells and fuse the viral envelope with the target cell's membrane releasing the viral contents into the cell and initiating the infectious cycle. As the sole viral

protein on the surface of the virus, the envelope protein is a major target for HIV vaccine efforts [10]. . Over half of the mass of the trimeric envelope spike is N-linked glycans. The density is high as the glycans shield the underlying viral protein from neutralisation by antibodies. This is one of the most densely glycosylated molecules known and the density is sufficiently high to prevent the normal maturation process of glycans during biogenesis in the endoplasmic and Golgi apparatus [10]. The majority of the glycans are therefore stalled as immature 'high-mannose' glycans not normally present on human glycoproteins that are secreted or present on a cell surface [11]. The unusual processing and high density means that almost all broadly neutralising antibodies that have so far been identified (from a subset of patients that have been infected for many months to years) bind to, or are adapted to cope with, these envelope glycans [12].

The molecular structure of the viral spike has now been determined by X-ray crystallography [13] and cryogenic electron microscopy [13,14]. These advances in structural biology were made possible due to the development of stable recombinant forms of the viral spike by the introduction of an intersubunit disulphide bond and an isoleucine to proline mutation (radical replacement of an amino acid) in gp41 [15,16]. The so-called SOSIP trimers not only reproduce the antigenic properties of the native viral spike, but also display the same degree of immature glycans as presented on the native virus [17]. Recombinant trimeric viral spikes are promising vaccine candidates as they display less non-neutralising epitopes than recombinant monomeric gp120, which act to suppress the immune response to target epitopes. The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and nine genes (gag, pol, and env, tat, rev, nef, vif, vpr, vpu, and sometimes a tenth tev, which is a fusion of tat, env and rev), encoding 19 proteins. Three of these genes, gag, pol, and env, contain information needed to make the structural proteins for new virus particles [17, 18]. For example, env codes for a protein called gp160 that is cut in two by a cellular protease to form gp120 and gp41. The six remaining genes, tat, rev, nef, vif, vpr, and vpu (or vpx in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease [19].

There were sudden rise of Kaposi Sarcoma in 1981, observed among young homosexuals (CDC,1982a) with infrequent lung Pneumocystis Carinii Pneumonia (PCP) discovered among homosexual men and drug abusers in that same period [14,15,16]. The initiator for these conditions, turned out to be the "Acquired Immunodeficiency Syndrome (AIDS)" which was the "terminal stage infection" by a retrovirus called the Human Immunodeficiency Virus (HIV) [20].HIV are of two types namely; HIV 1 and HIV 2.They are two different viruses. HIV 1 accounts for 95% of all infectious cases worldwide. HIV 2 is mainly seen in a few West African countries. Though HIV 2 progresses slowly than HIV 1 some antiretrovirals such as nevirapine and efavirens do not work against HIV 2. Structurally, they have genetic differences. The vpu gene found in HIV 1 is replaced by the vpx gene in HIV 2. Furthermore, the protease enzymes which are aspartic acid proteases share about 50% sequence identity. However, the enzymes have different substrates and inhibitor binding. Most prominent between the CGP 53820 inhibitory binding. HIV attacks the immune system, by infecting cells that have the CD4 receptor and the chemokine receptor CCR5 and CXCR4. It depletes CD4 cells and suppresses the immune system overtime. It is spread through blood products and body fluids, like contaminated injection "syringe, blood transfusion, sex and mother to child infections". As a major world health roblem till date and since 2008, HIV has killed approximately 2 million people, while infecting 2.7 million people [16,17,20].

HIV differs from many viruses in that it has very high genetic variability. This diversity is a result of its fast replication cycle, with the generation of about 10^{10} virions every day, coupled with a high mutation rate of approximately 3×10^{-5} per nucleotide base per cycle of replication and recombinogenic properties of reverse transcriptase [19,20].

This complex scenario leads to the generation of many variants of HIV in a single infected patient in the course of one day[21]. This variability is compounded when a single cell is simultaneously infected by two or more different strains of HIV. When simultaneous infection occurs, the genome of progeny virions may be composed of RNA strands from two different strains. This hybrid virion then infects a new cell where it undergoes replication. As this happens, the reverse transcriptase, by jumping back and forth between the two different RNA templates, will generate a newly synthesized retroviral DNA sequence that is a recombinant between the two parental genomes. This recombination is most obvious when it occurs between subtypes [21].

The closely related simian immunodeficiency virus (SIV) has evolved into many strains, classified by the natural host species. SIV strains of the African green monkey (SIVagm) and sooty mangabey (SIVsmm) are thought to have a long evolutionary history with their hosts. These hosts have adapted to the presence of the virus, which is present at high levels in the host's blood, but evokes only a mild immune response, does not cause the development of simian AIDS, and does not undergo the extensive mutation and recombination typical of HIV infection in humans [15]. In contrast, when these strains infect species that have not adapted to SIV ("heterologous" or similar hosts such as rhesus or cynomologus macaques), the animals develop AIDS and the virus generates genetic diversity similar to what is seen in human HIV infection [15]. Chimpanzee SIV (SIVcpz), the closest genetic relative of HIV-1, is associated with increased mortality and AIDS-like symptoms in its natural host [15]. SIVcpz appears to have been transmitted relatively recently to chimpanzee and human populations, so their hosts have not yet adapted to the virus [18] This virus has also lost a function of the nef gene that is present in most SIVs. For non-pathogenic SIV variants, nef suppresses T cell

activation through the CD3 marker. Nef's function in non-pathogenic forms of SIV is to downregulate expression of inflammatory cytokines, MHC-1, and signals that affect T cell trafficking. In HIV-1 and SIVcpz, nef does not inhibit T-cell activation and it has lost this function. Without this function, T cell depletion is more likely, leading to immunodeficiencY [18].

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. Some are mild, while others are more severe or even life-threatening [19,21]. 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. 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 [21].

Antiretroviral formulations blocks HIV at certain stages of the viral "life cycle" [21]. Processes such as "binding, fusion and entry, reverse transcription and integration, proviral transcription, cytoplasmic expression" are involved in the viral cycle [21], 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. The Moringa leaf are prepared for consumption either fresh, dried, or as extract of an aqueous solution [7,10,18,21]. 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 leaves [13]. They are also used to bring about milk production in lactating women [10,16,19,21], sediment impurities of water [22], detoxifies the system of free radicals [12,17,18], 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 blood cholesterol and 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 [23] 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).

Recruitment 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 x-rayed to the patients. The Longitudinal Randomized Comparative Trial (LRCT) was employed and used.

Procedure

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 glucose 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.

Ethical approval

Ethical approval was granted by the "University of Port Harcourt Research Ethics Committee"referenced as UPH/R&D/REC/---

Patient consent

In line with the ethical requirement documented by Didia (2008), the following ethical issues were considered while carrying out the 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.

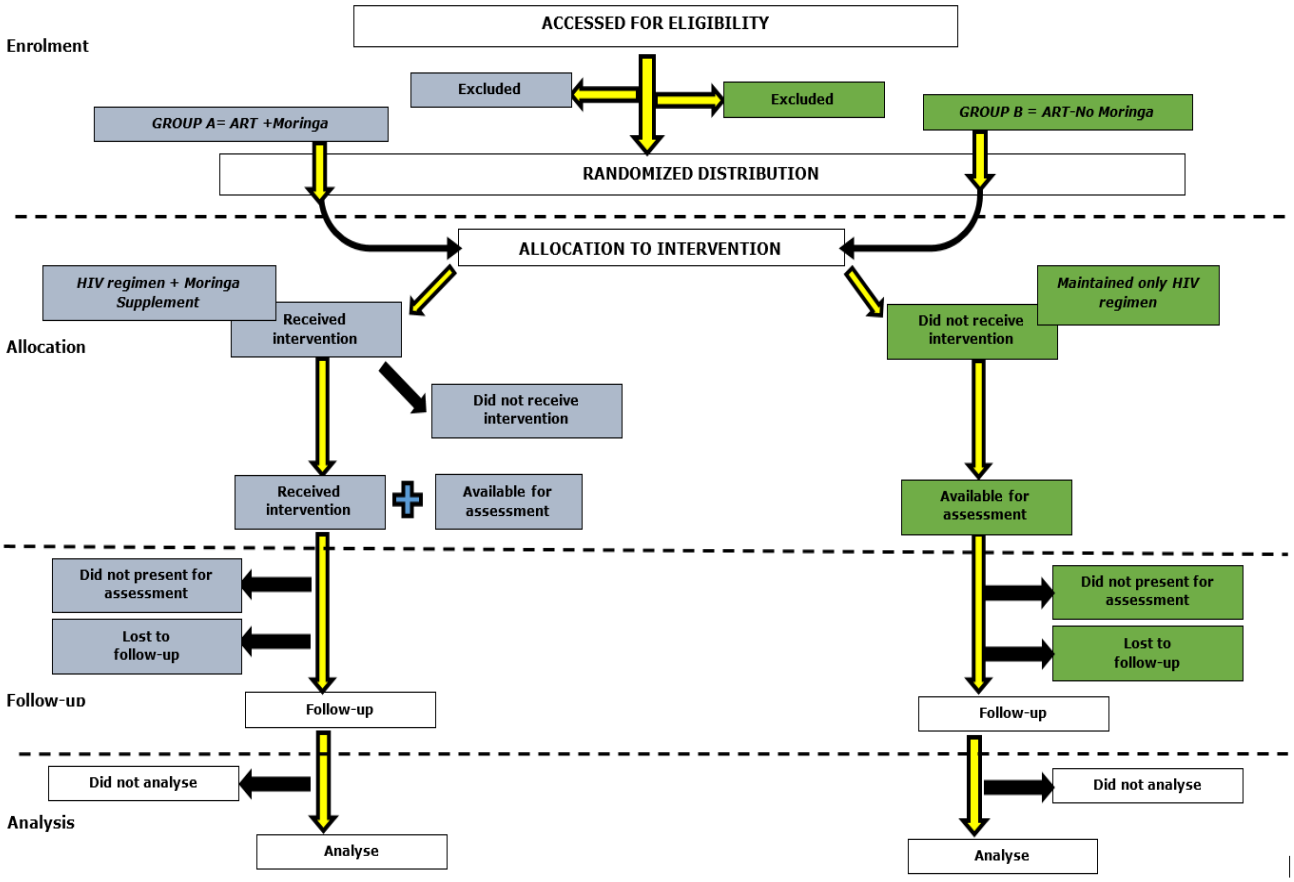

Figure 1: Schulz et al. (2010) model for Random Comparative Trail with modification by the Researcher

RESULT

Cholesterol 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 significant differences (P<0.001) observed in mean values of TLE/Moringa subjects between visit 0 and visit 1 in the level of serum cholesterol. 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 and 3)

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 cholesterol 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 cholesterol and triglyceride (3 and 4).

Table 1: Socio-demographic and anthropometric characteristics of the study population on visit 0

Sex		N	Mean±S.D	S.E
Age (yrs.)	Male	53	39.11±10.46*	1.43
	Female	87	35.63±8.33	0.89
	Total	140	36.01±9.41	0.77
Weight (kg)	Male	53	69.00±9.76	1.3
	Female	87	66.43±12.1	1.25
	Total	140	67.38±11.3	0.92
Height (m)	Male	53	1.71±0.09**	0.01
	Female	87	1.64±0.08	0.01
	Total	140	1.66±0.09	0.01
BMI (kgm^{-2})	Male	53	23.77±3.26	0.44
	Female	87	24.79±4.60	0.47
	Total	140	24.41±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±S.D**	**S.E**	**t-value**	**P-value**	**Inf**
CHOL (mmol/l)	Male	56	4.51±1.02	0.14	2.581	0.011	S
	Female	84	4.08±0.96	0.11			
	Total	140	4.25±1.00				
TG (mmol/l)	Male	56	1.43±0.49	0.07	2.305	0.023	S
	Female	84	1.24±0.47	0.05			
	Total	140	1.32±0.48				

Note: CHOL=Total cholesterol, TG=Triglyceride, 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 and test of mean differences of metabolic profile of the HIV Female patients on tenofovir based ART

Parameters	**Visits**	**Mean±S.D**	**Min**	**Max**	**S.E**	**95% C.I for Mean**	
						Lower Bound	**Upper Bound**
CHOL (mmol/l)	Visit 0	4.08±0.96	2.19	6.30	0.11	3.87	4.28
	Visit 1	3.99±0.78	2.03	6.42	0.08	3.82	4.16
	Visit 2	4.83±4.39	3.19	43.98	0.48	3.88	5.78
	Total	4.30±2.65	2.03	43.98	0.17	3.97	4.63
T.G (mmol/l)	Visit 0	1.24±0.47	0.30	2.30	0.05	1.14	1.35
	Visit 1	1.27±0.37	0.24	2.21	0.04	1.19	1.35
	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

Note: CHOL=Total cholesterol TDF/M=Tneofovir+Moniga, TDF/NM=Tneofovir alone S.D=Standard deviation, S.E=Standard error of mean, Min=Minimum, Max=Maximum, *^Post Hoc (Dunntte T3) multiple comparison (* Visit 0≠Visit 1, ^ Visit 0≠Visit 2, *^'P<0.001).

Table 4: Post Hoc (Dunnette T3) multiple comparison of the metabolic profile of HIV Male 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**
CHOL (mmol/l)	Visit 0	4.51±1.02*^	2.23	8.14	0.14	4.24	4.79
	Visit 1	4.03±0.81'	2.23	5.77	0.11	3.81	4.25
	Visit 2	3.75±0.72	1.91	5.18	0.10	3.56	3.94
	Total	4.10±0.91	1.91	8.14	0.07	3.96	4.24
T.G (mmol/l)	Visit 0	1.43±0.49*^	0.58	2.83	0.07	1.30	1.56
	Visit 1	1.25±0.40'	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

Note: CHOL=Total cholesterol TDF/M=Tneofovir+Moniga, S.D=Standard deviation, S.E=Standard error of mean, Min=Minimum, Max=Maximum, *^Post Hoc (Dunntte T3) multiple comparison (* Visit 0≠Visit 1, ^ Visit 0≠Visit 2, *^'P<0.001).

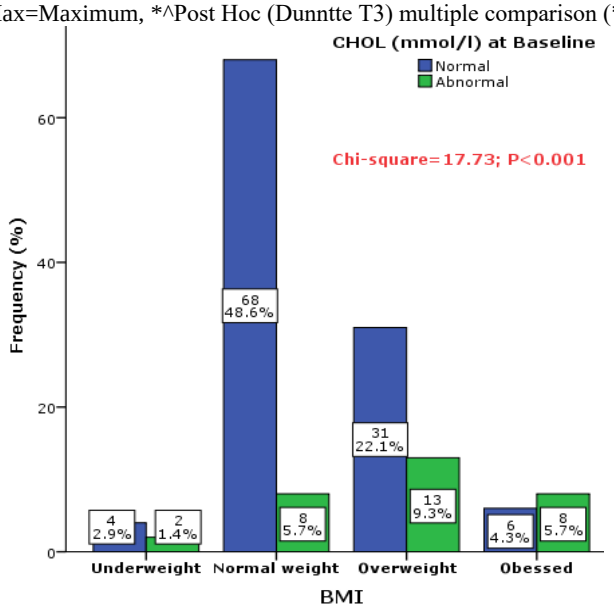

Figure 2: BMI associated cholesterol classification and distribution at Visit 0 (Baseline)

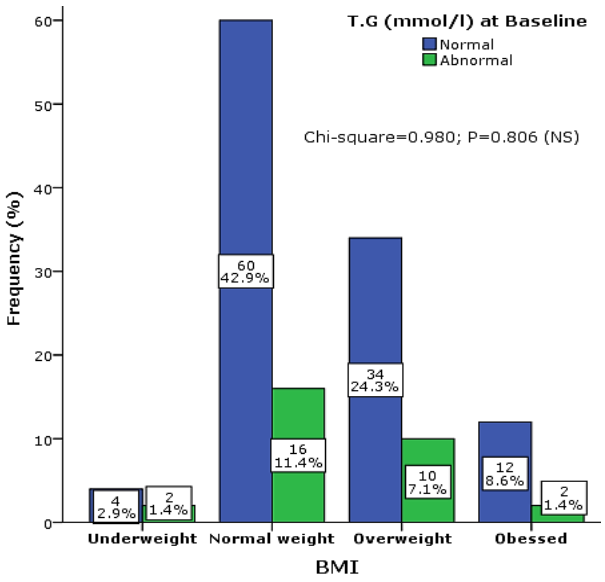

Figure 3: BMI associated triglyceride classification and distribution at Visit 0 (Baseline)

DISCUSSION

In the cross-sectional study, the metabolic profile of both HIV infected patients on Tenofovir (TDF) based regimen was determined [21,23]. The rationale for combining anti-HIV-1 agents is to provide more complete viral suppression, to limit the emergence of drug resistance during chronic viral replication, and to provide more effective antiretroviral treatment even when mixtures of drug-resistant and drug-sensitive strains are present [24,25].

It should be known that HIV patients in the current study have been on TDF ART for at least six months before commencement of this study and there are strong suggestions that the type of ARV-T, duration and application is significantly associated with "the severity of metabolic syndromes" [26].

The differences observed in the male and female values for CHOL and TG are indications of need for separation of reference values in clinical practice. At commencement of the study, the observed increased serum TC and TG are clear indications of metabolic interference of the TDF regimen; thus creating higher atherogenic risk [27]. Also, Tenofovir (TDF) based regimen which contains Efavirenz has been implicated with increasing lipid profile; as well as increases the susceptibility to hypercholesterolemia, mostly in patients with history of genetic dyslipidemia and other non-HIV associated metabolic syndromes. The non Moringa group showed no significant change in the serum level of the two metabolic parameters used in this work. But there were consistent improvement in the serum level of cholesterol and triglyceride from visit 1 and visit 2 compared to visit 0 blood analysis of subject in the moringa group. This suggests that Moringa oleifera may have helped ameliorate the metabolic side effect associated with taking Tenofovir/Lamivudine/efavirenz combination therapy. This may be due to various phytochemical constituents of Moringa oleifera.

These observations are in other studies [28,29,30,31,32,33] which they reported the "hypocholesterolemic and hypoglycemic" effect of M.oleifera. Kumar and Mandapaka[28] 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 some other findings [34,35,36,37,38]; 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 [39,40,41,42].

CONCLUSION

Results from this work suggests that consumption of Moringa oleifera, may have long time benefits in patients on antiretroviral regime. 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.

Acknowledgement

The authors of this work wishes to thank UNIPORT, UPTH and everyone involved in the success of this clinical research work.

Conflict of interest

There is no conflict of interest

REFERENCE

1. Eisinger RW, Dieffenbach CW, Fauci AS (2019). "HIV Viral Load and Transmissibility of HIV Infection: Undetectable Equals Untransmittable". JAMA. 321 (5): 451–452.
2. National Institutes of Health (NIH). (2010). The relationship between the Human Immunodeficiency Virus and the Acquired Immunodeficiency Syndrome: Understanding and Cause. Health & Research Topics. Available at http://www.niaid.nih.gov/topics/ hivaids/understanding/howhivcausesaids/pages/relationshiphivaids. as px
3. Estrella MC., Mantaring JBV., and David, GZ. (2000). A double blind, randomised controlled trial on the use of malunggay (Moringa oleifera) for augmentation of the volume of breastmilk among nonnursing mothers of preterm infants. Philipp Journal of Pediatrics, 49: 3–6. 12.
4. Ete T, Ranabir S, Thongam N, Ningthoujam B, Rajkumar N, and Thongam B. (2014). Metabolic abnormalities in human immunodeficiency virus patients with protease inhibitor-based therapy. Indian Journal of Sexually Transmitted Diseases, 35(2): 100– 103. 13.
5. Joseph OS , Builders M , Joseph O T , Famojuro TI, Ogira JO, Moses FD, Musa TL. (2021). Effect of the Demographic of Covid-19 on Different Countries; Using the USA for Comparism. International journal of multidisciplinary research and analysis. Volume 04 Issue 02. Page 193-203.
6. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). (2001). 285: 2486–2497.
7. Joseph O. T, Obianime. A. W., Siminialaye M. I., Joseph O. S., Anyanwu C. and Musa T. L. (2019). Clinical study on the effect of Moringa oleifera on serum level of glucose and tryglyceride in subjects taken tenofovir, lamivudine and efavirenz combination regimen. European Scientific Journal. Vol.15, (.21). Page 280 -293.
8. Choy SY, Prasad KMN, Wu TY, et al. Utilization of plant-based natural coagulants as future alternatives towards sustainable water clarification. J Environ Sci. 2014;26:2178–2189.
9. Chuang P-H, Lee C-W, Chou J-Y, et al. Anti-fungal activity of crude extracts and essential oil of Moringa oleifera Lam. Bioresour Technol. 2007;98:232–236.
10. Chumark P, Khunawat P, Sanvarinda Y, et al. The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of Moringa oleifera Lam. leaves. J Ethnopharmacol. 2008;116:439–446.
11. Joseph O. S., Builders M., Wazis C. H., Sabastine A. Z., Musa T. L. and Joseph O. T. (2019). Histological study of effect of ethanol stem extracts of Homalium letestui on thioacetamide - induced injury in albino rat, using various staining techniques**.** International Journal of Research and Scientific Innovation. Volume VI (Issue VII). Page 77 – 85.
12. LaRosa JC, Grundy SM, Waters DD. (2005). "Intensive lipid lowering with atorvastatin in patients with stable coronary disease," The New England Journal of Medicine, vol. 352, no. 14, pp. 1425–1435.
13. LaRosa JC, Grundy SM, Waters DD. (2005). "Intensive lipid lowering with atorvastatin in patients with stable coronary disease," The New England Journal of Medicine, vol. 352, no. 14, pp. 1425–1435.

14. Meraiyebu, A., Ogunwole, E., and Izuchukwu, NS. (2014). Effects of Aqueous Extract of Moringa oleifera Seeds on Alloxan Induced Hyperglycemia. Basic Sciences of Medicine, 3(3): 37-42.
15. Moore RD, Chaisson RE (1999). Natural history of HIV infection in the era of combination antiretroviral therapy. AIDS. 13 (14): 1933– 42.
16. Sabastine A. Z., Musa T. L., Joseph O. S., Builders M. and Joseph Opeyemi T. (2019). Histological study of effect of ethanol stem extracts of Homalium letestui in paracetamol induced injury in albino rat, using various staining techniques. American Journal of Biomedical Science & Research. 4(2). Page 82 – 89.
17. Joseph O.S., Builders M., Joseph O. T, Ariahu E. C., Zubairu S. A., Musa T. and Oyepata P.J. (2019). Toxicity study of ethanol leaf extract of ocimum canum on heart and lipid profile of wister rats. International Journal of Current Advanced Research. Volume 8. (Issue 05). Page 18800 – 18803.
18. Samson A. O., Joseph O. S., Samson O. A. and Emem R. A. (2019). Effect of Citrus Linton Juice and Tamoxifen on The oxidative activities of MCT-7 cell induced Bresat Cancer in Sprawgue Dawley Rats. Saudi Journal of Biomedical Research. Volume 8 (7). Page 76-92.
19. Perk G, De Backer H and Gohlke J. (2012). "European guidelines on cardiovascular disease prevention in clinical practice," European Heart Journal, vol. 33, no. 13, pp. 1635–1701.
20. Joseph O. S, Builders M., Isinkaye D. R., Sebastine A. Z., Musa T., Oyepata J. P., Joseph O. T. and Wazis C. (2019). Sub-Acute Toxicity Study of Ethanol Leaf Extract of Terminalia chebula On Brain, Stomach and Spleen of Wister Rats. American Journal of Biomedical Science & Research. 3(3). Page 277-282.
21. Kansal SK, Kumari A. Potential of M. oleifera for the treatment of water and wastewater. Chem Rev. 2014;114:4993–5010.
22. Joseph OS and Joseph O T (2021). Effect of Clinical Study of Moringa oleifera on Body mass index, Low density lipoprotein and Triglyceride level in Patients on Tenofovir/lamivudine/efavirenz Combination Therapy. Advanced Herbal Med. Vol. 6. Issue 1. Page. 14-27
23. Oyebadejo S. A, Joseph O. S, Adesite S. O and Omorilewa A.O. (2019). Effect of Citrus Limon Juice and Tamoxifen on the Tumour growth mass Indices, Cell Proliferation, Cell Viability and Cytogenetic (Mitotic Index) of Sprague Dawley Rats Induced MCF-7 Breast Cancer Cells. Saudi Journal of Biomedical Research. (4). Pg. 216 - 225.
24. Young TN, Arens FJ, Kennedy GE, Laurie JW, Rutherford GW (January 2007). Young T (ed.). "Antiretroviral post-exposure prophylaxis (PEP) for occupational HIV exposure". The Cochrane Database of Systematic Reviews (1):
25. Siegfried N, van der Merwe L, Brocklehurst P, Sint TT (July 2011). Siegfried N (ed.). "Antiretrovirals for reducing the risk of mother-to-child transmission of HIV infection". The Cochrane Database of Systematic Reviews (7): CD003510. doi:10.1002/14651858.CD003510.pub3. PMID 21735394.
26. "WHO HIV and Infant Feeding Technical Consultation Held on behalf of the Inter-agency Task Team (IATT) on Prevention of HIV – Infections in Pregnant Women, Mothers and their Infants – Consensus statement" (PDF). October 25–27, 2006. Archived (PDF) from the original on April 9, 2008. Retrieved March 12, 2008.
27. Horvath T, Madi BC, Iuppa IM, Kennedy GE, Rutherford G, Read JS (January 2009). Horvath T (ed.). "Interventions for preventing late postnatal mother-to-child transmission of HIV". The Cochrane Database of Systematic Reviews (1): CD006734. doi:10.1002/14651858.CD006734.pub2. PMC 7389566. PMID 19160297.
28. "WHO validates elimination of mother-to-child transmission of HIV and syphilis in Cuba". World Health Organization. June 30, 2015. Archived from the original on September 4, 2015. Retrieved August 30, 2015.
29. Reynell L, Trkola A (March 2012). "HIV vaccines: an attainable goal?". Swiss Medical Weekly. **142**: w13535. doi:10.4414/smw.2012.13535. PMID 22389197.
30. U.S. Army Office of the Surgeon General (March 21, 2011). "HIV Vaccine Trial in Thai Adults". ClinicalTrials.gov. Archived from the original on October 19, 2011. Retrieved June 28, 2011.
31. Modupe I. B., Oyepata S. J. and Akpobome R. V. (2019). Effect of Parkia biglobosa extract on open skin wound healing in dexamethasone - induced hyperglycaemia and histological assessment in rats. African Journal of Pharmacy and Pharmacology. Vol. 13(8), pp. 84-89.
32. Da Silva JPV, Serra TM, Gossmann M, et al. Moringa oleifera oil: studies of characterization and biodiesel production. Biomass Bioenergy. 2010;34:1527–1530. doi: 10.1016/j.biombioe.2010.04.002.
33. Builder M. I., Anzaku S. A. and Joseph S. O. (2019). Effectiveness of intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine against malaria in northern Nigeria. International Journal of Recent Scientific Research Vol. 10 (05), pp. 32295-32299.
34. . Doughari JH, Human SI, Bennade S, Ndakidemi PA (2009) Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. J Med Plant Res 3: 839- 848. 2.
35. Evans WC (2007) Pharmacognosy. (15th edtn), W.B Saunders, Edinburgh, Scotland. 3. Elujoba AA, Odeleye OM, Ogunyemi CM (2005) Traditional medicine development for medical and dental primary health care delivery system in Africa. Afr J Tradit Complement Altern Med 2: 46-61.
36. Edema AO (2012) Production of some common vegetables. Horticultural Research Instituite, Ibadan, Nigeria. 5. Fasuyi AO (2006) Nutritional potentials of some tropical vegetable leaf meals, chemical characterization and functional properties. African J Biotechnol 5: 49- 53. 6.
37. Akashi K,Traver D, Kondo M (1999) Lymphoid development from haematopoietic stem cells. Int J Haematol 69: 217-222. 7. Okochi YI, Akpotuzor J, Alli LA (2013) Comparism of an African herbal formula with commercially available haematinics. African J Biotechnol 2: 219-227.
38. Builders M. I., Joseph S. O. And B. Peter U. (2020). A Survey of Wound Care Practices by Nurses in a Clinical Setting. *International Journal of Healthcare and Medical Sciences*. Vol. 6, Issue. 5, Page 74-81.
39. Adedapo AA, Mogbojuri OM, Emikpe BO (2009) Safety evaluations of the aqueous extract of the leaves of Moringa oleifera in rats. J Med Plants Res 3: 586-591.
40. Adias TC, Ajugwo AO, Erhabor T, Nyenke CU (2013) Effect of pumpkin extract (Telfairia occidentalis) on routine haematological parameters in acetoneinduced oxidative stress albino rats. Am J Food Sci Technol 1: 67-69.
41. Flanagan J, Lessler MA (2000) Controlled phenylhydrazine-induced reticulocytosis in rat. The Ohio J Sci 70: 300-304. 12. Ganong W (2005) Review of Medical Physiology. (22nd edtn), Lange medical books/McGraw-Hill, New York, USA. 13. Sembulingam K, Sembulingam P (2010) Essentials of medical physiology. (5th edtn), Jaypee Brothers Medical Limited, India.
42. Joseph O. T, Obianime. A. W., Siminialaye M. I., Joseph O. S., Anyanwu C. and Musa T. L. (2019). Clinical study on the effect of *Moringa oleifera* on serum level of glucose and tryglyceride in subjects taken tenofovir, lamivudine and efavirenz combination regimen. *European Scientific Journal*. Vol.15, (.21). Page 280 -293.