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ADMINISTRATION OF A FORMULATED HERBAL MIXTURE (DIB) CAUSED A SIGNIFICANT INCREASE IN CD4 COUNTS AND REDUCTION IN HIV RNA OF VOLUNTEERED HIV PATIENTS ON ANTIRETROVIRAL THERAPY

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ABSTRACT

The scourge of HIV infection is yet to be brought under control. One major reason for this is the problem of resistance of the virus to available antiretroviral drugs. Another reason is the lack of access to these drugs in most rural communities where conventional treatment is non-existent. Therefore, the need for complementary and /or alternative treatments. In this study, a formulated herbal mixture (DIB) made from *Sorghum bicolor L.*, *Curcuma longa L.*, *Bridelia ferruginea B.* and honey was assessed as a complementary therapy for the management of HIV-positive individuals on antiretroviral therapy (ART). A total of 16 HIV-positive individuals attending a government-approved ART clinic in Nigeria who indicated interest in the investigation were recruited for this study. The volunteers were divided into two groups: A (12; administered DIB) and B (4; not administered DIB). The CD4 counts and the viral loads of these patients were assessed before the commencement of the administration of DIB and follow-up CD4 counts and viral load assay were carried out at the 3rd month and 6th month of continuous administration of DIB for Group A patients. The CD4 counts and the viral loads of group B patients which served as control were also assessed simultaneously. There was a progressive increase in the CD4 counts from the baseline (i.e., prior administration of DIB to the patients) to the sixth month of the administration of DIB to group A patients as compared to those in group B that were not administered DIB and there was reduction in viral load to an undetectable level (TND) in HIV patients administered DIB compared to patients not administered DIB who still recorded detectable HIV RNA at 6th month of the study. Thus, the progressive increase in CD4 count and the no detection of HIV RNA among patients administered DIB continuously for 6 months show the promise of DIB as a complementary therapy in the management of HIV patients. Therefore, DIB might be an option to consider as a complementary therapy in the management of HIV patients.

KEYWORDS: Herbal product, HIV, Viral load, CD4 count, Target-not-detected, Complementary therapy.

INTRODUCTION

Human immunodeficiency virus (HIV) is a chronic infection causing enormous socio-economic challenges by affecting the young and economically productive population in Africa.[1] World Health Organisation (WHO) estimates that over 75 million people globally have been infected with HIV, of which approximately 37 million are living with the infection[2]. The most affected is the Sub-Saharan Africa which accounts for 25.7% of the total cases.[3]

The infection of HIV targets majorly the immune cells which have a CD4 receptor on the surface. These cells include T-lymphocytes, monocytes, macrophages and dendritic cells. The CD4 receptor is used by the cell to signal to other parts of the immune system the presence of antigens. When HIV gets in contact with a CD4 cell, the gp120 spikes on the surface of HIV lock onto the CD4 receptor and another co-receptor, either CCR5 or CXCR4. The gp41 protein is used to fuse the HIV envelope with the cell wall. This process of fusion allows the HIV capsid to enter the CD4 cell.[4]

Several types of antiretroviral drugs have been developed to block different stages of the processes of attachment and entry. These include CCR5 inhibitor, Attachment inhibitor, Fusion inhibitor.[5] Although Antiretroviral therapy (ART) has been shown to be effective in managing the patients however, it is not without serious adverse events, which is especially evident in persons undergoing long-term treatment. For instance, the use of antiretroviral drugs has been associated with several acute and chronic toxicities and the appearance of body fat redistribution syndrome.[6,7] Another challenge is the

inability of the drugs to completely eradicate the virus.[8] This therefore has resulted in the virus becoming resistant to most of the available drugs. In a study carried out by Scott *et al.*[9] 55% of the HIV patients they assessed during a surveillance study showed HIV antiretroviral drug resistance. The incidence of resistance according to Elujoba *et al.*[10] can lead to persistent bacteremia. Therefore, new drugs are needed for effective management of HIV patients. In view of this, WHO suggested that ethnomedicines and other natural products should be systematically tested against HIV as they might yield effective and more affordable therapeutic agents.[11]

Plant materials have been used over the years in folklore medicine in the treatment of many human diseases. Many of these plants act as source of nutrients and also possess antimicrobial properties which help to protect against invasion by pathogens.[12] A couple of medicinal plants have been documented in literature to be used by HIV positive individuals in the management of HIV infection.[7,13] For instance, Mugomeri *et al.*[13] reported the following plants: *Hypoxis hemerocallidea* (African potato), *Hypericum perforatum* (St. John's wort), *Sutherlandia frutescens* (Sutherlandia) and *Allium sativum* (garlic) as the most used by HIV-positive people in Lesotho in the treatment of the infection. Furthermore, the synergistic use of some herbal therapies alongside conventional therapy among HIV patients who are undergoing HAART has also been reported in the search for complementary therapies. For instance, Shiferaw *et al.*[1] reported that 26.1% of HIV patients in their study were observed to be using herbal medicines while on ART. In other researches however, a couple of medicinal plants were actually evaluated for possible potential in treating HIV infection with positive results. For example, Onifade *et al.*[14] who worked on *Nigella sativa* observed seroconversion of HIV positive individuals to seronegative. Also, Elujoba *et al.*[10] reported the effectiveness of a concoction made from Garlic, Moringa and *Artemisia annua* in the treatment of HIV infection.

DIB is a formulated herbal product made from *Sorghum bicolor* L., *Curcuma longa* L, *Bridelia ferruginea* B and honey. Sorghum is a food grain that is grown across America and Africa. Sorghum species are known to have a high content of antioxidants, including simple phenolic acids, as well as polyphenols, particularly 3-deoxyanthocyanidins, such as luteolinidin and apigenidin.[15] The high content of these antioxidant compounds is present in sorghum-based beers and contributes to the inhibition of lipid peroxidation during mashing and boiling. The leaf sheath has a different chemical composition than the leaf blade.[16] Sorghum leaf sheaths have a very high concentration of dimeric 3-deoxy-anthocyanidins.[17] Sorghum extracts have a strong chemoprotective potential and inhibit proliferation of gastrointestinal cancer cell lines, and these effects are partially independent of their antioxidant content. The anticancer properties of sorghum are due in part to the high content of 3-deoxyanthocyanidins.[18] In addition to the high content of anti-inflammatory phenolic compounds, sorghum contains several groups of bioactive compounds with the capacity to induce proinflammatory immune responses. Water-soluble beta-glucans are found in sorghum that showed biologically active betaglucans capable of initiating macrophage activation.[19] According to Ali *et al.*[20], the therapeutic roles reported on sorghum are results of anti-inflammatory, anti-carcinogenic, antibiotic, antifungal, antiviral, hepatoprotective, anti-ulcer, anti-neoplastic, cholesterol-lowering and digestibility slowing properties. Such pharmaceutical functions are allied to the phytochemical contents of the plant such as phytosterols, policosanols, saponins, carotenoids and phenolic compounds, including flavonoids, tannins, and anthocyanins.[21] Different important flavonoids and phenolic acids identified in the leaf extracts of sorghum include gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, rutin, isoquercitrin, quercitrin, quercetin, and kaempferol.[22]

Honey is a natural sweet viscous fluid produced by honeybees from the pollen and nectar of flowering plants or from the nectar of blossoms which honeybees collect and transform by combining with their salivary secretions and deposit, dehydrate and store in the honeycomb to ripen.[23] Some honey substances are essential for human life such as sugars, proteins, vitamins, organic acids and minerals.[24]

Curcuma longa Linn. (Turmeric) is a tropical plant of South-eastern Asia and primarily found grown in tropical regions of Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, Phillipines and Nigeria.[25] It belongs to the family *Zingiberaceae*. It is locally known as Atale pupa in Yoruba; Gangamau in Hausa; Nwandumo in Ebonyi; Ohu boboch in Enugu (Nkanu East); Gigir in Tiv; Magina in Kaduna; Turi in Niger State and Onjonigho in Cross River (Meo tribe).[26] Turmeric has healthy influence on digestive system, and it also enhances the mucin secretion in the digestive tract. In classical literature, several actions of turmeric have been specified like antibacterial, antithelmintic anticancer, antiparasitic, antiseptic, anti-oxidative[27], anti- inflammatory, anti-rheumatic, anti-tumor, anti-phlegmatic, antiviral, astringent, aromatic, blood purifier, clear skin, neutralize free radicals. It has hepatoprotective[28] and nehroprotective properties against toxic agents-induced hepato-renal failure.[29,30] Also, it has protective activity against cytotoxic, teratogenic, and neurodegenerative diseases[31,32], anticoagulant and possess anti HIV activity to combat AIDS.[33]

Bridelia ferruginea Benth. (Euphorbiaceous) is commonly found in the Savannah regions.[34] Its bark extract has been used for the coagulation of milk and also lime juice for the formulation of a traditional gargle "Egun Efu". It is also reported of having potential for

water treatment and chemo preventive potentials.[35] Several phenolic compounds isolated from *B. ferruginea* stem bark were found to display radical scavenging and xanthine oxidase inhibition activities, therefore supporting the application of *B. ferruginea* in traditional medicine[36] as an anthelmintic, antiamebic, antianemic, antibacterial, anticonvulsant, antidiabetic, antidiarrhoeal, anti-inflammatory[37], antimicrobial[38], antiviral, hypoglycemic and for abdominal pain, cardiovascular, gynecological and sexual diseases[39] or anti-cancer.[40] Other reported activities of the bark extract include trypanocidal, molluscidal[41]

Although so much work has been done on the medicinal value of these plants (*Sorghum bicolor* L., *Curcuma longa* L, *Bridelia ferruginea* B and honey) individually, however none has been reported on the effectiveness of their mixture in the management of HIV positive individuals. Hence, based on the prevailing situation in the management of HIV/AIDS, this work was carried out to investigate the potentials of an herbal mixture compounded from *Sorghum bicolor* L., *Curcuma longa* L, *Bridelia ferruginea* B and honey in boosting the CD4 count and decreasing the viral load which may give way for the development of cheaper, easily available and effective alternative approach for therapeutic purposes.

MATERIALS AND METHODS

Research design

A parallel group randomized design was employed. In which each subject is randomized to one of two or more distinct treatment/intervention groups. Those who are assigned to the same treatment are referred to as a treatment group[42], following Standards of Reporting Trials Group.[43] Each participant had their whole blood samples collected for assessment of plasma HIV-RNA and CD4 counts prior commencement of the research. The follow-up sample collection was done at 3month and 6month of continuous administration of DIB.

Ethical Consideration Related to Data Collection

The research proposal was submitted and approved by the Research and Ethical review Committee at Ekiti State University Teaching Hospital (EKSUTH/A67/2019/10/009) and Department of Microbiology Federal of Technology Akure University. The participants were provided with background information on the study. The participants were also informed that their participation was voluntary and that they could withdraw their participation at any time. Also, the participants signed a written consent and confidentiality of their information was also assured.

Sampling Procedure

The inclusion criteria are age ≥ 18 years, patients on ART and who had undergone the mandatory adherence counseling classes organized by the ART clinic of the hospital. A total of 16 participants were enrolled based on these criteria.

The participants were then sub-grouped into 2 groups: Group A (12 HIV patients on ART which were administered DIB), Group B (4 HIV patient on ART not administered DIB).

Herbal Product

The formulated herbal product, DIB (D-immune booster) was compounded from the powder of *Sorghum bicolor* L., *Curcuma longa* L, *Bridelia ferruginea* B according to Ojo *et al*.[29]

Administration of DIB

One gram (1g) of the herbal powder is introduced into 0.3L hot water, 1gram of honey is added and taking as herbal tea once per day. The patients are monitored for adherence to the herbal therapy.

Bio-data collection

A standardized questionnaire was administered to capture data on socio-demographic characteristics of each participant.

Blood sample collection and analysis

Eight milliliter (8ml) whole blood was collected through antecubital fossa veins puncture into sterile EDTA tubes. Plasma was then separated from whole blood by centrifugation within 24 h of sample collection after which plasma was transferred to sterile cryovials and stored frozen at – 20°C at the Medical Microbiology laboratory at Ekiti State University Teaching Hospital. The processed samples were then transported to Nasarawa Viral Load Testing Laboratory under a cold-chain system. Plasma VL was done using COBAS Ampliprep /COBAS Taqman HIV-1 test[44]

CD4 count Assay

CD4 cell count level of the patients was determined using a freshly collected blood sample drawn into two separate 4 ml spray-dried dipotassium ethylenediaminetetraacetic acid (K_2EDTA) anticoagulant bottles and processed within 4 hours of collection. Samples for CD4 count were prepared and run on the Partec cyflow counter (Partec flow cytometer, GMBH, Germany) according to the manufacturer's instructions.[45]

Data analysis

A summarized approach with result evidence was used in this report according to Billingham *et al*.[46] This is because a small clinical trial is less likely to be self-contained, providing all of the necessary evidence to effectively test a particular hypothesis. Instead, it might be necessary to summarize all of the evidence from the trial and combine it with other evidence available from other trials or laboratory studies.[47]

RESULTS

The characteristics of participants in this study is as presented in table 1. Sixteen volunteers participated in the study of which 11 (68.75%) were females and 5

(31.25%) were males. The age distribution shows that majority were within age group 40-60 years (75.00%), followed by age group 20-40 years (18.75%). The least percentage of 6.25% was observed in age group, 61-80 years. The level of adherence of participants to DIB treatment shows that 8(50.00%) had adherence level greater that 95%, 4(25.00%) of the participants had adherence level between 80-95%. Duration of exposure of patients to ART is also shown in the table, 14 out of the 16 patients recruited for the study have been on ART for more than two (2) years. The longest duration of exposure to ART which was 12 years was observed in 5 (31.25%) of the participants while the least duration of ART exposure of less than a year was seen in 2 (12.25%) of the participants.

Table 1: Gender/Age distribution of participants.

Variables	Categories	Frequences	Percentage (%)
Gender (n=16)	Male	5	31.25
	Female	11	68.75
Age (n=16)	20-40	3	18.75
	40-60	12	75.00
	61-80	1	6.25
DIB therapy adherence (n=16)	≥ 95	8	50.00
	80-95	4	25.00
	Not Treated with DIB	4	25.00
Duration of ART (n=16)	Less than a year	2	12.50
	2-5 Years	6	37.50
	5-7 Years	3	18.75
	7-12 Years	5	31.25

The results of the baseline CD4 counts, the CD4 counts of the participants administered DIB after 3 months and 6 months of continuous administration and those not administered DIB are presented in table 2. The table shows a progressive increase in the CD4 counts from the baseline (i.e., prior administration of DIB to the patients) to the sixth month of the administration of DIB to group A patients as compared to those in group B that were not administered DIB. In the 3rd month of the administration of DIB to group A patients, 1(8.33 %) of the patients had increase in his CD4 count > 50.00% , 4(33.33 %) of the patients had increase in their CD4 $\geq$ 40.00 %, 6(50.00 %) of the patients had increase in their CD4 $\geq$ 30.00 %, 7(58.33 %) of the patients had increase in their CD4 $\geq$ 20.00 %, 9 (75.00 %) had $\geq$ 10.00% percentage increase in their CD4 counts while 12 (100.00 %) of the patients had increase in their CD4 $\geq$ 1.00 %. Two (50.00 %) of the HIV patients in group B that were not treated with DIB has percentage increase of $\leq$ 5.00 % in their CD4 counts while the remaining 2 (50.00 %) patients in group B had a percentage decrease in their CD4 counts which ranged from -28.00 to -1.00 %.

At 6 months of continuous administration of DIB, increase in CD4 count was observed in 7(87.50 %) out of the total number of 8 HIV patients that continued with the DIB treatment. Two (28.57 %) of the HIV patients had percentage increase in CD4 counts > 60.00 %, 3 (42.86 %) had CD4 counts increase $\geq$ 50. 00 %, 3 (42.86 %) had CD4 counts increase $\geq$ 40. 00 %, 4 (57.12 %) had CD4 counts increase $\geq$ 30. 00 %, 5 (71.43 %) had CD4 counts increase $\geq$ 20.00 %, 5 (71.43 %) had CD4 counts increase $\geq$ 10. 00 % and 7 (100.00 %) had CD4 count increase of $\geq$ 1.00 % CD4. The patients in group B which were not administered DIB had 2 (50.00 %) patients which had a CD4 count increase of < 20.00 % while the remaining 2 patients in that group had a percentage decrease of their CD4 counts which ranged from -18.00 % to -5.00 %.

Table 2: Effects of administration of DIB on the CD4 count (cells/mm^3) of participants.

Participants	Baseline	After 3months	PCD4G (%)	After 6months	PCD4G (%)
1HPT	408	559	37	ST	ST
2HPT	493	619	26	810	64
3HPT	650	919	41	ST	ST
4HPT	238	320	34	315	32
5HPT	1202	1876	56	ST	ST
6HPT	1069	1079	1	1100	3
7HPT	884	1008	14	908	3
8HPT	821	914	11	ST	ST
9HPT	297	317	7	450	52
10HPT	224	319	42	ST	ST

11HPT	417	604	45	673	61
12HPT	555	601	8	690	24
13HPNT	953	1002	5	782	-18
14HPNT	590	586	-1	701	19
15HPNT	596	601	1	668	12
16HPNT	805	582	-28	761	-5

Key: HPT (HIV positive treated with DIB), HPNT (HIV positive not treated with DIB), PCD4G (Percentage CD4 gain), ST (sample not analysed)

Viral load results in table 3 show that the baseline viral loads of the patients recruited for the study ranged from 20 - 553 copies/mL while 3 had TND (Target Not Detected). After the third month of administration of DIB to group A patients, the viral load of almost all of them 8(88.89 %) reduced in population with percentage reduction of > 50.00 % except in one of the patients that had a percentage reduction of only 15.27 %. However, for group B patients, the percentage reduction was < 40.00% in all the participants. After 6 months of administration of DIB, the viral load further reduced for the participants in group A that were tested. In fact, the viral RNA was not detected in 7(77.78 %) of the patients tested and the only patient (no. 11HPT) in whose blood the virus RNA was detected, the viral load reduced to 87.75 % of the original baseline value. The same trend of reduction was observed in group B patients except in one of the patients (no. 15HPNT) that had 89%* increase in his viral load instead of decrease in viral load.

Table 3: Effects of administration of DIB on viral load of recruited HIV patients.

Participants	Viral Load (copies/mL)				
	Baseline	After 3 months	%	After 6 Months	%
1HPT	214	21	90.19	ST	NA
2HPT	73	TND	NA	TND	NA
3HPT	105	21.2	79.81	ST	NA
4HPT	131	111	15.27	TND	NA
5HPT	313	110	64.86	ST	NA
6HPT	TND	TND	NA	TND	NA
7HPT	94	19	79.79	TND	NA
8HPT	115	13	88.70	TND	NA
9HPT	553	125	77.40	TND	NA
10HPT	102	18	82.35	ST	NA
11HPT	141	61	56.74	21.5	87.75 ↓
12HPT	TND	TND	NA	TND	NA
13HPNT	110	75	31.82	51	53.64
14HPNT	32	21	34.76	5	84.3 ↓
15HPNT	20	20	00.00	37.8	8 ↑
16HPNT	TND	20	NA	TND	NA

Key: HPT (HIV positive treated with DIB), HPNT (HIV positive not treated with DIB), ST (sample not analysed), % (percentage change), TND (Target not detected), ↓ (decrease), ↑ (Increase)

DISCUSSION

The effects of the use of a formulated herbal mixture (DIB) as a complementary therapy in the management of volunteered HIV patients on ART attending one of the government approved ART clinics in southwest, Nigeria was investigated. Majority of the recruited participants were females, and the predominant age group was 40-60 years (75 %). The level of adherence to ART was observed to be very high among the recruited participants. This is a good one because adherence to medication has been reported as one of the major contributors to drug effectiveness. According to Mkwala and Dambula[47] and Stafford *et al.*[48], adherence to antiretroviral therapy is critical to control viral replication and to prevent the development of drug-resistant mutations. However, despite their adherence, the baseline viral load of the volunteers recruited for this study was fairly high (ranged from 20 -553 copies/mL although 3 had TND) even though majorities of the patients have been on antiretroviral therapy for more than 3 years. This contradicts the report of Ripamonti *et al.*[49] who observed that 66% of HIV patients with HIV RNA ≥100,000 copies/mL and who are enrolled for ART, achieve HIV RNA < 5copies/mL at week 192 and 79% of the HIV patients with <100,000copies/mL baseline achieve < 5 copies/mL.

After the administration of DIB however to the patients in this study, there was a progressive decrease in the viral loads and progressive increase in CD4 counts as

compared with the control group. The progressive increase in the level of CD4 count of the patients treated with DIB shows the potential of the herbal mixture as an immune booster. An earlier work carried out by our team observed significant increase ($p<0.05$) in the immune cells of albino rats after the administration of DIB to them for 4weeks.[28] Therefore, the boosting of the immune system might be responsible for reduction in viral load of the patients administered DIB as compared to those not administered DIB. It is also possible that DIB has antiviral potential because of the observed viral load crash in almost all the patients that were administered DIB to undetected level by the 6th month of continuous administration. This however might require further studies to investigate possible virucidal potential of DIB.

The observed crash in HIV RNA load by the 6th month in almost all of the patients (7 out of 8 patients) treated with DIB in which there was no detectable HIV RNA is similar to the report of Onifade *et al.*[14] who observed seroconversion of HIV positive individuals on *Nigella sativa* to seronegative. The observed high viral load suppression potential in DIB complementary therapy could also be due to the presence of anti-inflammatory.[28]

The observation that DIB was also able to successfully maintain undetected HIV-RNA in patients who had TND viral load baseline in this study shows that the herbal drug does not have antagonistic effect on the conventional therapy (ART). As against many reports that herbal medicines like St John's wort and garlic with antiretroviral drugs can result in herb-drug interactions that may lead to treatment failure, drug resistance and toxicity.[51] In our previous study, DIB has been shown to have no observable toxic effect on major organs of albino rats rather it shows cardioprotective and hepatoprotective potentials.[28] This study shows DIB as a potential herbal mixture that can be exploited as a complementary drug for treatment of HIV patients on ART for better health management.

CONCLUSION

The present study showed that the administration of the formulated herbal mixture, DIB to HIV/AIDS patients on ART caused the reduction of the viral load in their blood to an undetected level within 6 months of administration and also caused an increase in their CD4 counts to normal levels.

Consent

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

Competing Interests

Authors declare that no competing interests exist.

Recommendation

Herbal treatment should be encouraged. It could serve as a complementary /supplementary treatment for the management of HIV patients especially in boosting their immune system.

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