

Original Research Article

**COMPARATIVE STUDIES ON THE EFFECTS OF ZIDOVUDINE,
NEVIRAPINE, LAMIVUDINE AND BIOCLEAN II ON FEMALE
HIV/AIDS CASES IN NIGERIA**

ABSTRACT

Triple drug therapy involving two nucleoside reverse transcriptase inhibitor (NRTIs) plus a protease inhibitor (PI) popularly known as highly active anti-retroviral treatment (HAART) currently is the gold standard in HIV/AIDS management universally. It is therefore, an acceptable reference point for other candidate tools of intervention in the HIV/AIDS cycle. The present study comparatively evaluates Bioclean II, a local herbal plant product. The effects of Zidovudine + Lamivudine + Nevirapine and Bioclean II on body weights, viral load, CD4⁺ T-cells and sero-status of female HIV/AIDS cases after one year (HAART) and 8 months standard procedures. The HAART group yielded 44.6kg – 48.6kg mean body weight, 299, 587.8 copies/ml – 199, 854.4 copies/ml mean viral load and 123.2 cells/ml – 461.2 cells/ml mean CD4⁺ T cells while the Bioclean II treatment group yielded 45.0kg – 61.4 kg mean body weights, 304161.8 copies/ml, mean viral loads and 123.4 cells/ml – 752.6 cells/ml mean CD4⁺ T cells respectively. The performance of Bioclean II was superior to that of HAART in the three parameters investigated, suggesting that Bioclean II has a potential as possible intervention tool in HIV/AIDS in the population investigated.

Key Words; HIV, AIDS, HAART, BIOCLEAN II, FEMALES, EXPOSURE

27 **INTRODUCTION**

28 Thirty-one years after the emergence of HIV/AIDS pandemic, definite cure still
 29 remains elusive. The epidemic seems not to be declining but shows pockets of
 30 slowing down or stabilizing in different parts of the world (UNAIDS, 2003). In
 31 Nigeria, multi-sectional approach has been adopted in fighting the HIV/AIDS
 32 epidemic: comprehensive antiretroviral drug treatment, prevention of mother to
 33 child transmission, voluntary counselling and testing and surveillance. These
 34 approaches have their major constraints. Take for instance, the antiretroviral
 35 drug treatment, the principal aim of the existing antiretroviral drug therapy is to
 36 suppress viral replication to as low as possible in order to delay disease
 37 progression but not to effect a cure. Consequently, the HIV/AIDS affected
 38 individuals would have to contend with a chronic, progressive infection and
 39 unpredictable outcome. The orthodox antiretroviral drugs can be categorised as
 40 follows: The reverse transcriptase inhibitors, protease inhibitors and fusion
 41 inhibitors (Ficshl et al., 1997). Each of these groups attack one point or the
 42 other in the life cycle of HIV such as inhibition of reverse transcriptase,
 43 integrase, transactivator (tat) transcription and protease. These drugs have
 44 started showing undesirable side effects ranging from bone marrow suppression,
 45 insomnia, malaise, headaches, rashes, abdominal pains, nausea, vomiting,
 46 pancreatic disorder and kidney damage (Merril et al., 1996). Some encourage
 47 resistance development in HIV (Larder et al., 1989). Thus making continued
 48 search for viable alternatives or even cure very necessary.

49 The present study is on Bioclean II, a natural product obtained from plants in
 50 Nigeria which was aired by African Independent Television (AIT) in December
 51 2012 in the episode titled, “Voyage of Discovery” and also reported by Ibeh et
 52 al. (2013). The efficacy of this product is to be evaluated comparatively with the
 53 standard regime of oral administration of antiretroviral in Nigeria (triple
 54 therapy) comprising 2 nucleoside reverse transcriptase inhibitor (NRTIs) plus a
 55 protease inhibitor (PI) (Zidovudine (AZT) + Lamivudine (3TC) + Nevirapine
 56 (NVP).

57 MATERIALS AND METHODS.

58 SAMPLE COLLECTION

59 Candidates on HAART were recruited from Central hospital, Stella Obasanjo
60 Hospital and University of Benin Teaching Hospital. The antiretroviral drug
61 naïve came from private hospitals/private practitioners and volunteers who
62 came on their own. Relevant documentations including informed consent forms
63 and questionnaires were administered to all of them.

64 SELECTION CRITERIA

65 The selection criteria include, prior exposure to antiretroviral drugs (Multiple
66 therapy), for not less than one year, female, age bracket of 25-40 years and
67 CD_4^+ T cells of less than 200×10^6 cells/L, sero status – HIV 1 and 2 positive for
68 the antiretroviral drug wise group (ARDW).

69 Antiretroviral drug naïve (single, double or triple therapy exposure) females, no
70 multiple infection e.g. TB, Syphilis, Age between 25 and 40 years for the
71 antiretroviral drug naïve group (ARDN).

72 The full profile of individuals in the ARDW group in this study are shown in
73 Tables 1 and 1a while Table 1b is for the drug naïve group.

74

75 **Table 1**

S/N	CODE	SEX	AGE	ARDW	DRUG EXPT in years	CD_4^+ cells count/ml
1	A	F	36	+	$1 \frac{1}{2}$	105
2	B	F	28	+	$1/2$	102
3	C	F	32	+	2	110
4	D	F	31	+	$1/2$	89
5	E	F	37	+	2	102

76 **Key:**ARDW = Antiretroviral drug Wise – Zidovudine + Lamuvidine +
77 Nevirapine

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79

80 **Table 1a**

S/N	Height (cm)	Body Weight (kg)	Viral load Copies/ml	Physical Appearance	Sero Status HIV 1 and 2
1	153	51	312,572	Gauntly with disorientation	+
2	149	43	308,314	Gauntly with disorientation	+
3	153	41	283,163	Gauntly with disorientation	+
4	152	48	340,218	Gauntly with disorientation	+
5	150	50	253,671	Gauntly with disorientation	+

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82 **Table 1b: Profile of HIV/AIDS Individuals in the Bioclean II Treatment**
83 **Group**

S/N	Height (cm)	Body Weight (kg)	Viral Load (copies/ml)	Sero status (HIV 1 and 2)	ARDW	CD4+ T cells (count/ml)	Sex	Age (years)
1	148	41	413,278	+	-	112	F	36
2	151	43	342,167	+	-	120	F	35
3	150	43	342,814	+	-	114	F	32
4	152	48	438,826	+	-	87	F	27
5	150	50	411,572	+	-	113	F	28

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86 **METHODS**

87 The selected individuals in the antiretroviral drug naïve group were placed on
88 10mls of Bioclean II twice daily for six months. After 3 months and at the end
89 of six months, blood samples were collected for analysis. The parameters

90 evaluated were the viral load, CD_4^+ T cell counts, viral antigen detection, body
91 weights and physical appearance of each candidate. Blood samples were
92 collected from the selected individuals in the antiretroviral drug wise group and
93 analysed for the same parameters.

94 **VIRAL ANTIGEN DETECTION**

95 Briefly, 4.0 ml of venous blood were collected from each subject into a sterile
96 vacutainer tube containing ethylene-diamine tetra acetic acid, 7.2 mg/4ml
97 solution (BDF Plymouth, PL67BP; UK). The tubes were arranged in racks and
98 allowed to stand at room temperature ($27\pm 1.0^\circ\text{C}$) for 15 minutes. Using
99 automatic pipettes, plasma was transferred from each tube into a pre-labelled
100 plain specimen vial for the detection of antibodies to HIV1 and 2 using HIV
101 rapid testing kits.

102 Each sample was screened with Determine and Unigold kits run in parallel.
103 Every sample that reacted with both kits was regarded as positive while a non-
104 reactive one was regarded as negative. A reaction with only one of the two kits
105 was regarded as discordant and stat pak was used as a tie breaker. A positive
106 result with stat pak confirmed positivity of the sample. All test kits were
107 evaluated for potency and shelf-life before use.

108 **CD_4^+ T CELLS COUNT**

109 Briefly, the reagents – PE antibody, partec buffer and count check beads stored
110 at 4°C were brought out to attain room temperature ($27\pm 1.0^\circ\text{C}$). Using an
111 automatic pipette (Eppendorf), 20 μl of PE was dispensed into a partec tube.
112 Then 20 μl of well mixed sequestered whole blood was added into the tube and
113 shaken gently to mix the content thoroughly. The mixture was incubated in the
114 dark at room temperature ($27\pm 1.0^\circ\text{C}$) for 15 minutes, mixing at 5 minutes
115 interval. Thereafter, 800 μl of CD_4^+ buffer was added to the tube and mixed
116 gently. Then the tube was plugged to the counter (Flow cytometer). During

counting, it was ensured that the CD₄⁺ cells monocytes and noise were well separated and gated. The count check beads were run as control.

VIRAL LOAD DETERMINATION

Viral load was determined by the Polymerase Chain Reaction method following the procedure outlined by Brown (2002). Briefly, the patient's blood and buffer were heated to a temperature of 95°C for the hydrogen bonds of the double helix of HIV DNA to be broken, resulting in single-stranded molecules to be used as temperate. This is the denaturation stage. This was followed by lowering the temperature to 50-56°C, to allow the primers to anneal to the appropriate single-stranded DNA template, forming short segments of double-stranded DNA where the polymerase attached to begin DNA synthesis. This was followed by the extension/elongation step at 72°C when the DNA polymerase (T_{aq}) synthesized new DNA strands which were complementary to the HIV DNA template strands. By repeating the cycle of denaturation-annealing-syntheses, copies of the viral DNA were amplified with their 5' and 3' ends both set by the primers.

BODY WEIGHT MEASUREMENT

The body weight of individuals were taken using the standing weighing balance (The Big Boss, Hana).

RESULTS

Table 2: EFFECTS OF ZIDOVUDINE + NEVIRAPINE + LAMIVUDINE AND BIOCLEAN II ON BODY WEIGHT OF FEMALE HIV/AIDS CASES

Item	Treatment (n=5)		Statistical T-test	Control
	Zid + Nev + Lam	Bioclean II		
Period (Days)	Body Weights (kg)		P Value	
0	43, 41, 40, 48, 51 (mean 44.6)	43, 43, 41, 48, 50 (mean = 45.0)	P > 0.05	58.2
90	45, 44, 43, 50, 50, 53 (mean= 47.0)	60, 51, 52, 58, 61 (mean = 56.4)	P < 0.05	60.5
180	46, 48, 45, 49, 55 (mean= 48.6)	62, 58, 62, 60, 65 (mean= 61.4)	P < 0.001	60.2

Table 3: EFFECTS OF ZIDOVUDINE + NEVIRAPINE + LAMIVUDINE AND BIOCLEAR II ON VIRAL LOAD OF HIV/AIDS CASES

Item	Treatment (n=5)		Statistical T-test	Control
	Zid + Nev + Lam	Bioclear II		
Time (in days)	Viral load (copies/ml)		P Value	
0	312572, 308314, 283163, 340218, 253671 (mean =299587.6)	324362, 315460, 290231, 346213, 247543 (mean=304161.8)	P > 0.05	ND
90	76, 340, 112326, 164706, 211327, 117, 471 (mean=136234)	12321, 7351, 5326, 2401, 11210 (mean=7721.8)	P < 0.001	ND
180	211370, 213052, 165725, 207682, 201443 (mean=199854.4)	ND, ND, 250, 5320, ND (mean=2785)	P < 0.001	ND

Key: ND = Not Detectable

Table 4: EFFECTS OF ZIDOVUDINE + NEVIRAPINE + LAMIVUDINE AND BIOCLEAR II ON CD₄⁺ T-cells of HIV/AIDS CASES

Item	Treatment (n=s)		Statistical T-test	Control
	Zid + Nev + Lam	Bioclear II		
Time (in Days)	CD ₄ ⁺ T-cells count (x/ml)		P-value	
0	112, 108, 180, 102, 114 (mean=123.2)	108, 116, 104, 182, 107 (mean =123.4)	P > 0.05	1102
90	326, 241, 372, 413, 425 (mean=355.4)	823, 706, 568, 604, 712 (mean = 682.6))	P < 0.001	1194
180	428, 392, 561, 524, 401 (mean= 461.2)	892, 756, 692, 785 (mean= 752.6)	P < 0.001	1011

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154 **RESULTS**

155 The profiles of the two groups of individuals investigated are shown in tables 1,
156 1a and 1b. There was a reasonable similarity in age, sex and body weights of
157 individuals in the two groups. The effects of treatment with Zidovudine +
158 Lamivudine + Nevirapine and Bioclean II on body weights of the individuals
159 investigated are shown on Table 2. There were increases in body weights of the
160 individuals in the two groups.

161 The effects of treatment with Zidovudine + Lamivudine + Nevirapine and
162 Bioclean II on Body weights of the individuals investigated are shown on Table
163 2. There were increases in body weights of the individuals in the two groups at
164 90 days and 180 days post treatment. The Bioclean II treatment group recorded
165 higher increases in body weight than the triple drug (Zid + Lam + Nev) group.
166 The differences in body weight gain at the two intervals were significant ($p <$
167 0.05 ; $p < 0.001$).

168 The effects of treatment on the viral load of individuals investigated are shown
169 on table 3. Triple drug treatment and Bioclean II produced viral load reductions
170 in the individuals investigated. The rate of viral load reduction in the Bioclean II
171 treatment group was higher than the HAART group.

172 Table 4 shows the effect of treatment on the CD_4^+ T cells of the individuals
173 investigated. There were increases in CD_4^+ T cells count at 90 and 180 days
174 treatment intervals. The Bioclean II treatment group recorded higher increase in
175 CD_4^+ T cells than the Zid + Lam + Nev treatment group. The differences in the
176 rate of CD_4^+ T cells increases by the two treatments were significant ($p <$
177 0.001).

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183 **DISCUSSIONS**

184 The effects of treatment with Zidovudine + Lamovudine + Nevirapine(HAART)
185 and Bioclean II on female HIV/AIDS cases in Nigeria were investigated.
186 Individuals in the two treatment groups were balanced in terms of sex, age,
187 body weight and CD₄⁺T cells at the beginning of the investigation (Tables 1, 1a
188 and 1b). HAART treatment was provided by approved Heart-to-Heart clinics in
189 Benin City and strict compliance was a selection criterion.

190 Treatment with triple drugs for over one year produced improvement in the
191 body weights of the HIV/AIDS cases investigated (Table 2). This finding is in
192 agreement with reports of other workers (Osayande, 2014). Bioclean II
193 produced a superior body weight gain by HIV/AIDS cases after 8 months
194 exposure (Table 2). This finding is in agreement with our earlier report on the
195 effect of Bioclean II on body weights of HIV cases (Ibeh et al., 2013). The
196 implication is that the two treatment methods had effect on the metabolic
197 activities of the individuals investigated which was translated to body weight
198 gain.

199 The HAART and Bioclean II treatment produced reductions in the viral load of
200 the HIV/AIDS cases investigated (Table 3). Reduction of viral load in
201 HIV/AIDS cases by HAART is already documented (Coutsoudis et al., 2010;
202 Anderson, 2012). The findings in the present study suggest that Bioclean II has
203 the potential to reduce the viral load of HIV/AIDS cases. That it did so at a
204 shorter exposure time than HAART and at a higher rate is very interesting
205 (Table 3). This result suggests that Bioclean II has an effective inhibitory effect
206 on the replication of HIV. The mechanism by which Bioclean II interferes with
207 the replication of HIV needs to be further investigated.

208 The effects of treatment with HAART and Bioclean II extended to the CD₄⁺ T
209 cells of the individuals investigated (Table 4). The two treatment methods
210 produced increases in the CD₄⁺ T cells count of the individuals investigated.
211 Improvement in CD₄⁺ T cells in HIV/AIDS cases exposed to HAART has been
212 documented (Coovadia, 2004; Krieke, 2007). The observed increases in CD₄⁺ T
213 cells of HIV/AIDS study is in agreement with our earlier report (Ibeh et al.,
214 2013). This finding suggests that Bioclean II is very effective in restoring
215 depleted CD₄⁺ T cells associated with HIV infection and progression to AIDS.
216 Although the mechanism of action of Bioclean II is not yet fully understood, it
217 is possible that there may be a direct arrest of CD₄⁺ cells destruction, abolition
218 of apoptosis of bystander cells, reduction of CD₈ cells, destruction of CD₄⁺ T

cells infected with HIV and neutralization of oxidative stress that destroys cells (Ibeh et al., 2014).

The performance of Bioclean II in this study suggests need for further studies to understand the mechanism of action of this local herbal product with a view to ascertain its value as an intervention tool in the cycle of HIV/AIDS disease.

REFERENCES

- Anderson, J. (2012) Women and HIV: Motherhood and More. *Curr. Opin. Infect. Dise.* 25: 58-65.
- B.A., Darby, G and Richman, D.D (1989). HIV with reduced Sensitivity to Zidovudine (AZT) isolated during prolonged therapy. *Science* 243: 1731-1734.
- Coutsoudis, A., Kwaan, I., Thomson, M. (2010) Prevention of vertical transmission of HIV-1 in resource limited settings. *Exp. Rev. Anti-infect. Ther.* 8: (10)1163-1175.
- Coovadia, H. (2004) Antiretroviral agents – how best to protect infants from HIV and save their mothers from AIDS. *N. Engl. J. Med.* 351(3): 289-292.
- Fischl, M.A., Richman, D.D., and Saag, M. (1997). Safety and antiviral activity of combination therapy with Zidovudine, Zalcitabine and two doses of interferon alphas in patients with HIV. AIDS Clinical Trials Group Study. *J. Acquire. Immune Defic. Syndr. Hum. Retroviral* 16: 247-253.
- Ibeh, I.N., Akanu, N., Mkpa, A.M., Isitua, C.C. and Ogefere, H.O. (2013) Evaluation of Anti-Human Immunodeficiency Virus (HIV) Properties of DXL (Decoction X-Liquid-Bioclean II)*J. Clin. Toxicol* S12: 004. Doi: 10.4172/2161-0495. S12-004
- Ibeh, I.N., Nnachi, A., Mkpa, A.M., Okungbowa, M.A and Ibeh, N.I (2014) Studies on the effects of Bioclean II on female HIV/AIDS cases in Nigeria (In Press).
- Kripke, C. (2009) Antiretroviral prophylaxis for occupational exposure to HIV. *Amer. Fam. Phys.* 76: 375-376.
- Merril, D.P., Moonis, M. and Chou, T.C. (1996). Lamivudine or Stavudine in two and three drug combinations against human immunodeficiency virus type 1 replication in-vitro. *J. Infect. Dis.* 173; 355-364.
- UNAIDS (2003) “Improved Methods and Assumptions for Estimation of the HIV/AIDS Epidemic and its impact: Recommendations of the UNAIDS Reference Group and estimations”. *AIDS* 16: 1-16.