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Effect of Treatment with First and Second Line Fix-Dose Antiretroviral Drugs on Hematological Indices and Markers of Oxidative Stress in Wistar Rats

Ezekiel U. Umoh
Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Science, University of Uyo, Akwa, Ibom State, Nigeria

Imaikpo U. Umoh
Department of Biochemistry, Faculty of Biological Sciences, University of Uyo, Nigeria

Eyong U. Eyong
Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Cross River State, Nigeria

Annotation: There may be negative consequence on the clinical outcome of HIV-infected individuals when using combined antiretroviral drugs, a resulting in metabolic complications. This study investigated the effect of first and second line fixed-dose combination (FDC) antiretroviral drugs on hematological indices and oxidative stress markers in Wistar rats. Thirty-five (35) male Wistar rats (Raths Novegicus) were divided into seven (7) experimental groups (A, B1, B2, C1, C2, D1 and D2). Group A received normal rat pellet and clean water. Group B1 received 17.14 mg/kgbwt/24h of fixed-dose EFV/3TC/TDF as first line regimen for 15 days, while Group B2 received same regimen for 30 days. Group C1 received 6.43 mg/kgbwt/12h of fixed-dose 3TC/ZDVt3.57 mg/kgbwt/12h of LPV/r as second line regimen for fifteen (15) days, while Group C2 received same regimen for 30 days. Group D1 received first line regime for 30 days then switched to second line regimen for 15 days (a total of 45 days), while Group D2 received first line regime for 30 days, then switched to second line regimen for another 30 days (a total of 60 days). First and second line regimens showed significant change ($P<0.05$) in markers of oxidative stress except for glutathione (GSH) which showed significant increase ($P<0.05$) in groups B2, C1, C2 and D1 compared to control. Hematological indices were not significant ($P>0.05$) in animals treated with both regimens in all the groups when compared with the control. Toxic effect of First and second line FDC antiretroviral drugs was significant on oxidative stress markers but no effect on hematological indices in rats; however repeated dose at long term use may be tolerated.

Keywords: Oxidative stress, Fix-dose, Antiretroviral drugs, Hematological indices, Free radicals.

INTRODUCTION

Human immunodeficiency virus (HIV) is a virus that attacks the body's immune system. It is an etiological agent of acquired immunodeficiency syndrome (AIDS), which is a serious global health threats of the present time (14). The earliest well documented case of human immunodeficiency virus (HIV) in human dates to 1959 (53). By 1983, the US CDC identified all major routes of HIV transmission, which does not include food, water, air, or personal contact. The first major scientific breakthrough against the disease occurred in 1983. Pasteur institute researchers, Francoise Barre-Sioussi and Luc Montagnier isolated a retrovirus, which they named lymphadenopathy-associated virus (LAV), from the lymph node of an individual with AIDS. In the same Issue on the scientific Journal of Science, National Cancer Institute researcher Robert Gallo described a retrovirus that he named the third of the human Tlymphotropic viruses, HTLV-III. The following year, Montagnier and

Gallo held a joint press conference to announce the two viruses, which were in fact the same virus and likely the cause of AIDS (53). Blood test for the virus was developed for the first time in 1985, allowing blood banks to begin testing for the virus. By this time, AIDS had been identified in all regions of the world, and 1.5 million people were estimate to have been infected. 1986, the international committee on Taxonomy of viruses (ICTV) officially named the retrovirus "human immunodeficiency virus" (HIV).

Antiretroviral drugs (ARV) are medications used for the treatment of diseases caused by retroviruses, primarily HIV. For management of HIV and AIDS, antiretroviral drugs have been introduced and this has help to reduce this chronic disease, increasing the life expectancy among HIV-infected patients (49). More than half of the global populations living with HIV (PLWH) were receiving ARV drugs, a record of 19.5 million people, as at 2017(49). Different classes of ARV drugs exist and these classes of drugs inhibits HIV replication at different stages in the HIV life cycle (38). These classes include; nucleoside and nucleotide reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), chemokine receptor (CCR5) antagonist, integrase strand transfer inhibitors (INSTIs) and post-attachment inhibitors (PAIs). Remarkable improvement in HIV prognosis has been reported in combined HIV therapy (38). Combined therapy is considered the most effective treatment for individuals with HIV infection (36).

Hematology involves the diagnosis, treatment and prevention of blood disorders and malignancies such as hemophilia, leukemia, lymphoma and anemia. Blood is made up of three major types of cells suspended in fluid called plasma. They include red blood cells (RBCs), white blood cells (WBCs) and platelets (PLTs). Several factors such as drugs and infection can cause an alteration in blood components. Hematological complications of HIV infection which include cytopenia of all major cell lines were recognized shortly after the first decryption of AIDS cases (31). Bone marrow suppression characterized by abnormal hematological indices is one of the serious adverse effects of antiretroviral drugs, especially the nucleoside reverse transcriptase inhibitors (40). Hematologic toxicity including neutropenia and anemia particularly in patients with advance HIV disease have been associated with the use of zidovudine, a nucleoside reverse transcriptase inhibitor. Anemia, one of the commonest manifestations of bone marrow suppression, often resolves when treatment is initiated with ARV drugs; however, studies have shown that anemia could get worse if NRTI, especially ZDV is a component in the anti-HIV regimen (Kerkhoff pecially ZDV is a component in the anti-HIV regimen (*21*).

Antioxidants are naturally occurring molecules that combat oxidative damage in biological entities by free-radical scavenging (7). A free radical is any chemical species with one or more unpaired electrons and capable of independent existence. Once formed, free radicals can react either with another molecule by different interactions or with another radical. Reactive oxygen species (ROS), which are free radicals, are potentially cytotoxic at higher concentration which can result in ROS-induced damage including cell death, mutations, chromosomal aberrations, and carcinogenesis (20, 44, 51). Studies have shown that antiretroviral drugs are associated with toxicological effects which could be associated with oxidative stress through the generation of oxidative radicals, depletion of antioxidants and antioxidant enzymes leading to mitochondria damage in the heart, kidney, liver, brain and other organs (20, 51). Didanosine has been reported to induce oxidative stress in the brain mitochondria of animal via increase in protein carbonyl (34). Zidovudine and indinavir are reported to be associated with some cardiovascular effect e.g. atherosclerosis which could be attributed to oxidative stress (19). Efavirenz which is known to be associated with central nervous system disorders was reported to induce oxidative stress in the intracranial visual relay centers of adult Wistar rats (2). Efevirenz has also shown evidence for their involvement of mitochondrial dysfunction and oxidative stress in its cellular toxicity in animals (4).

Significant increase in protein carbonyl content, decrease in glutathione and protein thiol, was observed in the kidney of tenofovir treated rats (39). Also decrease in the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione S transferase and glutathione reductase and a massive increase in myeloperoxidase activity were observed (25).

Damaged mitochondria could serve as a source of oxidative radicals involved in tenofovir induced kidney damage (39). Krambovis and colleagues also reported that tenofovir induced oxidative stress in the kidneys may be due to the overproduction of reactive oxygen species as well as the depletion of cellular antioxidant system (25). Available evidence has implicated anti-HIV drug of PIs class with endothelial dysfunction, including a decrease in endothelium-dependent vaso-relaxation, inhibition of nitric oxide synthase system, increase oxidative stress and induction of mitogen-activated protein kinases (46). Also, study reports that oxidative stress can disrupt endothelial homeostasis by dysregulating the balance between pro-and anti-atherogenic factors (30). PIs have also been shown in many studies to induce endoplasmic reticulum stress in many cell types including hepatocytes, macrophages and intestinal epithelial cells probably via the generation of oxidative radicals (50, 52). To date, numerous reports show that NRTIs, NNRTIs as well as PIs drug class, trigger massive ROS production in various cell types (9, 48). Treatment of HIV patients with fixed-dose antiretroviral drugs containing ZDV/3TC/NVP and also fixed-dose regimen containing ZDV/3TC/EFV was reported to decrease selenium concentration in some patients and increased production of free radicals (5). MDA, SOD, GPx and CAT are markers of oxidative stress. This study assessed the effects of treatment with first and second line fix-dose antiretroviral drugs on hematological indices and markers of oxidative stress in Wistar rats.

MATERIALS AND METHODS

Materials

Chemicals and Reagents

The assay kit for the determination of activities of superoxide dismutase (SOD), catalase (CAT) Glutathione (GSH) glutathione peroxidase (GPx) and concentration of serum malondialdehyde (MDA) were obtained from Fortrees Diagnostic Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS, United Kingdom. Hematological analysis was conducted using Automated Hematological Analyzer, Model MSLBA56, Med Singlong Medical equipment and glass wares used in this s

Experimental Animals

Experimental Animals (male albino Wistar rats) used for this study were purchased from the Animal House, faculty of Basic Medical Science, University of Uyo. The animals were kept in standard plastic cages and housed in a good atmospheric condition under a 12-hour day/light cycle. They were allowed free access to rat pellet and clean water *adlibilum.* The feeding lasted for a period of one month to get the desired weight of 200 g and above. During this period, the rats got acclimatized to the environment prior to the commencement of the experiment. Body weight of the animals was taken at baseline and weekly throughout the experimental period.

Drug Sample

The following fixed-dose combination (FDC) antiretroviral drugs (first and second line regimens) manufactured by Mylan Laboratories Limited, India were obtained from University of Uyo Teaching Hospital (UUTH) for the study.

1. First line Regimen: FDC of TDF/3TC3EFV (Symfi® or Telura®) containing two (2) NRTIs [Tenofovir Diisoproxil Fumarate (TDF)/Lamivudine (3TC)] and one (1) NNRTI [Efavirenz (EFV)] in one table. Thus, a single dose of TDF/3TC/EFV contains 300mg of TDF, 300mg of 3TC and 600mg of EFV.
2. Second Line Regimen: FDC of 3TC/ZDV (Combivir®) containing two (2) NRTI [Lamivudine (3TC)/Zidovudine (ZDV] in one table, co-administered with boosted Lopinavir (LPV/r) (Kaletra®). A single dose of 3TC/ZDV contains 150mg of 3TC and 300mg of ZDV, while a dose of LPV/r is made up of 200 mg of LPV co-formulated with 50 mg of ritonavir (r).

Experimental design

A total of thirty-five (35) male albino rats (*Rattus novegicus*) of the Wistar strain weighing between two hundred (200) and two hundred and fifty (250) grams were used in the study. The rats were divided into four groups (A, B, C and D). Group A which had five (5) rats served as control. Groups B, C, and D had ten (10) rats each; they were sub-divided into B1, B2, C1, C1, D1 and D2. This gave a total of seven (7) experimental groups of five (5) animals each. The cages were labeled accordingly and drug administration carried out as follows:

S/N	Groups	Specification
I	A	Normal animal fed with rat pellets and distilled water, received no treatment.
II	B_1	Received 17.14mg/kg/bwt/24h of fixed-dose 3TC/TDF/EFV as first line regimen for fifteen (15) days.
III	B_2	Received 17.14mg/kg/bwt/24h of fixed-dose 3TC/TDF/EFV as first line regimen for thirty (30) days.
IV	C_1	Received 6.43mg/kg/bwt/12h of fixed-dose 3TC/ZDV + 3.57mg/kg/bwt/12h of LPV/r as second line regimen for fifteen (15) days.
V	C_2	Received 6.43mg/kg/bwt/12h of fixed-dose 3TC/ZDV + 3.57mg/kg/bwt/12h of LPV/r as second line regimen for thirty (30) days.
VI	D_1	Received 17.14mg/kg/bwt/24h of fixed-dose 3TC/TDF/EFV as first line regimen for thirty (30)) days, then switched to 6.43mg/kg/bwt/12h of fixed-dose 3TC/ZDV + 3.57mg/kg/bwt/12h of LPV/r as second line regimen for fifteen (15) days (a total of 45 days).
VII	D_2	Received 17.14mg/kg/bwt/24h of fixed-dose 3TC/TDF/EFV as first line regimen for thirty (30) days, then switch to 6.43mg/kg/bwt/12h of fixed-dose 3TC/ZDV + 3.57mg/kg/bwt/12h of LPV/r as second line regimen for another thirty (30) days (a total of 60 days). Note: Bwt= Body weight

Preparation of Stock Solution

Drugs used in the study were all presented in tablet form. Therapeutic dosage of the drugs for human adult weighing seventy (70) kg were 1200 mg of fixed-dose EFV/3TC/TDF; 450 mg of fixed-dose 3TC/ZDV and 250 mg of LPV/r respectively. To obtain the corresponding therapeutic dosage for the rat models one tablet each of 3TC/TDF/EFV (1200 mg) and 3TC/ZDV (450 mg) were crushed with pestle and mortar, dissolved in 100ml of distilled water to get stock solution of concentration of 12 mg/ml and 4.5 mg/ml respectively. Equally, two tables of LPV/r (500 mg of 250 mg each) were crushed and dissolved in 100ml of distilled water to give a concentration of 5.0 mg/ml. required dosage for each of the rats were calculated based on the body weight then measured as aliquot and administered to the animals through oral intubation.

Collection of Blood Sample, Preparation of sera and Tissue Sample

At the end of administration period (15, 30, 45 and 60 days), the experimental animals were fasted overnight and anaesthetized by dropping each in a transparent glass jar saturated with chloroform fumes. Blood sample was collected from each animal by cardiac puncture after dissection using sterile needles and syringes into a labeled sample bottle. Sample from each animal was divided into two; one part was put in anticoagulant bottles, well mixed with EDTA to avoid coagulation, and used to evaluate hematological indices, while the other part was put into plain sample bottles. The later was

centrifuged at 3000 rpm for 10 minutes using a bench top centrifuge. The serum collected was preserved in the refrigerator for biochemical analysis which was carried out promptly.

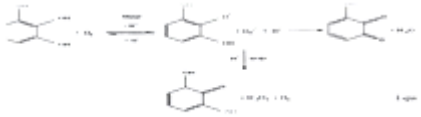
Assessment of Hematological Indices

Automated Hematology Analyzer was used to carry out hematological analyses. The instrument utilizes both whole blood and pre-diluted (PD) blood samples. When using PD mode the dilution is done manually before putting the samples into the transducers. The transducer chamber has a minute hole called aperture. On both sides of the aperture there are electrodes between which flow direct current. Blood cells suspended in the diluted samples pass through the aperture causing direct current resistance to change between the electrodes. As direct current changes, blood cells, size is detected as electric pulses. Blood cell count is then calculated by counting the pulses; a histogram of blood cells sizes is plotted by determining the pulse sizes. Analyzing a histogram makes it possible to obtain various analysis data including differential whole blood count, red cells, indices and derived values.

Assessment of Activity of Antioxidant Enzymes/markers of Lipid Peroxidation

The following antioxidant enzymes/lipid peroxidation markers were assessed in this study: Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione (GSH) and malondialdehyde (MDA).

Serum superoxide dismutase (SOD) activity was determined using a simple and rapid method, based on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol. The principle of this method is based on the competition between the pyrogallol auto-oxidation by O_2 and the dismutation of this radical by SOD.



Serum catalase (CAT) activity was assayed using the method described by

Foassati (1980). The method is based on the measurement of the hydrogen peroxide substrate remaining after the action of catalase. Catalase reacts with a known quantity of hydrogen peroxide and converts it to water and oxygen (catalytic pathway).

$$2H_2O_2 \xrightarrow{CAT} O_2 + 2H_2O$$

An aliquot of the reaction mixture is then assayed for the amount of hydrogen peroxide remaining by a colorimetric method. In the colorimetric method, a substituted phenol (3,5-dichloro-2-hydroxybenzesulfonic acid), which couples oxidatively to 4-aminoantipyrine in the presence of hydrogen peroxide and horseradish peroxidase (HRP) was used. The reaction gives a red quinoneimine dye (N-(4-antipyryl)-3-chloro-5-sulfonatep-benzoquinone-monoimine) (Equation 35) that absorbs at 520 nm.

$$2H_2O_2 \text{ (Left over) } + \text{DHBS} + \text{AAP} \xrightarrow{HRP} \text{Quinoneimine Dye} + 4H_2O$$

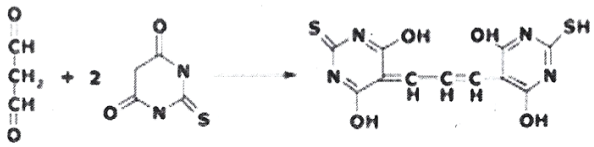
The color intensity measured at A_{520} is inversely proportional to the catalase activity in the original sample.

Serum GPx activity was assayed using an indirect determination method published by Mannervik, (1985). In this method GPx activity was measured indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of hydroperoxide by GPx is recycled to its reduced state by glutathione reductase (GR) and NADPH (B-Nicotinamide Adenine Dinucleotide Phosphate –Reduced).

$$R\text{-}O\text{-}O\text{-}H + 2GSH \xrightarrow{GPx} R\text{-}O\text{-}H + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GPxGR} 2GSH + NADP^+$$

The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance observed at 340 nm. Under condition in which GPx is rate limiting, the rate of decrease in A_{340} is directly proportional to the GPx activity in the sample.



Equation 3.38

MDA TBA $MDA\text{-}TBA_2$

Serum glutathione was determined based on enzymatic recycling method using glutathione reductase and Ellman's reagent (DTNB) (Ellman, 1959), for the quantification of GSH, Glutathione reductase reduces GSSG to GSH. Ellman's reagent, DTNB (5-5'- dithiobis [2-nitrobenzoic acid]) reacts with thee sulfhydryl group of GSH to form yellow color chromophore, 5-thionitrobenzoic acid (TNB) and GS-TNB. The mixed disulfide, GS, TNB that is concomitantly produced is reduced by glutathione reductase to recycle the GSH and produce more T.

NB.

$$GSSH \xrightarrow{\text{Glutathione Reductase}} 2GSH$$

The rate of TNB production is directly proportional to this recycling reaction which is in turn directly proportional to the concentration of GSH in the sample. Measurement of the absorbance of TNB at 415nm provides an accurate estimation of GSH in the sample.

Concentration of serum MDA was determined based on the reaction of MDA with chromogens

Malondialdehyde (MDA) in the sample reacts with a chromogenic reagent, 2-thiobarbituric acid (TBA) at 25^0C, to generate a pink $MDA\text{-}TBA_2$ adduct, a chromophore that absorbs strongly at 532 nm.

Statistical analysis

Data were analyzed using SPSS statistical software package version 20.0 and results expressed as mean ± standard error of mean (SEM). Analysis of Variance (ANOVA) and Least Significant Difference (LSD) multiple post hoc comparison tests were carried out on the data and Mean difference between groups were considered statistically significant at $p<0.05$.

Ethical consideration

Prior to the commencement of this study approval was sought and granted by Faculty of Basic Medical Sciences Ethical Committee, University of Uyo, Nigeria.

RESULTS AND DISSCUSION

Result

Assay of Hematological Indices

Table 1 shows the mean ± SD of concentration of hematological indices such red blood cells (RBC); hemoglobin (HGB); hematocrit (HCT); mean corpuscular volume (MCV); mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet

(PLT), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW), white blood cells. (WBC), lymphocytes (LYM), monocytes (MON) and granulocytes (GRA) of male Wistar rats treated with first and second line FDC antiretroviral drugs. From the result, statistically insignificant ($p<0.05$) increase in some hematological parameters (RBC, WBC, HCT, PDW and GRA) was observed in Group C_1 and C_2 (rats treated with second line regimen for 15 and 30 days respectively) when compared with the control group. Conversely, concentration of WBC in groups B_1 and B_2 (rats treated with first line regimen for 15 and 30 days) insignificant ($p<0.05$) decrease compared to control. Serum concentration of PLT differed insignificantly with elevated levels in groups B_1, B_2, C_1 and C_2 when compared with control. Also, non-significant decrease ($p<0.05$) of LYM levels was observed in groups C_1 and C_2 compared to control. Other hematological profile in this study also showed non-significant change in the treated groups when compared with the control group.

Table 1: Effect of treatment with first and second line FDC antiretroviral drugs (17.14mg/kgbwt/24h of EFV/3TC/TDF and 6.43mg/kgbwt/12h of 3TC/ZDV + 3.57MG/KGBWT/12h of LPV/r) on markers of oxidative stress in male albino wistar rats

GROUP (n=5)	RBC (10^6/UL)	HB (g/dl)	PVC (%)	MCV (um^3)	MCH (pg)	MCHC (g/dl)	RDW (%)	PLT (10^3um)	MPV (um^3)	PCT (%)	PDW (%)	WBC (10^3/ul)	LYM (%)	MON (%)	GRA (%)
A (CONTROL)	9.39±0.46	17.8±1.08	48.85±3.17	51.9±1.06	51.9±1.06	36.5±0.17	17.6±0.21	1007.50±36.02	7.55±0.22	0.76±0.03	19.65±1.01	18.40±0.74	73.78±1.59	12.18±1.35	14.05±2.09
B_1 (1^{st} line) 15 days	8.70±0.17	16.65±0.36	35.10±9.66	51.90±0.69	51.90±0.69	36.90±0.13	15.43±0.23	1059.00±73.68	7.05±0.32	0.75±0.05	17.05±0.58	7.10±1,38	78.73±3.02	11.73±1.43	9.55±1.17
B_2 (1^{st} line) 30 days	8.64±0.37	16.80±0.67	44.55±1.79	51.58±0.28	51.58±0.28	37.73±0.21	16.75±0.67	1225.50±131.73	7.03±0.07	0.87±0.09	17.43±0.18	9.88±1.12	72.30±0.80	11.20±1.08	16.50±1.54
C_1 (2^{nd} line) 15 days	10.17±0.17	18.98±0.48	50.53±1.02	49.65±0.39	49.65±0.39	37.55±0.36	18.10±0.54	1132.00±55.26	7.15±0.16	0.82±0.05	20.9±0.49	21.80±3.31	68.58±7.39	12.20±2.21	19.23±5.20
C_1 (2^{nd} line) 30 days	9.86±0.33	18.75±0.61	50.73±1.81	51.43±0.60	51.43±0.60	36.98±0.13	18.13±0.53	1158.00±104.06	7.30±0.26	0.84±0.05	20.20±1.07	19.43±2.94	68.95±5.28	13.01±1.14	18.05±4.55
D_1 (B_2 to C_1) 45 days	9.83±0.37	18.75±0.38	49.65±1.16	51.53±0.54	51.53±0.54	37.80±0.44	17.75±0.48	1027.25±24.06	6.63±0.06	0.68±0.01	18.05±0.67	13.00±1.51	75.65±1.38	10.43±0.85	13.93±2.02
D_2 (B_2 to C_2) 60 days	9.78±0.16	18.45±0.47	48.88±1.40	49.98±0.81	49.98±0.81	37.78±0.38	19.68±1.14	947.25±127.88	7.05±0.19	0.66±0.08	22.48±1.60	15.53±2.24	71.90±1.21	11.30±0.72	16.80±1.70

Values are presented as Mean ± Standard Error of Mean (SEM).

Source: Computed by the researcher from raw data of hematological analysis (2019).

Legends: RBC = Red Blood Cells; HB = Hemoglobin; PCV = Packed Cell Volume; MCV = Mean Corpuscular Volume; MCH = Mean Cell Haemoglobin; MCHC = Mean Cell Hemoglobin Concentration; RDW = Red Cell Distribution Width; PLT = Platelet; MPV = Platelet Volume; PCT = Plateletcrit; PDW = Platelet Distribution Width; WBC = White Blood Cells; LYM = Lymphocytes; MON = Monocytes; GRA = Granulocytes.

Assay of the Activities of Antioxidant Enzymes

Table 2 shows the Mean ± SD of activities of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) of male albino Wistar rats treated with first and second line FDC antiretroviral drugs. The result showed significant increase ($p<0.05$) in serum SOD in Groups B_2 (rats treated with first line anti- HIV regimen for 30 days) and D_2 (rats treated with first line anti-HIV regimen for 30 days then switched to second line regimen for another 30 days) compared with Group A (control) and in between mean with Group B_1. Serum levels of CAT differed insignificantly ($p<0.05$) in all the treated groups compared with the control. Significant increase ($p<0.05$) was observed in serum GSH in Groups B_2, C_1 (rats treated with second line regimen for 15 days), C_2 (rats treated with second line regimen for 30 days) and D1 (rats treated with first line regimen for 30 days then switched to second line regimen for 15 days) compared with the second group and in between with Group B1. Serum GSH in Group D2, also showed significant increase ($p<0.05$) when compared in between mean with Groups B_2, C_1 and D_1. No significant change ($p<0.05$) was observed in serum GPX in all the treated groups compared with the control. There was a significant increase ($p<0.05$) in serum MDA.

Table 2: Effect of treatment with first and second line FDC antiretroviral drugs (17.14mg/kgbwt/24h of EFV/3TC/TDF and 6.43 mg/kgbwt/12h of 3TC/ZDV + 3.57MG/KGBWT/12h of LPV/r) on markers of oxidative stress in male albino Wistar rats

GROUP (n=5)	SOD (U/ml)	CAT (u/ml/mins)	GSH (mM)	GPx (U/L)	MDA (u/M)
A (Control)	1.01 ± 0.21	16.20 ± 0.40	1.86 ± 0.20	3.07 ± 0.63	2.36 ± 0.41
B_1 (1^{st} Line) 15 days	0.99 ± 0.11	16.16 ± 0.43	1.60 ± 0.19	3.63 ± 1.00	4.45 ±0.64[a]

B_2 (1st Line) 30 days	1.77 ± 0.15[ab]	16.35 ± 0.12	3.21 ±0.08[ab]	3.12 ± 0.14	2.71 ±0.68[b]
C_1 (2nd Line) 15 days	1.51 ± 0.27	16.24 ± 0.36	3.21 ±0.32[ab]	3.69 ± 0.29	2.67 ±0.024[b]
C_2 (2nd Line) 30 days	1.25 ± 0.26	16.977± 0.27	3.10 ±0.20[ab]	2.39 ± 0.48	3.53 ± 0.63
D_1 (B_2 to C_1) 45 days	1.47 ± 0.31	16.50 ± 0.54	2.94 ±0.26[ab]	3.18 ± 0.62	3.54 ± 0.75
D_2 (B_2 to C_2) 60 days	1.81 ± 0.18	15.80 ± 1.16	2.03±0.23[cdet]	3.66 ± 0.68	1.60 ± 0.53[bet]

Values are presented as Mean ± Standard Error of Mean (SEM).

Source: computed by the research from raw data of biochemical analysis (2019).

Legends: SOD = superoxide dismutase; CAT = catalase; GSH = Glutqthione; GPx = Glutathione peroxidase; MDA = Malonaldehyde; [a] = significantly different when compared to Group A ($p<0.05$); [b] = significantly different when compared to Group B_1 ($p<0.05$); [c] = significantly different when compared to Group B_2 ($p<0.05$); [d] = significantly different when compared to Group C_1 ($p<0.05$); [e] = significantly different when compared to Group B_2 ($p<0.05$); [d] = significantly different when compared to Group C_1 ($p<0.05$); [e] = significantly different when compared to Group C_2 ($P<0.05$); [f] = significantly different when compared to Group D_1 ($p<0.05$); n = number of animals per group.

Discussion

Blood is the only tissue that flows throughout the body system. It carries oxygen and nutrients to all part of the body and waste products back to the lungs, kidney and liver for disposal. It remains an essential part of the immune system, crucial to fluid temperature balance, a highway for hormonal message and a hydraulic fluid for several functions in the body. Blood disorders referred to as hematological abnormalities ranges from anemia, neutropenia, thrombocytopenia to serious cases such as venous thrombo-embolism, hemophagocytic syndrome, and altered coagulation mechanisms etc. these abnormalities are associated with various diseased conditions and are common complications of human immunodeficiency virus infection (10).

Anemia is the most common hematological abnormalities in human immunodeficiency virus (HIV) patients; hence, a HIV disease progresses, the prevalence and severity of anemia also increase (35). Thrombocytopenia, which could occur at any stage of HIV infection, is the second most frequent complication of human immunodeficiency virus infection. Chronic infection with HIV is well-characterized causes of chronic immune thrombocytopenic pupura (ITP) (27). The possible mechanisms that have been reported are immune-mediated destruction of platelets by antibodies, cross-reacting antibodies that are directed toward HIV proteins, particularly gp120 and p24. However, this type of platelet destruction is characterized by very low platelet counts with normal packed cell volume and white blood cell count (3). Also, neutropenia, which occur at the advanced stage of the infection, is the most common leucopenia occurring in HIV infected individuals (10). HIV infection suppresses the bone marrow and leads to decreased levels of granulocyte colony-stimulating factor (the factor that stimulates production of white blood cells in the bone marrow) and affects the granulocyte-macrophage lineage, resulting in leucopenia and neutropenia. These abnormalities increase as the disease advances as sequelae of HIV-related opportunities infection or malignancies. Different types of hematological abnormalities have been reported in both antiretroviral-treated and untreated individuals as oppose to HIV-naïve subjects (32).

In this study, the changes observed in most hematological parameters were similar to the findings in various other studies. ARV drugs from PIs class especially RTV have been shown to directly activate platelets concentrations in HIV-treated individuals (24, 28) which is in agreement with this study. Also, in two studies carried out independently by Kibaru *et al.* (2015) and Ifeanyichukwu *et al.* (2016) it was observed that the WBC in HIV-positive subjects on ARV drugs showed insignificant decrease compared to the controls. Again, Kibaru *et al.* (2015) reported that following six (6) months of treatment with fixed-dose ARV drug, hematological reconstitution occurred progressively for all blood lineages. Therefore, it was opined that the reduced leucocyte profile might thus be due to generalized pancytopenia as a result of chronic infection like HIV and the efficacy of antiretroviral drugs to improve immunity leading to less infection. Hence, the pancytopenia observed in HIV/AIDS patients

may probably be due to either the virus infection or other latent malignancies not as a result of administration of ARV drugs. According to Shruthi *et al.* (2017) initiation of FDC antiretroviral drugs has led to reversal of most hematological complications that are the direct result of HIV infection.

Other studies have confirmed the ability of FDC antiretroviral drugs to correct or improve anemia and other hematological parameters of HIV infection. Kusfa *et al.* (2017) observed a rise to normal in hematological level among HIV-positive patients six (6) months after commencement of ARV drugs. Equally, in an earlier study by Mocroft *et al.* (1999) it was found that the use of D4T/ZDV/3TC as FDC antiretroviral drugs was statistically associated with improved in hemoglobin levels. They suggested that the positive effect of the combination anti-HIV drugs is probably due to the reduction in viral load, decreased destruction of mature hematopoietic cells of multiple lineages and an improvement in the blunted erythropoietin response which could be also have led to decreased incidences of opportunities infections. A report by Subodh *et al.* (2018) observed that an HIV-positive patient on FDC of ZVD/3TC/NPV was presented with some adverse effects of ZDV on diagnosis, but surprisingly the patient had no hematological toxicities which are considered sine qua non ZDV toxicity. However, Ukoha *et al.* (2015) and other researchers have reported cases of ZDV- associated impairments in hematological function in rats which is at variance with this study.

In a line with the present study, Thomas *et al.* (2012) observed that albino Wistar rats treated with fixed-dose ARV drug showed reduction in total WBC on day ten (10), but no statistically significant difference was observed in all the parameters evaluated when the test groups were compared with the control on day twenty-five (25). The probable explanation could be that the animals must have adapted or adjusted to its normal body physiology through a negative feedback mechanism, hence must have compensated for the reduction in some blood parameters at the start of drug administration which later normalize on repeated doses of the drug. Hence, findings from this study showed that FDC anti-HIV drugs were associated with greater likelihood of correcting hematological abnormalities when used for longer periods of time which corroborate with the work reported by Enawgaw *et al.* (2014) that HIV-positive patients who were on fixed-dose ARV drug had greater number of blood cells within six months of beginning treatment and hematological disorders were corrected.

Oxidative stress is a physiological condition where there is an imbalance between concentration of reactive oxygen species (ROS) and antioxidants, Reactive oxygen species (ROS) are highly reactive forms of oxygen, free radicals (FR), which are short-lived intermediates containing one or more unpaired electrons. They include an array that has superoxide anion (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), nitric oxide (NO), hypochlorous acid (ClOH), peroxide (ROOH) and peroxynitrite (ONOO-) (33). Oxidative stress arises from the inability of antioxidant defenses to effectively clear the reactive oxygen species (ROS) produced from oxidative metabolism (17).

Excessive ROS accumulation will lead to cellular injury, such as damage to DNA, proteins, and lipid membranes. These highly reactive species are generated in a number of conditions by cellular and a cellular mechanism and have been implicated as an aetiological factor of a wide range of disease including cancer, diabetes, cardiovascular disease, atherosclerosis, neurodegenerative diseases, HIV infection and anti-HIV drug-associated toxicity (6, 8).

Series of studies have reported an increase in oxidative stress characterized by persistent redox imbalance associated with HIV infection manifested by an increase in oxidants and a decrease in antioxidant serum levels (11). Specifically, a study done in eighty four (84) HIV-infected patients during a 6-month period of ARV drug demonstrated a significant increase in serum peroxidation potential, total hydroperoxide, MDA, and advanced oxidation protein product levels as well as a change in glutathione level, compared to their levels before the treatment and to healthy controls (16).

Studies relating oxidative stress with ARV drugs through mitochondrial dysfunction were reported by some scholars. Treatment of HIV patients with FDC antiretroviral drugs containing ZDV/3TC/NVP and ZVD/3TC/EFV was reported to decrease selenium levels in some patients and increased production of free radicals (5). Numerous line of evidence has shown that protease inhibitors (PIs)

induced endoplasmic reticulum (ER) stress in many cell types including hepatocytes, macrophages and intestinal epithelial cells probably via the generation of oxidative radicals (45, 50).

ZDV and IDV are reported to associate with some cardiovascular effects e.g. atheroseclerosis, which could be attributed to oxidative stress (19). EFV which is known to be associated with central nervous system disorders was reported to induce oxidative stress in the intracranial visual relay centers of adult wistar rats (2). EFV has also shown evidence for the involvement of mitochondrial dysfunction and oxidative stress in its cellular toxicity in animals (4). HIV transgenic mice exposed to TDF showed ultrastructural mitochondrial abnormalities and decreased proximal tubular DNA, but no optical microscopical changes were observed. Mitochondria damage is reported to be associated with TDF induced nephrotoxicity in humans and animals (23). This mitochondria DNA inhibition may cause energy deprivation and increase reactive oxygen species formation (39). Krambovis (2005) and colleagues also reported that TDF-induced oxidative stress in the kidneys may be due to the overproduction of reactive oxygen species as well as the depletion of cellular antioxidant system (20). Nitroso-oxidative stress and NFKB activation was also reported to contribute to TDF induced renal damage in rats.

In this study, increase level in serum activity of SOD and concentration of GSH was observed in animals treated with fixed-dose TDF/3TC/EFV and 3TC/ZDV + LPV/r which is at variance with report by Adikwu *et al.* (2014), In earlier study conducted by Muthu *et al.* (2008) it was found that HIV-infected individuals receiving antiretroviral drugs experienced normal levels or GPx over time, which is in agreement with the current study.

Also, high concentration in serum MDA was observed in animals treated with fixed-dose TDF/3TC/EFV (the first 15 days) suggesting lipid peroxidation and initiation in oxidative stress process; however, this anomaly was shown to normalize in subsequent treatment. This is in consonant with study carried out by Elias *et al.* (2014) who observed elevated level in cardiac MDA in animals treated with LPV/r, but no synergistic elevation in cardiac MDA level was observed when LPV/r was co-administered with another agent. This implies that repeated administration of FDC anti-HIV drugs may not have toxic effect in biochemical parameters.

Enzymatic antioxidants protect organs from drug-induced oxidative stress and decreases in their levels connote organ injury (47). Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of cellular antioxidant enzymes and other redox molecules. The initial step in the detoxification process is the disputation of oxygen radicals to hydrogen peroxide by superoxide dismutase. Catalase protects cells from oxidative stress of hydrogen peroxide by its cleavage to water and oxygen (15). Glutathione peroxidase facilitate the conjugation of hydrogen peroxide to glutathione (reduced) leading to the generation of water and oxidized glutathione (3). GSH is an important biomolecule against chemically induced toxicity and can participate in the elimination of reactive intermediaries by reduction of hydroperoxides in the presence of GSH dependent enzymes; it functions as a free radical scavenger; it generates a – tocopherol; and also play important role in the maintenance of protein sulfhydryl group (37). MDA is a useful index of lipid peroxidation being a major breakdown product of lipid peroxides. The engagement defenses and induction of lipid peroxidation evidence in elevation of MDA level (3). This multiple defense mechanisms developed by mammalian cells for neutralization (scavenging) of ROS help protect themselves against oxidation of biological molecules.

Conclusion

The introduction of antiretroviral (ARV) drugs for the management of HIV and AIDS has reduced HIV this disease and significantly increased the life expectancy among HIV-infected patients. The advent of drug expansion programs by pharmaceutical companies, research institutions and state agencies, has led to modification of antiretroviral drugs to a fixed-dose combination (FDC), currently used in HIV treatment. FDC antiretroviral drugs containing TDF/EFV/3TC and 3TC/ZDV + LPV/r were used in this study. From this study, treatment with these drugs causes a significant change in GSH level coupled with insignificant increase in serum activities of SOD and GPx. However, there may be

temporary drug-induced lipid peroxidation which was indicated by abrupt increase in serum MDA observed at the start of the treatment which seems to normalize in subsequent repeated dosages to rule out oxidative stress. The regimens had no deleterious effect on the hematological parameters in the treated animals. Switching from first to second line regimen did not expose the animals to negative effect of these drugs, therefore, these regimens can be used in HIV/AIDS management and treatment.

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