Asian Journal of Biochemistry, Genetics and Molecular Biology

Volume 16, Issue 12, Page 150-164, 2024; Article no.AJBGMB.128888
ISSN: 2582-3698

Biochemical Serum Indices of Hepatic and Renal Functions of Rats in First and Second Line Fixed Doses of Anti-retroviral Drugs Versus First Line With Switch To Second Line Combination

Ezekiel U. Umoh [a*], Efreti F. Effiong [b] and Chinasa J. Onyeulor [c]

[a] Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Science, University of Uyo, Akwa, Ibom State, Nigeria.
[b] Department of Biochemistry, Faculty of Biological Sciences, University of Uyo, Nigeria.
[c] Department of Biochemistry, Faculty of Science, Kingsley Ozumba Mbadiwe University, Ogboko, Imo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajbgmb/2024/v16i12431

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/128888

Original Research Article

Received: 22/10/2024
Accepted: 24/12/2024
Published: 28/12/2024

ABSTRACT

HIV/AIDS is a condition in human in which the immune system begins to fail, leading to life threatening opportunistic infections. This study investigated the effect of first and second line fixed-dose combination (FDC) antiretroviral drugs on some biochemical parameters in Wistar rats. Thirty-

**Corresponding author: E-mail: ezekielumoh@uniuyo.edu.ng;*

Cite as: *Umoh, Ezekiel U., Efreti F. Effiong, and Chinasa J. Onyeulor. 2024. "Biochemical Serum Indices of Hepatic and Renal Functions of Rats in First and Second Line Fixed Doses of Anti-Retroviral Drugs Versus First Line With Switch To Second Line Combination". Asian Journal of Biochemistry, Genetics and Molecular Biology 16 (12):150-64. https://doi.org/10.9734/ajbgmb/2024/v16i12431.*

five (35) male Wistar rats (Raths Novegicus) were divided into seven (7) experimental groups (A, B_1, B_2, C_1, C_2, D_1 and D_2). Group A received normal rat pellet and clean water. Group B_1 received 17.14 mg/kgbwt/24h of fixed-dose EFV/3TC/TDF as first line regimen for 15 days, while Group B_2 received same regimen for 30 days. Group C_1 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 C_2 received same regimen for 30 days. Group D_1 received first line regime for 30 days then switched to second line regimen for 15 days (a total of 45 days), while Group D_2 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 serum level of hepatic parameters observed in 15, 30 and 45 days, while animals treated for 60 days was insignificant ($P>0.05$) in all the parameters compared to control. Non-significant change ($P>0.05$) was observed in renal indices of rats treated with these regimens, while significant increase ($P<0.05$) First and second line FDC antiretroviral drugs exerted toxic effect on hepatic in rats; however repeated dose at long term use may be tolerated.

Keywords: Anti-retroviral; hepatic function; renal function; fixed-dose.

1. INTRODUCTION

"Human immunodeficiency virus (HIV) is characterized and identified as an etiological agent of acquired immunodeficiency syndrome (AIDS), which remains one of the most serious global health threats of the present time" (Faria et al., 2014). "HIV/AIDS is a condition in human in which the immune system begins to fail, leading to life threatening opportunistic infections" (Douek et al., 2009). "It is the fourth leading cause of mortality in the world" (Guha and Sardar, 2011). "According to World Health Organization/United Nation AIDS (WHO/UN AIDS) program and estimated 36.9 million (30.8 million – 42.9 million) people worldwide are living with HIV with 1.8 million new infections every day; and about 35 million people have died of AIDS since the epidemic began" (WHO/UN AIDS Programme, 2018).

"In sub-sharan Africa, more than 23.5 million people were living with HIV as at 2017; thus, the WHO African region remains most severely affected, with nearly 1 in every 25 adults (4.1%) living with HIV and accounting for nearly two-thirds of the people living with HIV worldwide" (Kharsany and Karim, 2017). Currently, Nigeria has the second largest HIV epidemic rate in the world, second to South Africa.

"Anti-retroviral (ARV) drugs are medications used for the treatment of diseases caused by retroviruses, primarily HIV. The introduction of antiretroviral (ARV) drugs for the management of HIV and AIDS has reduced HIV and significantly increased the life expectancy among HIV-infected patients" (World Health Organization, 2015). "As at 2017, more than half of the global populations living with HIV (PLWH) were receiving ARV drugs, a record of 19.5 million people" (World Health Organization, 2018). "They are of different classes including; the nucleoside and nucleotide reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), chemokine receptor (CCR5) antagonist, integrase strand transfer inhibitors (INSTIs) and post-attachment inhibitors (PAIs). Each of these classes of drugs inhibits HIV replication at different stages in the HIV life cycle" (Lundgren et al., 2015; Pinola et al., 2010).

"The liver and kidney are highly specialized tissues which consists of cells that regulate a wide variety of high-volume biochemical reactions. Drugs or toxic compounds, injury, an autoimmune process, or a genetic defect can cause liver and kidney damage". (Kaspar et al., 2017). "Hepatotoxicity occurs with most antiretroviral regimen, and it has been shown to be more common with the order classes of antiretroviral drugs. In particular, studies show that ARV drugs from PI class such as RTV and IDV are more likely to cause liver toxicity compared to other agents" (Bruno et al., 2006).

Liver function tests (LFTs) are carried out to detect the presence of liver disease. The most important biochemical analytes of the liver significant in diagnosing drug-induced hepatotoxicity and other disease of the liver include: Transaminase (Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), Total Protein (T-Prot), Albumin (ALB), Total and direct bilirubin (T-Bil and D-Bil).

"Antiretroviral (ARV) drugs have been associated with chronic kidney disease, and the major drugs implicated in this include indinavir, atazanavir, and tenofovir (Atta et al., 2008; Patil et al., 2015; Samuels et al., 2017). A number of observational studies have documented tenofovir-associated nephrotoxicity following its widespread use in patients with multiple co-morbid conditions" (61). "Ritonavir-boosted PIs are reported to have an increased propensity of causing renal injury, and approximately 70 % of the published cases of TDF- induced nephrotoxic effects are observer with concomitant use of low-dose ritnonavir" (Kiser et al., 2008). "An interaction between lopinavir-ritonavir combination therapy and TDF, which manifests as a decrease in the renal clearance of TDC, has been identified" (Kiser et al., 2008). Some biochemical tests for the assessment and evaluation of renal function include test for plasma concentrations of the waste substances such as urea, creatinine as well as electrolytes. This study assessed biochemical serum indices of hepatic and renal functions of rats in first and second line fixed doses of anti-retroviral drugs versus first line with switch to second line combination.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and reagents

All chemicals and reagents used for this study were of analytical grade. The assay kit for the determination of activities of alanine amino transaminase (ALT), aspartate amino transaminase (AST) and alkaline phosphate (ALP); concentration of total protein (TP), total bilirubin (T-Bil), albumin (ALB), serum urea (U), creatinine (Cr), electrolytes were obtained from Fortrees Diagnostic Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS, United Kingdom.

2.1.2 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.

2.1.3 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.

i. 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.

ii. 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).

2.2 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 B_1, B_2, C_1, C_1, D_1 and D_2. 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:

List 1. Experimental Design

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: Bw= Body weight

2.3 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.

2.4 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 were emptied into plain sample bottles and 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.

2.5 Biochemical Analysis

Sera obtained after centrifugation were used in the assessment of the following biochemical parameters viz: activities of hepatic enzymes (ALT, AST, and ALP); renal indices (urea and creatinine); serum electrolytes (sodium, potassium, chloride and bicarbonate).

ALT and AST activities were determined using the method modified and recommended by International Federation of Clinical Chemistry, IFCC (1986). In the reaction, ALT catalyzes the reversible transamination of L-alanine and a-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD.

L-alanine + a-ketoglutarate $\xrightarrow{\text{ALT}}$ Pyruvate + L-glutamate

Pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ L-lactate + NAD^+

Activity of ALP was determined using the method described by Bowers and McComb (1966), which involved measuring the rate of hydrolysis of various phosphate esters in a buffered system. In

the reaction, ALP catalyzes the hydrolysis of the colorless organic phosphate ester substrate p-Nitrophenyl phosphate (p-NPP) to the yellow colored product p-Nitophenol and inorganic phosphate. This reaction occurs at an alkaline pH of 10.3

$$\text{p-NNP} + H_2O \xrightarrow{\text{ALP}} \text{p-Nitrophenol} + H_3PO_4$$

Serum total protein was analyzed using a method known since 1878 as Biuret color reaction in which the protein molecules react with cupric ions.

$$\text{Alkali Protein} + Cu^{++} \longrightarrow \text{Copper-protein chelate (colored complex)}$$

Bilirubin level in the serum was determined using the Jendrassik-Grof principle (Jendrassik and Grof, 1938). Total bilirubin (unconjugated + conjugated) concentration was determined in the presence of a catalyst, where bilirubin reacts with diazotized sulfanilic acid in acidic medium to form azobilirubin.

Determination of serum albumin was based on bromocresol green colorimetric method modified by Doumas, Watson and Biggs, (1971). In the assay, albumin binds to the indicator dye bromocresol green (BCG) at a pHof 4.1 to form a blue-green colored complex

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH 4.1}} \text{Albumin-BCG complex}$$

Indices of renal function were also assessed. Serum urea was assessed based on enzymatic procedure for the determination of urea using the coupled urease/glutamate dehydrogenase (GLDH) enzyme system published by Talke and Schubert in 1995. Urea is hydrolyzed in presence of urease to produce ammonia and CO_2. The ammonia produced reacts with 2-oxoglutarate and NADH in presence of GLDH to yield glutamate and NAD.

$$\text{Urea} + H_2O + 2H^{2+} \xrightarrow{\text{Urea}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{2-Oxoglutarate and NADH} \xrightarrow{\text{GLDH}} H_2O + 2NAD^+ + \text{Glutamate}$$

Serum creatinine was determined based on Jaffe (1886) method (kinetic alkaline picrate) modified by Fabiny (1971). It is a kinetic procedure which does not require deproteinization of the sample and is formulated to reduce the interference of serum proteins. A precise volume of sample is introduced into a reaction flask containing an alkaline picrate solution. At an alkaline pH, creatinine in the sample reacts with picrate.

$$\text{Creatinine} + \text{Alkaline picrate} \longrightarrow \text{deep yellow colored complex}$$

The rate of increase in absorbance taken at 500 nm due to the formation of this complex is directly proportional to the concentration of creatinine in the sample.

Serum electrolytes such as Sodium, Potassium, Chloride and Bicarbonate were assayed.

Serum Sodium (Na^+): Concentration of sodium in serum was assayed using the method described by trinder (1951) and Maruna (1958). The reaction is based on the ability of sodium to react with specific chromogens to produce an increase absorbance with the increase in concentration of sodium ions in the sample material. In this method, sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocynaide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Serum Potassium (K^+): Serum potassium was determined suing photometric measurement of potassium ions in a protein-free filtrate which was combined with sodium tetraphenylboron as described by Sunderman (1958). In this method, the amount of potassium is determined using sodium tetraphenylboronin a specially prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range 2-7 mEq/L.

Serum chloride (Cl): Concentration of chloride in serum was determined using colorimetric method described by Zall *et al.* (1956). The chloride ion displaces thiocyanate from non-ionized mercuric thiocyanate to form mercuric chloride and thiocyanate ions. The released thiocyanate ions react with ferric ions to form a red colored complex.

$$Hg(SCN)_2 + 2Cl \longrightarrow HgCl_2 + 2SCN^-$$

$$3SCN^- + 2Fe^{3+} \longrightarrow Fe(SCN)_3 \text{ (red complex)}$$

The colored complex formed absorbs light at 480 nm. The intensity of the color produced is directly proportional to the chloride concentration.

Serum bicarbonate ($HCO)_3$: This assay utilizes the enzymatic method developed by Forrester *et*

al. (1976). In this method, bicarbonate (HCO_3^-) and phosphoenolpyruvate (PEP) are converted to oxaloacetate and phosphate in the reaction catalyzed by phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MDH) catalyzed the reduction of oxaloacetate to malate with the concomitant oxidation of reduced nicotinamide adenine dinucleotide (NADH) (Equation 18).

$$PEP + hco_3^- \xrightarrow[\text{Magnesium}]{\text{PEPC}} \text{Oxaloacetate} + H_2PO_4^-$$

$$\text{Oxaloacetate} + NADH^+H^+ \xrightarrow{\text{MDH}} \text{Malate} + NAD^+$$

This oxidation of NADH results in a decrease in absorbance of the reaction mixture measured at 405 or 415 nm, which is proportional to the concentration of bicarbonate in the sample being assayed.

2.6 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 $p<0.05$.

2.7 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.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Assay of Hepatic Enzymes and Concentration of Serum Activities

Table 1 shows the Mean ± SD of activities of Alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and concentration of albumin (ALB), total protein (TP) and total bilirubin (T-Bil) of male albino Wistar rats treated with first and second line FDC antiretroviral drugs. From the result, it was observed that rats administered with first line regimen differed significantly ($p<0.05$) with high activity of serum ALT observed at 15 days (Group B_1) and 30 days (Groups B_2) respectively.

Then switched to second fixed dose, when compared with control (Group A). Animals in Group A showed time-dependent increase in ALT activity when compared with Group B_1. Serum ALT activity of rats treated with second line regimen for 15 days (Group C_1) and 30 days (Group C_2) did not differ significantly ($p<0.05$) in serum ALT in Groups $D_1$and D_2 (rats treated with first line for 30 days then switched to second line for 15 and 30 days respectively) when compared with rats in control group.

No significant difference ($p>0.05$) in AST activity was observed in rats treated with first line regimen for 15 days (Group B_1), but high activity of Serum AST was observed with significant increase ($P<0.05$) in rats treated with first line regimen for 30 days (Group B_2).

Then switched to second fixed dose line regimen for 45 days (Group D_1 - 45 days) when compared with control. There was also a significant ($P<0.05$) increase in serum AST in Groups B_2, C_1, C_2, and D_1 when compared with Group B_1. However, significant reduction ($P<0.05$) in serum AST was observed in rats treated with first line

3.1.2 Assay of Renal Enzymes Concentration and Serum Activities

Table 2 shows the mean ±SD of concentration of urea (U), creatinine (Cr), Sodium (Na^+), Potassium (K^+) Chlorine (Cl^-) and Bicarbonate (HCO_3^-) of male albino wistar rats treated with first and second line FDC antiretroviral drugs. The result showed significant increase ($p<0.05$) in serum urea in rats treated with first line regimen for 15 days (Group B_1) compared with control, but no significant change was observed in rats treated with same regimen for 30 days (Group B_2) when compared with the control. Equally, significant increase ($P<0.05$) in serum urea was also observed in rats treated with second line regimen for 15 days (Group C_1) compared with the control Groups C_2, D_1 and D_2showed significant difference ($p<0.05$) in serum Sodium (Na^+) in Group B_1 compared with Group B_2.

Table 1. Effect of treatment with first and second line FDC antiretroviral drugs (17.14/kgbwt/24hof EFV/3TC/TDF and 6.43 mg/kgbwt/12h of 3TC/ZDV + 3.57mg/kgbwt/12h of LPV/r) on hepatic function parameter in male albino Wistar rats

GROUPS (n=5)	ALT (UL)	AST (UL)	ALT/AST	ALP (UL)	ALB (g/L)	TP (g/dl)	T-BIL (umol/L)
A (Control)	48.83 ± 1.88	127.80 ± 10.07	2.84 ± 0.39	22.00 ± 1.15	28.05 ± 1.40	7.99 ± 0.28	3.63 ± 0.31
B_1 (1st line) 15days	59.05 ± 3.66[a]	95.37 ± 3.74	2.37 ± 0.50	72.50 ± 7.79	28.13 ± 0.83	8.63 ±0.11	12.05 ± 0.99
B_2(2nd line) 30 days	63.90±6.98[ab]	139.37 ± 8.92[b]	3.05 ± 0.53	158.50 ± 8.66	27.90 ± 2.12	8.09 ± 0.37	12.87± 1.62[ac]
C_1(2nd line) 15days	45.08 ± 3.55[c]	148.50 ± 5.53 [b]	3.59 ±0.80	178.50 ± 6.45	27.55 ± 0.99	7.84 ± 0.21	8.67 ± 0.97[ab]
C_2(2nd line)30 days	51.87 ±2.90[d]	154.85 ± 18.05[b]	4.15 ± 0.44	166.75 ± 7.49	25.25 ± 0.06	7.06 ± 0.45[abd]	11.30 ± 1.23[a]
D_1 (B_2 to c_1) 45 days	53.83 ± 2.80	192.75 ± 11.90[abcde]	3.79 ± 0.85	153.67 ± 11.05	23.78±0.84[abcd]	8.24 ± 0.39[e]	[7.23] ± 0.75[abcde]
D_2(B_2 to c_2) 60 days	51.90 ± 1.98	137.73 ± 6.60[bf]	3.55 ± 1.17	127.25 ± 5.56	26.15 ± 0.84	7.65 ±0.23	4.88±0.98[bcde]

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

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

Legends: ATL – Alanine amino transaminase; AST – Aspartate amino transaminase; ALP – Alkaline Phosphates; TP-Total Protein; ALB- Albumin; T-BILL – Total Bilirubin.[a] = significantly different when compared to Group A (p<0.05);[b] = significantly different when compared to GroupB_1 (p<0.05); [c] = significantly different when compared to 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.

Table 2. Effect of treatment with first and second line FDC antiretroviral drugs (17.14/kgbwt/24h of EFV/3TC/TDF and 6.43mg/kgbwt/12h of 3TC/ZDV + 3.57mg/kgbwt/12h of LPV/r) on renal function indices in male albino Wistar rats

GROUPS (n=5)	UREA (U) (mmol/l)	CREATININE (Cr) (umol/l)	HCO_3^- (mmol/l	Na^+ (mmol/l)	K^+ (mmol/l)	Cr (mmol/l
A (Control)	0.86 ± 0.29	82.88 ± 4.21	27.97 ± 0.69	111.91 ± 2.04	9.42 ± 0.24	73.66 ± 1.40
B_1 (1st line) 15days	2.31 ± 0.50[a]	101.14 ± 5.62	29.10 ± 0.35	110.98 ± 4.06	10.37 ± 0.99	76.56 ± 3.57
B_2 (2nd line) 30 says	1.81 ± 0.58	95.52 ± 2.29	30.03 ± 0.39[a]	132.69 ± 3.42[ab]	10.48 ± 1.38	78.46 ± 3.22
C_1 (2nd line) 15days	2.00 ± 0.23[a]	98.33 ± 6.69	28.78 ± 0.77	132.55 ± 6.42	7.41 ± 0.69[c]	80.68 ± 4.82
C_2 (2nd line) 30 days	1.14 ± 0.17[b]	82.88 ± 9.56	28.94 ± 1.01	135.00 ± 3.02[ab]	8.03 ± 0.41[d]	77.70 ± 5.59
D_1 (B_2 to c_1) 45 days	0.56 ± 0.13[bcd]	101.15 ± 9.73	29.60 ± 0.10	132.58 ± 3.98[ab]	7.43 ± 1.01[bd]	90.45 ± 5.87[abe]
D_2(B_2 to c_2) 60 days	0.44 ± 021[bcd]	98.33 ± 11.81	30.86±0.33[abce]	122.45 ± 3.37[c]	7.81 ± 0.12[bd]	88.76 ± 2.67[a]

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

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

Legends: CREAT – Creatinine; Na^+ - Sodium; K^+- Potassium; CL^- - Chloride; HCO_3^- - Bicarbonate. [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 C^2 (p<0.05);n = number of animals per group.

There was no significant difference ($p<0.05$) in serum creatinine in all the treated groups compared with the control. Also, there was no significant different ($p<0.05$) is serum Sodium (Na^+) in Group B_1 compared with the control, but significant increase ($p<0.05$) was observed in when compared with rats in control group and Group B_1. However, Na^+ level in Group D_2 differed significantly ($P<0.05$) compared with Group D_1. No significant change ($p>0.05$) was observed in serum potassium (K^-) level in Groups B_1 and B_2 compared with control, but significant decrease in this electrolyte was observed in Groups, D_1 and D_2 when compared with Group B_1 and B_2 respectively. Serum Chlorine (Cl^-) level was not significant ($p>0.05$) in Group B_1, B_2, C_1 and C_2 compared with Group A(control), but a significant increase ($p<0.05$) was recorded in Groups D_1 and D_2 when compared with Group A. significant increase ($p<0.05$) in serum bicarbonate (HCO_3^-) level was observed in Groups B_2 and D_2 compared with Group A, 27.97± 0.60, but HCO_3^- levels in other treated groups were not significant ($p>0.05$) when compared with the control group.

3.2 Discussion

"The liver is a critical organ and remains the main metabolic hub of the human body. It performs an array of functions that help support metabolism, immunity, digestion, vitamin, storage, detoxification, biotransformation of drugs among other functions" (Almazroo et al., 2017). "The liver is intertwined with nearly every system in the body; hence, it is prone to a variety of pathologies" (Si-Tayeb et al., 2010)**. "**Several enzymes that trigger important chemical reactions in the body are produced and found within the cells of the liver, however, damage or injury to the liver, can cause elevations in the liver enzyme activities" (Ozer et al., 2008). Studies have shown that in patients commencing fixed-dose combination (FDC) antiretroviral drugs, fourteen to twenty percent (14-20%) will experience elevations in hepatic enzymes (Chu et al., 2010; Lucien et al., 2010; Teklay et al., 2013) indicating hepatic injury.

"Several studies have associated increase serum activities of transaminases with ARV drug-induced liver injury. Efavirenz (EFV), in isolation has been linked independently with hepatotoxicity in both human and animal studies" (Elias et al., 2015; Kayode et al., 2011; Mugusi et al., 2012; Peter and Edagha, 2016). Increased serum ALT and AST levels in animals treated with EFV are reported in many cases (Edelman et al., 2012). Other cases have reported hepatotoxicity associated with FDC antiretroviral drugs with hepatotoxicity attributed to EFV component of the agent (Dufor et al., 2000a; Echeniqu and Rich, 2013). Although the mechanisms involved are not clearly stated, EFV associated hepatotoxicity has been attributed to the actions of EFV and its synthetic 8-hydroxy efavirenz (8-OHEFV) metabolite (Bumpus, 2011); also recent evidence has pointed to a specific mitochondrial action of EFV accompanied by the induction of n endoplasmic reticulum (ER) stress/unfolded protein response in human hepatocytes (Polo et al., 2015). In a case report documented by Patil *et al*. (2015), EFV component of fixed-dose FTC/TDF/EFV was implicated in hepatotoxicity, with remarkably elevated transaminases.

"In line with these documented evidence, the elevated activity of serum ALT observed in the present study is likely due to the effect of EFV component of TDF/3TC/EFV (first line anti-HIV regimen) on the liver cells. Serum ALT activity is the most accurate biomarker of hepatotoxicity since by virtue of its location in the liver plays a vital role in amino acid metabolism and gluconeogenesis" (Dufor et al., 2000a). "ALT being hepato-specific (principally found in the cytosol of hepatocytes), its estimation is more specific for liver abnormalities" (Nathwani et al., 2005).

Equally, zidovudine (ZDV), a component of second line regimen used in this study is implicated in cases of hepatic injury in advanced AIDS patients. In an earlier study by Freidman *et al.,* (1993), it was reported that patients treated with ZDV developed massive hepatomegaly and micro vesicular steatosis which progressed to fulminant hepatic failure. ZDV-induced hepatic damaged was also observed among HIV positive patients on ARV drugs administered as a monotherapy (Pandit et al., 2012). "ZDV caused acute hepatitis due to hypersensitivity, which resolved approximately few days after the therapy was replaced with didanosine and zalctitabine without recurrence of toxicity" (Wassef and Kelser, 1992). Recently, a similar case was reported by Subodh *et al*. (2018) in which adverse effect was reversed on replacement of FDC antiretroviral drug containing ZVD/3TC/NPV with TDF/3TC/EFV. According to the report, the adverse effect was attributed to the ZDV component of the drug. High activity of serum AST was observed in Group D_1 (rats

treated with first line regimen for 30 days then switched to second line regimen for 15 days). This could be (by inference) due to combine actions of EFV and ZDV from both lines of antiretroviral regimens administered to animal in this group.

"AST is found in the liver and other organs including heart, muscle, brain and kidney. Thus, injury to any of these tissues can cause an elevated activity of this enzyme" (Dufor et al., 2000a). "When damage to heart or liver cells occurs, AST being intracellular enzyme is released into the peripheral blood. Though it is considered a less specific biomarker enzyme for hepatocellular injury, its significant activity in the serum could be a sign for hepatocellular necrosis" (Ozer et al., 2008). "However, the ratio of serum AST to ALT can be used to differentiate liver damage from other organs damage" (Nathwani et al., 2005). "Thus, the significant increase in activities of transaminases observed in this study may be an evidence of hepatotoxicity which may be as a result of leakage from the cells through peroxidative damage of membranes and loss of functional integrity of cellular membrane in liver" (Iniaghe et al., 2008)

Conversely, activity of serum ALP observed in this study was statistically insignificant ($p<0.05$) with low activity in all the treated groups compared to the control. Though some studies have implicated high activity of serum ALP along with ALT and AST in cases of hepatic dysfunction associated with antiretroviral (ARV) drugs, the present findings is in consonant with the report documented by Oladipo *et al*. (2016), where serum ALP activity was significantly reduced in male albino Wistar rats treated with ARV drug. While the activities of transaminases were high.

Also, Abubakar *et al.* (2014), reported that "AST and ALT activities of HIV positive patients treated with antiretroviral drugs were significantly higher ($p<0.05$) compared with non-treated group, while ALP activity was significantly lower ($p<0.05$) in the same group compared with non-treated group". Similarly, Goni *et al.* (2013) observed that "there was no statistically significant difference between the mean serum activity of ALP enzyme among the study group of HIV/HBV co-infected and HIV mono-infected patients on ARV drugs compared with ALT and AST activities which were significantly high and moderate respectively". Furthermore Patil *et al.* (2015) observed that the activities of transaminases (ALT and AST) were remarkably high while ALP activity was significant, and the patient did not develop signs of hepatic encephalopathy. In earlier study carried out by Cello (1989), unexplained high activity of ALP was observed while the transaminases activities remained insignificant in an ARV drug naïve HIV-positive patient. This raised the suspicion of a co-existing HIV infection, cholecystitis, which was hitherto undetected in the subjects with AIDS. By these findings, ALP was noted as a fruitful maker for identifying more underlying opportunistic infections in HIV-positive patients. According to Burke (2002), high activities of ALT and AST with lower or normal ALP favors cell necrosis whereas the reverse points to cholestasis. Also, Travlos *et al.* (1996) in a research on ALP enzymes in a rat model stated that the major circulating form of ALP in rats is the intestinal rather than the liver isoenzyme, and though total serum ALP activity is commonly measured, it is generally minimally increased in association with intrahepatic cholestasis in rat models.

Serum albumin observed in this study differed insignificantly ($p<0.05$) in all the treated groups compared to the control. However, serum total protein was statistically significant ($p<0.05$) with low concentration observed in Group C_2 (rats treated with second line regimen for 30 days) when compared with control. In line with this, many studies including Umar *et al*. (2008) had reported lowered level of albumin and total protein in rats treated with ARV drugs. Albumin is a protein primarily synthesized by the hepatic parenchymal cells. Its primary function is to maintain colloidal osmotic pressure in the vascular and extravascular areas of the body and prevent oedema. Hypoalbuminemia occurs in gastrointestinal (GI) malabsorption, glomerulonephritis, nephritic syndrome, cirrhosis, severe burns, neoplasms, and autoimmune diseases. Additionally, albumin is a carrier transport protein. Serum albumin depends on an adequate supply of amino for its synthesis. Thus, low level of serum protein slows mRNA synthesis of albumin and results in lower or insignificant serum levels of albumin (Friedman and Fedem, 2010). Protein is primarily synthesized in the liver and consists mainly of albumin and few globulins. Changes in total protein levels are due mostly to changes in albumin concentration (O'Connell et al., 2005). Albumin and total protein concentration are the markers of biosynthetic capacity of the liver (Dey et al., 2013). Thus, defect in synthesis of albumin and

total protein generally reflects defective metabolic capacity of the liver. Therefore, the observed reduction in albumin and total protein levels in this study may suggest hepatic dysfunction and suppression of the synthesis function of the liver (Ogunro et al., 2005) with regards to these parameters.

"On the contrary, there was a significant increase ($p<0.05$) in serum total bilirubin in all the treated groups when compared with the control. This is agreement with other studies which have associated drugs from NNRTLs class (EFV and NPV) with elevated levels of total bilirubin" (Umar et al., 2008;Umoren et al., 2014; Fevery, 2008). "Bilirubin is a tetrapyrole produced during the normal breakdown of haemoglobin" (Doumas and Wu, 1991). "In the liver, uridine diphosphate (UDP)-glucuronyl transferase converts bilirubin, to a mixture of monoglucuronides and diglucuronides referred to as conjugated bilirubin, which is then secreted into the bile by an ATP-dependent transporter" (Dey et al., 2013; Muchowski, 2014). "The bile in turn serves as a means for excretion of bilirubin from the body; thus, bilirubin level serves as a measure of liver and bile tract function. Total bilirubin includes both the conjugated and unconjugated (free) forms of bilirubin and, if elevated, is usually indicative of liver damage or hemolysis" (Fevery, 2008). "Accumulation of bilirubin or its conjugates in body tissues results in a disease condition called jaundice, which is characterized by high plasma bilirubin levels and deposition of yellow bilirubin pigments in skin, sclera mucous membranes, and other less visible tissues" (Wolkoff and Berk, 2012). Therefore, impaired metabolism of bilirubin indicates compromised excretory capacity of the liver (Muchowski, 2014); hence, the significant increase in levels of total bilirubin seen in this study.

"The kidneys play a vital role in the excretion of waste products and toxic such as urea, creatinine and uric acid, regulation of extracellular fluid volume, serum osmolality ad electrolyte balance" (Damiatati, 2019). "It controls the composition and volume of the body fluids. The regulatory function of the kidneys maintains the stable environment of the cells necessary for them to perform the various activities. The kidneys also function by filtering the plasma and removing substances from the filtrate at variable rates according to the needs of the body" (Roling et al., 2006). Earlier findings had reported that electrolytes deficiencies are prevalent in both treated and HIV-naïve infected populations (Cheesbrough, 2005) and have been associated with accelerated replication of the virus, depletion of $CD4^+$ T-Cell, impaired immune responses, (Damiatati, 2019) frequent opportunistic infections, renal impairment/dysfunction and a greater incidence of HIV- related mortality (Onodugo et al., 2014).

"Use of ARV drugs, especially from NRTIs and PIS have been associated with various kidney syndromes including various electrolyte and acid-base disorders, acute kidney injury (AKI), lactic acidosis, and chronic kidney disease (CKD). These injuries occur via multiple mechanisms, including direct tubular toxicity, allergic reactions, and precipitation of insoluble drug crystals within renal tubular lumens" (Perazalla, 2010).

"Treatment with TDF containing regimen has been implicated in kidney dysfunction and serum electrolytes imbalance in both human and animal studies. Reports from animal studies show that TDF causes mitochondrial DNA (mtDNA) depletion and mitochondrial toxicity" (Adikwu et al., 2014). "Renal proximal tubules from HIV positive transgenic mice exposed to TDF showed ultrastructural mitochondrial abnormalities (irregular shapes with sparse, fragmented cristae) and decreased mtDNA levels, which paralled the ultrastructural mitochondrial abnormalities" (Herlitz et al., 2010).

"An interaction between lopinavir-ritonavir (LPV/r) combinations therapy and TDF, which manifests as a decrease in the renal clearance of TDF, has been identified" (Kiser et al., 2008). "TDF is actively taken up into the proximal tubules and secreted into the lumen via multidrug resistance-associated protein-4, MRP4. Inhibition of MRP4 by LPV/r leads to increased intracellular TDF levels that may increase its nephrotoxicity effects" (Imoka et al., 2007). Roling, (2006) has also reported involvement of 3TC and didanosine as monotherapies with tubular dysfunction.

In this study, significant increase in serum urea were observed in animals treated for 15 days with first line and second line regimens (Groups B1 and C1) respectively, whereas creatinine level differed insignificantly in all the treated groups. This is in line with studies conducted by Kateregga *et al.* (2018) and other documented cases earlier stated. Serum levels of urea and creatinine are essential clininal endpoints for the assessment of renal function (Kumar et al., 2005). "Urea is the major nitrogen-containing metabolic product of protein metabolism. It is the

primary vehicle for removing toxic ammonia from the body. Urea is synthesized in the liver from the ammonia produced from the catabolism of amino acids via the hepatic urea cycle. The conversion from ammonia to urea is regulated by N-acetylglutamate (NAG), which activates carbanoyl phosphate synthetase in the urea cycle. Urea is transported in the blood to the kidneys where it is excreted in the urine. In addition to its role as a carrier of waste nitrogen, urea also has a role in the countercurrent exchange system of the nephrons in which water and ions are reabsorbed from excreted urine" (Cheesbrough, 2005). "It is freely filtered by the glomeruli and partially passively resorbed as filtrate transverses the renal tubules. Urea reabsorption is inversely proportional to urine flow rate. Consequently, urea concentration depends on protein intake, protein catabolism, and kidney function" (Kohler et al., 2011). Therefore, elevated serum levels of urea could be correlated with impairment in renal function (Ganong, 2009) due to mitochondrial toxicity associated with TDF since the rate determining step in urea cycle takes place in the mitochondria.

Creatinine is synthesized primarily in the liver from the methylation of glycoyamine and is removed from the blood, chiefly by the kidneys, through glomerular filtration. According to Cheesbrough (2005) "creatinine is a better indicator of over-all renal function (Glomerular filtration rate, GFR) since its levels are less affected than urea levels by age, diet and dehydration. Thus, insignificant level of serum creatinine observed in the current study implies less impaired renal function".

Serum sodium, chlorine and bicarbonate though significant increased, were observed in the present study not to exceed the normal reference range. Ramamoorthy *et al.* (2012) had treated rats with monotherapy of TDF for 35 days and reported that TDF was associated with metabolic acidosis that occurs due to defective bicarbonate reabsorption in the proximal tubule. Other reported cases of TDF-induced renal toxicity have been characterized by serum electrolytes imbalance (Patel et al., 2010; Tourret et al., 2013).

4. CONCLUSION

FDC antiretroviral drugs containing TDF/EFV/3TC and 3TC/ZDV + LPV/r were used in this study. These combinations are standard antiretroviral drugs recommended by WHO as preferred first and second line regimens respectively for management and treatment of HIV/AIDS. Findings from this study have revealed that the regimens exhibit toxic tendency on hepatic tissues of the tested animals. However, the regimens showed no deleterious effect on renal function of these animals. This study has also demonstrated that switching from first to second line regimen did not expose the animals to untoward consequences of severe drug effect, but rather there was a weight gain which may rule out toxic effects of the regimens on long term repeated dosage. Thus, the use of these regimens in the management and treatment of HIV/AIDS should be encouraged while hepatic functions of the recipients should be monitored.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICS APPROVAL

Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

HUMAN AND ANIMAL RIGHTS

The care and use of animals in this study was in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH, 1996).

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Abubakar, M. G., Abduljalil, M. M. & Nasiru, Y. I. (2014). Changes in Liver Function

Enzymes of HIV/AIDS Patients Treated with Antiretroviral Drugs (ARVS) in Specialist Hospital, Sokoto, Nigeria. *Nigerian Journal of Basic and Applied Science,* 22(3/4): 85-89.

Adikwu, E., Deo, O., Zidafamor J. & Obele. R. (2014). Effect of co-administered lopinavir/ritonavir and sulfamethoxazole/ trimethoprim on cardiac function and architecture of albino rats. *International Journal of Basic and Clinical Pharmacology,* 3(5): 817-823.

Almazroo, O. A., Miah, M. K. & Venkataramanan, R. (2017). Drug metabolism in the liver. *Clinical Lilver Disease, 21*(1):1-20

Atta, M. G., Deray. G. & Lucas, G. M. (2008). Antiretroviral nephrotoxicities. *Seminars in Nephrologoy,* 28(6): 563-575.

Bowers, G. N. & McComb, R. B. (1966). A continous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Journal of Clinal Chemistry*, 12(2): 70-89.

Bruno, R., Sacchi, P., Maiocchi, L., Patruno, S. & Filice, G. (2006). Hepatotoxicity and antiretroviral therapy with protease inhibitors: A review. *Journal of Digestive and Liver Disease,* 38(6): 363-373.

Bumpus, N. N. (2011). Efavirenz and 8-hydroxyefavirenz induce cell death via a JNK- and BimEl- dependent mechanism in primary human hepatocytes. *Toxicology and Applied Pharmacology,* 257(2): 227-234.

Burke, M. D. (2002). Liver function. *Human Pathology,* 6: 237-286.

Cello, J. P. (1989) Acquired immune-deficiency syndrome cholangiopathy: spectrum disease. *American Journal of Medicine,* 86: 539-546.

Cheesbrough, M. (2005). *District laboratory practice in tropical countries part.* (2nd ed.). New York, USA: Cambridge University Press.

Chu, K. M., Boulle, A. M., Ford, N., Goemaere, E., Asselman, V. & Van, C. G. (2010). Nevirapine-associated early hepatotoxicity: incidence, risk factors, and associated mortality in a primary care ART programme in South Africa. *PloS one,* 5(2*)*: 9183-9190.

Damiatati, S. (2019). A pilot study to assess kidney functions and toxic dimethyl-arginines as risk biomarkers in women with low vitamin D levels. *Journal Medical Biochemistry,* 38(2): 145-152.

Dey, S. K., Indranath, G., Debojyoti B., Praveen, A., Sumanta, J., Anindya, D. & Sukanta K. D. (2013). Liver function profile anomalies in HIV seropositive tuberculosis. *Journal of Clinical and Diagnostic Research,* 7(6): 1068-1072.

Douek, D. C., Roederer, M. & Koup, R. A. (2009). Emerging concepts in the immunopathogenesis of Aids. *Annual Review of Medicine,* 60: 471-484.

Doumas, B T. & Wu, T. W. (1991). The measurement of bilirubin fractions in serum. *Critical Reviews in Clinical Laboratory Sciences,* 28(5-6): 415-445.

Doumas, B. T., Watson, W. A. & Biggas, H. G. (1971). Albumin standard and the measurement of serum albumin with bromocresol green. *International journal of Clinical Chemistry,* 31: 87-96.

Dufor, D. R., Lott, J. A., Nolte, F. S., Gretch, D. R. & Koff, R. S. (2000a). Diagnosis and monitoring of hepatic injury: II. Recommendations for use of laboratory tests in screening, diagnosis and monitoring. *Clinical Chemistry,* 46(12): 2027-2049.

Echeniqu, I. A. & Rich, J. D. (2013). EFV/FTC/TDF-associated hepatotoxicity: A case report and review, *AIDS Patient Care and STDs,* 27(9): 493-497.

Edelman, E. J., Gordon, K. & Rodriguez-Barradas, M. C. (2012). Patients-reported symptoms on the antiretroviral regimen efavirenz/emtricitabine/tenofovir," *AIDS Patient Care and STD.* 26(6): 312-319.

Elias, A., Nelson, B., Igbans, R. O. & Onyedenyifa, O. J (2015). Hepatotoxic Assessment of co-treatment with isoniazid and efavirenz in albino rats. *Advances in Pharmacology and Pharmacy*, 3(4): 75-81.

Fabiny, D. L. Rambant, A., Suchard, M. A., Baele, G., Bedfored, T., Ward, M. J., Tatem, A. J., Sousa, J. D., Arinaminpathy, N., Pepin, J., Posada, D., Peters, M., Pybus, O. & Lemey P.(2014). HIV epidemiology: The early spread and epidemic ignition of HIV in human populations. *Science,* 346: 56-61.

Faria, N. R., Rambant, A., Suchard, M. A., Baele, G., Bedford, T., Ward, M. J., Tatem, A. J., Sousa, J. D., Arinaminpathy, N., Pepin, J., Posada, D., Peters, M., Pybus, O. & Lemey P. (2014). HIV epidemiology: The early spread and epidemic ignition of HIV in human populations. *Science*, 346: 56-61.

Fevery, J. (2008). Bilirubin in clinical practice: A review. Liver international: *Official Journal of the International Association for the study of the Liver,* 28(5): 592-605.

Forrester, J. S., Wataji, L. G., Silverman, D. A. & Pierre, K. J. (1976). Enzymatic method for determination of CO_2 in serum. *Journal of Clinical Chemistry,* 22(2): 243-261.

Freidman, J., Helfert, K., Harmrell, M. & Stein, D. (1993). Hepatomegaly with sever steatosis in HIV-seropositive patients. *AIDS*, 7: 379-385.

Friedman, A. & Fedem, S. (2010). Reassessment of albumin as a nutritional marker in kidney disease. *Journal of the American Society of Nephrology,* 21: 223-230.

Ganong, W. F. (2009). *Review of medical physiology.* (23rd ed). New York: McGraw Hill.

Goni, B. W., Yusuph, H., Mustapha, S. K., Sahabi, M. A., Gwalabe, S. A., Tahir, A., Bakki, B. & Kida, I. M. (2013). Hepatic transaminase and alkaline phosphatase enzyme levels in HIV/HBV co-infected and HIV mono-infected patients in Maiduguri, Nigeria. *Nigerian Journal of Clinical Practice,* 16: 530-534.

Guha, P. & Sardar, P. (2011). Prevalence of paediatric HIV infection in Eastern India-first report. *Journal of AIDS and Clinical Research,* 2: 127-135.

Herlitz, L. C., Mohan, S., Stokes, M. B., Radhakrishnan, J., D'Agati, V. D. & Markowitz, G. S. (2010). Tenofovir nephrotoxicity: Acute tubular necrosis with distinctive clinical, pathological, and mitochondrial abnormalities. *Kidney International,* 78: 1171-1177.

Imoka, T., Kusuhara, H., Adachi, M., Schuertz, J. D., Takeuchi, K. & Sugiyama, Y. (2007). Functional involvement of multidrug resistance-associated protein 4 (MRP4/ABCC4) in the renal eliminating of the antiviral drugs adefoviral and tenofovir. *Molecular Pharmacology,* 71(2): 619-627.

Iniaghe, O. M., Malomo, S. O. & Adebayo, J. O. (2008). Hepatroprotective effect of the aqueous extract of leaves of *Acalypha racemosa* in carbon tetrachloride treated rats. *International Research Journal of Plant Science,* 2: 301-305.

International Federation of Clinical Chemistry, IFCC (1986). Method for the measurement of catalytic concentration of enzymes. *Journal of Clinical Chemistry and Biochemistry,* 24: 498-507.

Jendrassik, L. & Grof, P. (1938). Estimation of total serum bilirubin level by Spectrophotometrically in serum and plasma. *Biochemistry Journal,* 297: 82-89.

Kaspar, D. L., Brawald, E., Farci, A. S., Hauser, S. L., Longo, D. L., Loscalzo, J. & Jameson, J. L. (2017). *Harrison's principles of internal medicine.* (19th ed.). New York: McGraw-Hill, 2770Pp.

Kateregga, J. N., Atuheire, C., Nalunga, J. & James, N. (2018). Effect of antiretroviral drugs on the renal function of HIV seropositive patients attending ISS clinic of Mulago Hospital, Uganda. *Annals of Biotechnology,* 4: 1016-1028.

Kayode, A. A., Kayode, O. T., Aroyeun, O. A. & Stephen, M. C. (2011). Haematologic and hepatic enzymes alterations associated with acute administration of antiretroviral drugs. *Journal of Pharmacology and Toxicology,* 6: 293-302.

Kharsany, A. B. M. & Karim, Q. A. (2017). HIV Infection and AIDS in sub-saharan African: Current status, challenges and opportunities. *Open AIDS Journal,* 10: 34-48.

Kiser, J. J. Carten, M. L. & Aquilante, C. L. (2008). The effect of lopinavir/ritonavir on the renal clearance of tenorfovir in HIV-infected patients. *Journal of Clinical Pharmacology and Therapeutics,* 83(2): 265-272.

Kohler, J. J., Hosseni, S. H. & Green, E. (2011). Tenofovir renal proximal tubular toxicity is regulated by OATI and MRP4 transporters. *Laboratory Investigation,* 91: 852-859.

Kumar, R. S., Sivakumar, T., Sunderan, R. S., Gupta, M., Mazumdar, U. K. & Gomathi, P. (2005). Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. Stem bark. *Brazilian Journal of Medical and Biological Research,* 38: 1015-1024.

Lucien, K. F. H., Clement, A. N. J., Fon, N. P., Weledji, P. & Ndikvu, C. P. (2010). The effects of antiretroviral treatment on liver function enzymes among HIV-infected out patients attending the central hospital of Yaounde, Cameroon. *African Journal of Clinical and Experimental Microbiology,* 11(3): 174-178.

Lundgren, J. D., Babiker, A G., Gordin, F., Emery, S. & Grund, B. (2015). Initiation of antiretroviral therapy in early asymptomatic HIV infection. New England Journal of Medicine, 373: 795-807.

Muchowski, K. E. (2014). Evaluation and treatment of neonatal hyperbilirubinemia.

American Family Physician, 89(11): 873-878.

Mugusi, S., Ngaimisi, E., Janabi, M., Minzi, O., Bakari, M. & Riedel, K. D. (2012). Liver enzyme abnormalities and associated risk factors in HIV patients on efavirenz-based HAART with or without tuberculosis co-infection in Tanzania. *Plos One,* 7(7): 40180-40187.

Nathwani, R. A., Pais, S., Reynolds, T. B. & Kaplowitz, N. (2005). Serum alanine aminotransferase in skeletal muscle diseases. *Hepatology*, 41: 380-382.

O'Connell, T., Horita, T. & Kasravi, B. (2005). Understanding and interpreting serum protein electrophoresis. *American Family Physician,* 71(1): 105-112.

Ogunro, P. S., Oparinde, D. P. & Okesina, A. B. (2005). Liver function tests in HIV-I infected asymptomatic patients and HIV-I AIDS patients without hepatomegaly in Lagos, Nigeria. *African Journal of Clinical and Experimental Microbiology,* 6(1): 40-45.

Oladipo, E. K., Afolabi, A. Y., Omomowo, I. O., Oloke, J. K. & Awoyelu, E. H. (2016). Biochemical markers of the liver of male wistar rats on oral administration of nevirapine suspension. *African Journal of Clinical and Experimental Microbiology,* 17(1): 53-61.

Onodugo, O. D., Chukwuka, C., Onyedum, C., Ejim, E., Mbah, A., Nkwo, P., Ugwu, G., Ukoma, U., Okafor, C., Anyene, C., Nwabueze, E., Obi, F., Akabueze, J. Uche, I., Ekochin, F. & Nnoruka, E. (2014). Baseline in South East Nigeria. *Journal of International Association of Providers of AIDS Care,* 13(5): 476-480.

Ozer, J., Ratner, M., Shaw, M., Bailey, W. & Schomaker, S. (2008). The current state of serum biomarkers of hepato-toxicity. *Toxicology*, 245: 194-205.

Pandit, A., Sachdeva, T. & Bafna, P. (2012). Drug-induced hepatotoxicity: A review. *Journal of Applied Paharmacological Science,* 02(05): 233-243.

Patel, K., Patel, A. K., Rajan, R. R. & Patel, A. R. (2010). Tenofovir associated renal dysfunction in clinical practice: An observation cohort study. *Indian Journal of Sexual Transmitted Disease,* 31: 30-34.

Patil, R., Mel, A., Ona, P, H., Carey, J., Moshenyat Y., Alhaddad, A. & Sury, A. (2015). Acute Liver Toxicity due to Efavirenz/ Emtricitabine/ Tenofovir. *Case Reports in Hepatology,* 2-9.

Perazalla, M. A. (2010). Tenofovir-induced kidney disease: And acquired renal tubular mitochondriopathy. *Kidney International,* 78(11): 1060-1063.

Peter, A. I. & Edagha, I. A. (2016). Histoipatahological effect of co-administration of efavirenz and vitamin E on the liver, and biochemical parameter in wistar rats. *Asian Journal of Medical Sciences,* 7: 5-17.

Pinola, M., Lazzarin, A., Antinori, A., Carosi, G. & Di Perri, G. (2010). Lopinavir/ritonavir + tenofovir dual therapy versus lopinavir/ritonavir-based triple therapy in HIV-infected antiretroviral naïve subjects: The Kalead study. *Journal of Antiviral and Antiretroviral,* 2: 056-062.

Polo, M., Alegre, F. & Funes H. A. (2015). Mitochonddrial (dys) function - a factor underlying the variability of efavirenz-induced heptotoxicy?" *British Journal of Pharmacology*, 172: 1713-1727.

Ramamoorth, H., Issac B. & Abraham, P. (2012). Evidence for the roles of oxidative stress, nitrosative stress ad Nf-Kb activation in Tenofover Disoproxil Fumarate (TDF) induced renal damage in rats. *Journal of infectious Disease,* 12:1-6.

Roling, J., Schmid, H., Fischereder, M., Draenert, R. & Goebe, F. D. (2006). HIV-associated renal diseases and highly active antiretroviral therapy (HAART)-induced Nephropathy. *Clinical Infectious Disease,* 42: 1488-1495.

Samuels, R., Bayerri, C. R. & Sayer, J. A. (2017). Tenofovir disoprocxil fumarate-associated renal tubular dysfunction: Noninvasive assessment of mitochondrial injury. *AIDS,* 31: 1297-1306.

Si-Tayeb, K., Lemaigre, F. P. & Ducan, S. A. (2010). Organogenesis and development of the liver. *Development Cell,* 18(2): 175-89

Sunderman, F. W. (1958). Studies in serum electrolytes xxii: A rapid, reliable method for serum potassium using tetraphenylboron. *American Journal of Clinical Pathology,* 2:92-103.

Talke, H. & Schubert, G. E. (1995). Enzymatic determination of urea using the coupled urease/glutamate dehydrogenase (GLDH) enzyme system. *Mediators of Inflammation,* 43: 174-176.

Teklay, G., Legesse, B. & Legesse, M. (2013). Adverse effect and regimen switch among patients on antiretroviral treatment in a

resource limited setting in Ethiopia. *Journal of Pharmaco-Vigilance,* 1: 115-128.

Tourret, J., Deray, G. & Isnard-Bagnis, C. (2013). Tenofovir effect on the kidneys of HIV-infected patients: A double edged sword? *Journal of American Society of Nephrology,* 24: 1519-1527

Travlos, G. S., Morris, R. W., Elwell, M. R., Duke, A., Rosenblum, S. & Thompson, M. B. (1996). Frequency and relationships of clinical chemistry and liver and Kidney histopathology findings in 13week toxicity studies in rats. *Toxicology*, 107: 17-29.

Trinder, P. (1951). A rapid method for the determination of sodium in serum. *Analyst*, 76: 596- 599

Umar, R. A. Hassan, S. W., Ladan, M. J., Matazu, I. K., Shehu, B., Shehu, R, A., Muhammed, L. G. & Molabo, F. I. (2008). Adverse hepatic effects associated with administration of antiretroviral drugs (nevirapine, lamivudine and stavudine) to albino rats; Implication for management of patients with HIV/AIDS. *Asian Journal of Biochemical,* 3: 19-25.

Umoren, E. B., Obeme, A. O., Odo, M. O. & Osim, E. E. (2014). Effect of nevirapine administration of antiretroviral on biliary secretion/its biochemical composition in wistar rats. *Journal of Antivirals and Antiretroviral,* 6:045-049.

Wassef, M. & Kelser, P. (1992). Hypersensitivity to ziodovudine: Report of a case and review of the literature. *American Journal of Medicine,* 93: 94-96.

WHO/ UN AIDS Programme (2018). Fact sheet – Latest statistics on the status of AIDS epidemic. www. unaids. org. Archived from the original on July 13, 2017. @ htt://www.who.int/medacare/factsheets/fs360/en/ (Retrieved July 21, 2018).

Wolkoff, A. W. & Berk, P. D. (2012). Bilrubin metabolism and jaundice. In E. R. Schiff, W. C. Maddrey, and M. F. Sorrell (Eds). *Schipff's diseases of the liver II.* New Jersey, USA: Wiley Publisher, Hoboken, 120-151Pp.

World Health Organization (2015) *Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV.* Geneva: WHO.

World Health Organization (2018). New WHO recommendations: Antiretroviral therapy for adults and adolescents. Geneva: WHO.

Zall, D. M., Donald, F. & Garner, M. Q. (1956). Photometric determination of chloride in water. *Journal of Analytical Chemistry,* 28(11): 1665-1668.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/128888