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Acute and Chronic Toxicity Studies of Antiretroviral Regimens in Albino Rats

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Abstract

The acute and chronic oral toxicity studies of antiretroviral regimens were evaluated in albino rats. For chronic toxicity study, the antiretroviral drugs were administered singly and in combination (CMB). Lamivudine® (3TC), Zidovudine® (AZT) and Nevirapine® (NVP) at single doses of 5000 mg/kg each produced treatment related signs of toxicity in tested animals during the first few hours but returned to normal after 48 hours therefore the 48 hours LD_{50} of each drug was estimated to be more than 5000 mg/kg. Chronic toxicity showed an initial percentage increase in weight between 0-6 weeks and a corresponding decrease in weight from 6-13 weeks was observed. There was no significant ($P>0.05$) change in serum electrolyte levels in the treated groups when compared to the control group. Aspartate transaminase (AST) was significantly ($P<0.05$) increased in groups administered with 3000 mg/kg of CMB, 4000 mg/kg of CMB and at all administered doses of NVP.

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Similarly all administered doses of NVP caused a significant (P<0.05) increase in the level of 5' nucleotidase (5'NT). At administered doses of 2000 mg/kg and 4000 mg/kg body weight, NVP caused a significant (p<0.05) increase in total cholesterol (TC) upon comparison with the control. Administered doses of CMB and NVP, except at 1000 mg/kg body weight respectively, showed a significant (p<0.05) increase in the level of malondialdehyde (MDA) when compared with the control group. Catalase activity recorded a significant (P<0.05) decrease at doses of 3000 mg/kg and 4000 mg/kg body weight of CMB and NVP. Histopathological examination of the liver showed changes in the treated group when compared with the control group. These changes were corroborated by the non-invasive results. The above results indicate that NVP was the potent hepatotoxic agent, followed by CMB, AZT and 3TC. The hepatotoxicity was also dose dependent.

Keywords: Antiretroviral regimen; Antioxidants; Hepatotoxic; Histopathology; Lipid profile; 5'-nucleotidase

1. Introduction

Acquired Immune Deficiency syndrome (AIDS) has been defined as a disease of the human immune system caused by the human immunodeficiency virus (HIV). An individual can also be said to be infected with HIV if he has a CD4+ T-cell count below 200cells/µl [1,2].AIDS progressively reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic infections and tumors [3,4]. A clinical diagnosis of HIV may be made based on the presenting symptoms and signs but many of these are non-specific hence the need for accurate and specific techniques. There are two major assay methods for diagnostic testing of HIV infection. These testing methods are virological testing and serological testing [5,6].

The origin of HIV/AIDS has been a subject of controversy among scientists [7,8]. Numerous theories have been put forward regarding this subject. These theories can be separated into two major groups. The first group proposed that HIV/AIDS is man-made while the other proposed that the virus arose out of genetic modifications. The discovery of the causative agent of AIDS (HIV virus) in 1983 was credited to Luc Montagnier. The first documented case of AIDS occurred in the U.S.A. in the early 1980s [9,2].

AIDS as a pandemic has been spreading geographically [10]. NACA (National Agency for the Control of AIDS) estimates that 2.98 million people in Nigeria are currently infected. Estimates from the Joint United Nations Program on HIV/AIDS (UNAIDS) show a rise of 400,000 in the number of people living with HIV/AIDS in Nigeria between 2001 and 2008. The prevalence of HIV showed an increase from 1.9% in 1991 to 5.8% in 2001. There was a decline to 5.0% in 2003 and further to 4.4% in 2005 before rising to 4.6% in 2008. All the 36 states and F.C.T have HIV prevalence above 1%. In the North-Western region, Sokoto, Zamfara and Kebbi States have a prevalence of 3.3%, 2.1% and 1.0% respectively [11].

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV. When three or four of such antiretroviral drugs are taken in combination, the approach is known as Highly Active Antiretroviral Therapy (HAART) [12]. The introduction of HAART, a cocktail of nucleoside and non-nucleoside analogues capable of inhibiting reverse transcriptase and protease has led to well documented reductions in the risk of AIDS related mortality. A survey carried out by UNAIDS in 2010 showed a reduction

in the number of deaths due to AIDS-related causes. In 2010 alone, 700,000 AIDS-related deaths were averted due to the introduction of HAART [13].

Toxicity studies are usually carried out to determine the dosage at which the effects of a substance are toxic, non-toxic or beneficial. In this research, the toxicological effects (biochemical and histopathological) of antiretroviral regimens when administered to rats over a long period was studied extensively. Furthermore the drugs were administered in single and combined doses after their LD_{50} had been determined. In the chronic toxicity studies carried out, the average weight change of the animals over the administration period was also recorded.

The advent of HAART can be said to be a two-sided coin. On one side are the numerous benefits it has offered the world in its fight against a deadly virus. On the other side are the problems it poses to the HIV-infected individuals. These problems, whether real or perceived have diminished the earlier enthusiasm generated by HAART and has raised in its place a state of fear and confusion [14]. The purpose of this study is to evaluate the toxicity profiles of antiretroviral regimens which are currently part of the HAART cocktail in order to determine whether they are toxic or non-toxic despite being beneficial.

This study was designed to give valid answers to the following questions:

- What is the LD_{50} of the antiretroviral drugs?
- Are the drugs toxic or non-toxic when administered over a long period of time?
- When administered as a monotherapy, which of the antiretroviral regimens is the most toxic, moderately toxic and least toxic?
- When administered as a combination therapy, are the toxicological effects ameliorated?
- Which is the safest combination?

Over the years, the number of deaths resulting from HIV/AIDS has fallen dramatically [15]. Thanks to antiretroviral treatment, more people are now living with HIV. At the end of 2010, an estimated 34 million people were living with HIV worldwide, up 17% from 2001[13]. The benefits that have been derived from the use of HAART have been numerous. Some of these include decreased mortality and morbidity among HIV-infected patients [16,17]. The emergence of HAART has also helped in prevention of mother to child transmission (PMTCT) [18]. However certain issues such as antiretroviral toxicity and resistance have led to impaired compliance and premature discontinuation of HAART among HIV-infected individuals.

Reports have associated HAART with toxicities amongst which are nephrotoxicity, hepatotoxicity and mitochondrial toxicity. These toxicities may impair patients' adherence to treatment thus compromising viral control [19,20,21]. More light has to be shed on this issue in order to remove doubt, fear and a deep feeling of uncertainty in the infected patients regarding the safety of these drugs. Some HIV-infected individuals especially in the developing countries have turned to the use of herbal remedies which may have more adverse effect on their already endangered health especially in the advent of a drug-drug interaction and other unknown side-effects.It is therefore paramount that research be carried out in establishing the safety of these drugs.

To the best of my knowledge, there are few reports on chronic toxicity profiles of HAART. The current study therefore, evaluates the *in vivo* acute and chronic effects of antiretroviral agents, singly and in combination, on liver and kidney parameters of *albino* rats. It also evaluates the *in vivo* chronic effects of selected antiretroviral drugs (HAART) singly and in combination on serum lipid profiles and oxidative stress markers using albino rats. Assay of 5'-nucleotidase amongst other liver function markers was carried out. Assay of 5'-nucleotidase has been reported to be one of the best enzyme indices of liver damage because of its specificity [22,23]. In addition, the liver and kidney tissues were examined histopathologically as a means of knowing if there was tissue damage and the extent of the damage.

2. Materials and Methods

All chemicals and reagents used for this study were of analytical grade. The antiretroviral drugs used for the study were obtained from the Anti-AIDS Pharmacy, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. Zidovudine was manufactured by Cipla Ltd, Verna Industrial Estate, Goa, India with Batch No: X11241; nevirapine was manufactured by Aurobindo Pharma Ltd, Andhra Pradesh, India with National Agency for Food Drug Administration and Control (NAFDAC) reg No: 04-7679 and lamivudine was manufactured by Hetero Drugs Ltd, Hyderabad, India Batch No: E110243B. These are the components of HAART cocktail prescribed and recommended for HIV-infected persons in Sokoto State.

A total of one hundred and forty-four (144) albino rats (wistar strain) of either sex were used in the research and were obtained from the Animal House, Department of Biological Science, Usmanu Danfodiyo University Sokoto. The animals were kept in metal cages in a well ventilated room and allowed to acclimatize for fourteen (14) days. They were allowed free access to clean water and feed *ad libitum* throughout the experiment, except for the short fasting period where the drinking water was still in free access but no food supply was provided twelve (12) hours prior to the treatment.

Acute Oral Toxicity was performed using the up and down procedure of the organisation for economic and cultural development [24]. This procedure involves the limit Test, which was also used for the selection of a starting dose as well as determination of LD_{50}ofthe testing material.For chronic toxicity study, one hundred and twenty-four (124) rats were weighed and subsequently divided into four (4) treatment groups. Members of each treatment group were further divided into four sub-groups (n= 6-9 rats per group). Each group was labeled to represent the drug to be administered. The animals in group 1 received distilled water as placebo. Animals in group 2 were administered a combination therapy (CMB) of lamivudine, zidovudine and nevirapinewith each subgroup administered 20%, 40%, 60% and 80% LD_{50} of CMBrespectively. Group 3, 4 and 5 animals were administered nevirapine (20%,40%,60% and 80% LD_{50} of nevirapine), lamivudine (20%,40%,60% and 80% LD_{50} of lamivudine) and zidovudine (20%,40%,60% and 80% LD_{50} of zidovudine) respectively.

The doses of the regimens were calculated based on the body weight of the animals. The drugs were administered in 1ml distilled water via a standard orogastric cannula for 90 days to represent chronic dosing. This helped in studying the long-term effects of the drugs. The body weight changes were monitored throughout

the experimental period weekly and the percentage change in body weight on the sixth (mid-period) and thirteenth week (concluding period) was calculated.

$$\text{Percentage change in weight} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

The rats were anaesthetized by dropping each of the animals successively in a transparent plastic jar saturated with chloroform vapour. The animals were removed from the jar and blood samples were collected through cardiac puncture. The blood samples were allowed to clot for 30 minutes, before centrifugation for 10 minutes at 500g and the serum collected was used for biochemical assays including assessment of serum 5'-nucleotidase enzyme activity.

Subsequently the animals were sacrificed by decapitation; the organs (liver and kidney) were removed. They were perfused with cold saline to completely remove all the red blood cells. One gram (1g) portion of each liver was used to prepare homogenate in ice cold potassium chloride (KCl) solution (0.86 %). The required amount was weighed and homogenized using Teflon homogenizer. The homogenate was centrifuged at 700g for 10 minutes to remove the nuclear fraction. The supernatant was used for the estimation of antioxidant enzymes, gluthathione reductase, superoxide dismutase and catalase. The level of lipid peroxidation was determined by estimation of malondialdehyde content by measuring the concentration of thiobarbituric acid reacting substrate (TBARS) in the liver homogenate.

The following liver function tests were determined. Serum 5'-nucleotidase (5'-NT) activity was determined using Bertrand- Buret method [25] while colorimetric method of Sood [26] was used for measurement of serum alkaline phosphatase activity. Serum alanine aminotransferase (ALT) activity and serum aspartate aminotransferase (AST) activity were determined using Reitman-Frankel method [27]. To determine the total protein in the blood, the biuret method of Gomall and his colleague [28] was employed. In addition, the dye binding technique utilizing bromocresol green (BCG) as modified by Doumas and his colleagues[29] was employed in the determination of serum albumin concentration (ALB).Colorimetric method by Jendrassik and Grof [30] was employed for determination of serum total and conjugated (direct) bilirubin concentration.

The following methods were used for kidney function tests. Serum urea was determined by Diacetyl monoxime Method using thiosemicarbazide [31] while colorimetric method with deproteinization was used in the determination of serum creatinine [32].Sodium and potassium concentration were determined using flame photometer [33]. Furthermore serum chloride was determined by colorimetric method [34].

Total cholesterol was determined using Trinder's method while colorimetric method of Tietz was used for measurement of serum triglyceride level [35,34]. HDL cholesterol level was determined using the method described by Lopez-Virella *et al* [36]. A TBARS (thiobarbituric acid reactive substance) assay was used for the quantification of the end product of lipid peroxidation, to be specific, malondialdehyde (MDA)[37].The alloxan "305" method of Patterson and Lazarow [38] was modified and used for determination of glutathione while

enzymic assay of catalase was carried out by the method described by [39]. Superoxide dismutase activity was assayed by the method of McCord and Fridovichwith some modifications [40].

Three animals from each group were selected randomly, dissected through a central abdominal incision. The liver and kidney samples were collected and immediately fixed in 10% formal-saline in labeled plastic bottles. The tissues were dehydrated in graded concentrations of xylene, embedded in molten paraffin wax and sectioned at 5µ. Tissue sections were fixed on glass slides and stained with hematoxylin and eosin for light microscopy at 400X [41]. Photomicrographs of some of the tissues were taken using a microscope fitted with a camera unit.

3. Results

The results were presented as Mean ± Standard Deviation of the mean. The results were analysed using Bonferroni's multiple comparison test. Values with P-values less than 5% were considered significant. GraphPad InStat software (San Diego U.S.A) was employed for the analysis.In the limit test, the animals in the control group exhibited normal signs and behavior. The breathing and heart beat was normal while they remained active throughout the course of the study. In contrast, the nevirapine-administered animals refused taking food or water and they remained inactive for six (6) hours following administration of a test dose of 5000 mg/kg. Normal activity was observed after six (6) hours as shown in Table 1. No mortality was observed for 48 hours. Thus the LD_{50} of nevirapine is greater than the test dose (5000 mg/kg).

After administration of 5000 mg/kg of lamivudine to the animals, there was refusal of food and water for an hour after which normal activity was observed as shown in Table 2. No mortality was observed for 48 hours. Thus the LD_{50} of lamivudine is greater than the test dose (5000 mg/kg).Likewise, following administration of 5000 mg/kg of zidovudine to the animals, there was refusal of food and water for two hours initially. There was also an increased rate of heart beat but these changes were back to normal after (2) two hours as shown in Table 3. No mortality was observed for 48 hours. Thus the LD_{50} of zidovudine is greater than the test dose (5000 mg/kg).

The effect of oral administration of the antiretroviral regimens on the ratio of organ weight: body weight, organ body index as well as percentage change in body weight on the sixth and thirteenth week are shown in Table 4. Administration of CMB resulted in significant increase in the ratio of kidney weight: body weight when compared with the control group. However, only a higher dose of 3000mg/kg had a significant ($p<0.05$) effect on the ratio of liver weight: body weight. There was also a significant ($p<0.05$) increase in percentage weight gain at 6 weeks with the exception of 2000mg/kg body weight of CMB. Higher doses of NVP had no significant ($p<0.05$) effect on the ratio of liver to body weight. However there was a significant ($P< 0.05$) increase in the percentage weight gain at 6 weeks. There was also a decrease in the percentage weight loss from 6 to 13 weeks although the change was not significant when compared with the control. On the other hand, the lowest administered doses of 3TC had no significant effect on any of the above parameters. This was also observed with the lower administered doses of 1000mg/kg and 2000mg/kg body weight of AZT respectively.

Table 1. General appearance and behavioral observation for control and nevirapine® treated groups

	Control						Treatment					
Observation	1hr	2hrs	4hrs	6hrs	48 hrs	14 days	1hr	2hrs	4hrs	6hrs	48 hrs	14 days
Food/water refusal	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Present	Present	Absent	Absent
Aggressiveness	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Eyes and ears	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convulsions	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salivation	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Activity	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Active	Active
Breathing	Normal	Normal	Normal	Normal	Normal	Normal	Fast	Fast	Fast	Normal	Normal	Normal
Heart beat	Normal	Normal	Normal	Normal	Normal	Normal	Fast	Fast	Fast	Normal	Normal	Normal
Diarrhea	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Injury	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Mortality	No	No	No	No	No	No	No	No	No	No	No	No

Table 2. General appearance and behavioral observation for control and lamivudine® treated groups

	Control						Treatment					
Observation	1hr	2hrs	4hrs	6hrs	48 hrs	14 days	1hr	2hrs	4hrs	6hrs	48 hrs	14 days
Food/water refusal	Absent	Absent	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent
Aggressiveness	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Eyes and ears	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convulsions	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salivation	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Activity	Active	Active	Active	Active	Active	Active	Inactive	Active	Active	Active	Active	Active
Breathing	Normal	Normal	Normal	Normal	Normal	Normal	Fast	Normal	Normal	Normal	Normal	Normal

Heart beat	Normal	Normal	Normal	Normal	Normal	Normal	Fast	Normal	Normal	Normal	Normal	Normal
Diarrhea	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Injury	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Mortality	No	No	No	No	No	No	No	No	No	No	No	No

Table 3. General appearance and behavioral observation for control and zidovudine® treated groups

	Control						Treatment					
Observation	1hr	2hrs	4hrs	6hrs	48 hrs	14 days	1hr	2hrs	4hrs	6hrs	48 hrs	14 days
Food/water refusal	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
Aggressiveness	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Eyes and ears	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convulsions	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salivation	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Activity	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Active	Active
Breathing	Normal	Normal	Normal	Normal	Normal	Normal	Fast	Fast	Normal	Normal	Normal	Normal
Heart beat	Normal	Normal	Normal	Normal	Normal	Normal	Fast	Fast	Normal	Normal	Normal	Normal
Diarrhea	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Injury	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Mortality	No	No	No	No	No	No	No	No	No	No	No	No

Table 5 presents the effect of oral administration of antiretroviral regimens on serum sodium ion, potassium ion, chloride ion, urea, creatinine and uric acid. Only the higher doses of NVP at 3000mg/kg and 4000 mg/kg body weight caused increased serum urea and creatinine significantly ($p<0.05$) when compared with the control. However, there was a significant ($p<0.05$) decrease in the level of serum creatinine at 4000 mg/kg body weight of AZT. This dose also had a significant ($p<0.05$) effect on the serum uric acid level when compared with the control.

Table 4. Effect of oral administration of antiretroviral regimens on the ratio oforgan weight: bodyweight of rats and percentage change in bodyweight of rats

Group(N)	Kidney: Body Wgt	Liver: Body Wgt	Change in Body Wgt at 6 wks (%)	Change in Body Wgt at 13 wks (%)
CONTROL(8)	0.0016±0.0003	0.018±0.002	12.1±6.3	10.0±4.9
CMB 20(5)	0.0027±0.0007*	0.032±0.008	55.5±32.2*	-1.1±9.1
CMB 40(4)	0.0034±0.0006*	0.039±0.007	35.3±7.5	-12.9±3.0*
CMB 60(5)	0.0034±0.0006*	0.040±0.016*	62.0±5.3*	-14.7±13.9*
CMB 80(5)	0.0027±0.0005*	0.035±0.008	56.5±21.9*	-10.1±12.3*
NVP 20(4)	0.0027±0.0007*	0.029±0.004	33.1±9.3	5.0±5.1
NVP 40(4)	0.0033±0.0004*	0.036±0.009	17.3±8.2	-5.4±4.5
NVP 60(5)	0.0020±0.0003	0.030±0.009	50.1.±14.4*	-1.5±12.8
NVP 80(4)	0.0020±0.0005	0.030±0.009	61.3±23.2*	-9.5±8.3
3TC 20(5)	0.0020±0.0003	0.020±0.009	38.8±9.7	6.5±8.2
3TC 40(5)	0.0020±0.0004	0.020±0.004	31.8±13.7	-3.4±12.0
3TC 60(4)	0.0030±0.0002*	0.040±0.006*	25.8±7.0	-0.2±2.6
3TC 80(3)	0.0030±0.0003*	0.030±0.005	12.56±10.0	1.3±8.9
AZT 20(5)	0.0020±0.0003	0.019±0.004	36.2±17.3	5.9±8.4
AZT 40(5)	0.0020±0.0001	0.019±0.004	57.4±11.3*	2.2±3.0
AZT 60(4)	0.0030±0.0006*	0.027±0.005	23.0±11.7	2.9±14.9
AZT 80(5)	0.0020±0.0004	0.021±0.006	29.2±12.4	-2.9±9.8

Values are expressed as mean ± S.D. The level of significance was set at *P<0.05 when compared with control at 0 mg/kg body weight

The effect of oral administration of antiretroviral regimens on some serum enzyme indices in rats is shown in Table 6. All doses of administered nevirapine caused a significant ($p<0.05$) increase across all the parameters when compared with the control. CMB administered group at 3000 mg/kg and 4000 mg/kg body weight also caused a significant ($p<0.05$) increase across all parameters as well when compared with the control group.

With the exception of ALP, there was a significant ($p<0.05$) increase in AST, ALT and 5'- NT upon administration of 3000 mg/kg body weight of AZT upon comparison with the control. However all parameters showed significant ($p<0.05$) increase at an administered dose of 4000 mg/kg body weight of AZT. Furthermore, it was observed from the result that 3TC at all administered doses showed no significant ($p>0.05$) increase in ALP and 5'-NT when compared with the control.

Table 5. Effect of oral administration of antiretroviral regimens on some kidney function parameters in rats

GROUP(N)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	Urea (mmol/l)	Creatinine (mg/dl)	Uric Acid (mg/dl)
CONTROL(8)	140.8±2.3	4.7±0.7	98.6±2.6	6.4±0.8	0.7±0.01	4.0±0.62
CMB 20(5)	138.2±5.5	4.8±0.6	105.6±4.9	6.8±1.1	0.8±0.10	4.1±1.28
CMB 40(4)	140.8±3.9	4.5±0.7	108.8±2.9	7.9±0.3	1.0±0.19	4.3±2.44
CMB 60(5)	136.8±7.6	5.1±0.8	100.8±1.5	7.6±1.1	0.8±0.10	5.4±0.99
CMB 80(5)	140.8±3.2	4.7±0.8	97.4±9.1	8.4±1.2	1.0±0.17	5.1±1.01
NVP 20(4)	137.5±7.2	4.5±0.7	90.3±8.8	7.7±0.3	0.9±0.09	3.2±1.90
NVP 40(4)	137.3±8.7	4.5±0.7	93.5±5.3	8.4±0.5	1.0±0.20	4.1±0.24
NVP 60(5)	135.0±11.4	4.6±0.7	94.2±11.2	9.8±1.4*	1.2±0.34*	4.5±0.64
NVP 80(4)	141.0±3.0	4.9±0.7	94.0±3.5	11.3±1.5*	1.6±0.22*	6.4±0.47
3TC 20(5)	139.6±3.9	4.0±0.4	108.8±7.0	7.1±1.1	0.7±0.04	4.4±0.52
3TC 40(5)	139.0±6.8	5.0±0.7	99.4±5.2	7.8±0.4	0.7±0.04	4.1±1.50
3TC 60(4)	138.0±2.6	4.2±0.8	105.8±10.0	8.0±2.0	0.8±0.08	4.4.±0.66
3TC 80(3)	139.7±7.6	4.7±0.5	99.3±5.9	8.0±0.6	1.0±0.29	6.8±1.46
AZT 20(5)	137.6±7.6	4.6±0.6	93.4±4.1	7.2±0.9	0.8±0.11	3.5±2.90
AZT 40(5)	141.0±2.7	4.2±0.7	92.0±10.6	8.1±1.6	0.8±0.17	4.7±3.12
AZT 60(4)	142.8±1.7	4.3±0.3	104.0±4.8	8.1±0.8	0.9±0.18	6.4±1.64
AZT 80(5)	141.2±2.9	4.7±0.7	94.2±3.0	7.7±2.3	0.3±0.24*	8.0±1.05*

Values are expressed as Mean ± SD. The level of significance was set at *P<0.05 when compared with control at 0 mg/kg body weight

Table 6. Effect of oral administration of antiretroviral regimens on some serum enzyme indices in rats

GROUP(N)	AST (U/l)	ALT (U/l)	ALP (U/l)	5' NT (U/l)
CONTROL(8)	11.9±1.2	4.4±0.5	175.6±33.7	36.0±9.5
CMB 20(5)	13.1±1.5	7.1±0.7*	234.4±47.8	138.2±5.5
CMB 40(4)	15.0±1.1*	9.3±1.0*	255.7±49.3	56.3±8.5*
CMB 60(5)	16.9±0.5*	10.9±0.3*	348.7±102.5*	78.0±11.8*
CMB 80(5)	21.4±1.5*	12.6±0.6*	438.9±54.9*	90.2±8.7*
NVP 20(4)	16.8±0.6*	10.1±1.2*	330.7.6±78.2*	74.0±9.5*
NVP 40(4)	21.5±1.6*	13.3±0.3*	443.0±39.6*	109.7±10.5*
NVP 60(5)	23.4±2.4*	14.7±0.5*	499.1±117.1*	129.2±10.5*
NVP 80(4)	25.8±1.0*	15.9±1.6*	612.6.6±27.2*	145±7.0*
3TC 20(5)	12.7±1.3	5.6±1.6	198.8±9.9	39.6±3.9
3TC 40(5)	14.8±0.7*	8.3±0.9*	204.3±10.0	42.3±0.3

3TC 60(4)	15.7±0.9*	10.5±0.4*	258.5±73.0	55.5±6.6
3TC 80(3)	18.6±2.4*	11.2±0.3*	297.4±92.4	62.6±12.0
AZT 20(5)	12.6±1.5	8.0±3.1*	201.0±18.1	34.8±9.1
AZT 40(5)	13.8±0.9	10.9±0.4*	233.3±41.8	59.8±10.4*
AZT 60(4)	17.1±0.8*	12.1±1.1*	300.8±35.1	84.4±29.4*
AZT 80(5)	18.9±1.1*	14.1±0.6*	344.7±70.4*	98.7±9.5*

Values are expressed as Mean ± SD. The level of significance was set at *P<0.05 when compared with control at 0 mg/kg body weight.

The effect of oral administration of antiretroviral drugs on serum non-enzyme indices is shown in Table 7. Nevirapine administered at 2000 mg/kg, 3000 mg/kg and 4000 mg/kg body weight caused significant ($p<0.05$) decrease in the level of total protein and significant ($p<0.05$) increase in total bilirubin level when compared with the control group. CMB and AZT at all administered doses had no significant ($p>0.05$) effect on albumin and direct bilirubin upon comparison with the control group. However, oral administration of lamivudine at 4000 mg/kg body weight caused a significant ($p<0.05$) increase in total bilirubin. Other administered doses of lamivudine showed no significant ($P>0.05$) effect across all parameters when compared with the control.

Table 7. Effect of oral administration of antiretroviral regimens on serum non-enzyme indices in rats

GROUP(N)	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
CONTROL(8)	14.7±1.0	4.4±1.2	0.15±0.05	0.023±0.01
CMB 20(5)	13.9±0.5	3.5±0.4	0.26±0.04	0.054±0.02
CMB 40(4)	13.2±1.0	3.5±0.5	0.31±0.03	0.078±0.02
CMB 60(5)	11.1±0.8*	3.1±0.2	0.47±0.04*	0.083±0.02
CMB 80(5)	7.3±0.3*	3.0±0.3	0.64±0.20	0.110±0.04
NVP 20(4)	14.2±1.6	3.1±0.3	0.34±0.05	0.005±0.03
NVP 40(4)	9.7±2.5*	3.0±0.4	0.80±0.07*	0.100±0.02
NVP 60(5)	7.0±0.5*	3.1±0.5	1.03±0.20*	0.080±0.03
NVP 80(4)	5.4±0.6*	1.8±0.4*	1.65±0.50*	0.350±0.43*
3TC 20(5)	13.6±2.2	4.8±0.2	0.28±0.06	0.004±0.02
3TC 40(5)	13.6±1.6	4.1±1.1	0.45±0.06	0.005±0.03
3TC 60(4)	11.2±0.8	4.0±0.3	0.43±0.12	0.004±0.05
3TC 80(3)	11.5±1.3	3.1±1.1	0.73±0.01*	0.009±0.02
AZT 20(5)	13.3±1.3	4.4±1.0	0.28±0.07	0.003±0.02
AZT 40(5)	13.0±1.8	2.9±0.7	0.42±0.20	0.005±0.05
AZT 60(4)	10.3±0.7*	3.0±1.0	0.57±0.10*	0.006±0.03
AZT 80(5)	8.1±1.5*	2.7±1.1	0.98±0.10*	0.006±0.06

Values are expressed as Mean ± SD. The level of significance was set at *P<0.05 when compared with control at 0 mg/kg body weight

Table 8 presents the effect of oral administration of antiretroviral regimens on serum lipid profile. It was observed that NVP had no significant ($p>0.05$) effect on LDL and HDL: LDL when compared with control. At 2000 mg/kg and 4000 mg/kg body weight, NVP caused a significant ($p<0.05$) increase in total cholesterol upon comparison with the control. Furthermore, nevirapine had a significant effect on vLDL cholesterol at all doses. However at an administered dose of 1000 mg/kg body weight, nevirapine caused no significant ($p>0.05$) effect on total cholesterol, triglyceride and HDL cholesterol when compared with the control.

Lamivudine at all doses administered had no significant ($p>0.05$) effect across all parameters. Also, zidovudine had no significant ($p>0.05$) effect on the level of triglyceride, HDL cholesterol, LDL cholesterol, vLDL cholesterol and HDL: LDL at all administered doses. At administered doses of 1000 mg/kg, 2000 mg/kg and 3000 mg/kg body weight, CMB had no significant ($p>0.05$) effect across all parameters when compared with the control group.

Table 8. Effect of Oral Administration of Antiretroviral Regimens on Serum Lipid Profiles in Rats

GROUP(N)	Total Chol (mg/dl)	Triglyceride (mg/dl)	HDL Chol (mg/dl)	LDL Chol (mg/dl)	vLDL Chol (mg/dl)	HDL: LDL
CONTROL(8)	99.1±12.9	112.0±14.2	72.0±9.1	11.0±13.3	22.4±2.8	4.0±5.1
CMB 20(5)	118.8±16.2	96.0±25.3	99.4±16.4	11.4±12.5	19.2±5.1	3.8±4.6
CMB 40(4)	139.0±21.3	70.0±17.7	107.0±35.4	20.6±13.8	14.0±3.6	2.5±1.6
CMB 60(5)	162.8±18.7	78.0±30.2	124.0±14.1	23.2±18.8	15.6±6.0	14.2±18.2
CMB 80(5)	230.8±29*	56.0±18.8*	161.4.±19.2*	45.4±40.1	11.2±3.8*	9.3±10.3
NVP 20(4)	143.5±39.6	58.0±20.0	116.3±15.5	24.9±42.4	8.9±3.7*	3.0±5.2
NVP 40(4)	206.5±50.4*	56.0±17.3*	132.0±21.3	69.3±50.7	11.2±3.5*	1.0±0.7
NVP 60(5)	187.6±55.7*	59.2±25.0*	162.2±22.6*	30.8±35.1	11.8±5.0*	2.3±2.9
NVP 80(4)	287.0±24.1*	38.0±13.7*	236.0±65.3*	43.5±48.7	7.6±2.7*	15.2±27.5
3TC 20(5)	109.4±19.3	94.4±20.7	74.6±33.0	25.9±17.6	18.9±4.1	1.8±1.5
3TC 40(5)	91.6±15.4	107.2±22.3	96.0±51.5	2.6±6.0	21.4±4.5	-
3TC 60(4)	119.5±18.6	92±15.3	73.5±34.3	39.9±26.9	18.4±3.1	0.8±0.6
3TC 80(3)	174.3±29.6	59.3±12.1	87.3±44.7	76.3±73.0	11.9±2.4	0.4±0.5
AZT 20(5)	114.4±37.6	105.6±22.2	67.0±13.5	29.7±40.3	21.1±4.4	6.5±10.9
AZT 40(5)	113.6±19.2	99.2±36.9	81.8±19.7	20.7±18.2	19.8±7.4	4.9±7.1
AZT 60(4)	176.0±6.3*	100.0±33.0	73.3.±11.9	82.8±14.1	20.0±6.6	0.9±0.3
AZT 80(5)	179.2±69.0*	99.2±36.0	97.2±28.4	57.3±72.4	19.9±7.3	4.4±7.3

Values are expressed as Mean ± SD. The level of significance was set at *P<0.05 when compared with control at 0 mg/kg body weight.

The effect of antiretroviral regimens on liver enzymatic and non-enzymatic antioxidant levels is shown in Table 9. Administered doses of CMB and NVP except at 1000 mg/kg body weight respectively caused a significant ($p<0.05$) increase in the level of MDA when compared with the control group. However there was no significant ($p>0.05$) effect in the level of reduced gluthatione at all doses of administered drugs except at 4000 mg/kg body weight of nevirapine. Also zidovudine at administered doses of 1000 mg/kg and 2000 mg/kg respectively caused no significant ($p>0.05$) effect across all parameters.

Administered doses of 4000 mg/kg body weight of lamivudine caused a significant ($p<0.05$) decrease in catalase level when compared with the control group. Also 2000 mg/kg body weight of lamivudine administered orally caused a significant ($p<0.05$) decrease in the level of superoxide dismutase upon comparison with the control. However other administered doses of lamivudine had no significant ($p>0.05$) effect across all parameters when compared with the control.

Table 9. Effect of oral administration of antiretroviral regimens on liver-enzymatic and non-enzymatic antioxidants levels in rats

GROUP(N)	MDA (nmoles /g tissue)	Reduced Gluthatione(ng GSH/g tissue)x 10^6	Catalase (units/g tissue)	Superoxide Dismutase (units/g tissue)
CONTROL(5)	0.77±0.08	0.10±0.011	3.7±0.7	14.1±1.6
CMB 20(5)	1.21±0.22	0.09±0.004	3.2±0.6	12.3±1.3
CMB 40(4)	1.66±0.25*	0.08±0.015	2.1±0.4	12.2±1.7
CMB 60(5)	2.02±0.09*	0.08±0.016	1.9±0.3*	10.0±1.8*
CMB 80(5)	2.25±0.28*	0.07±0.011	1.2±0.5*	8.8±1.1*
NVP 20(4)	0.86±0.09	0.09±0.006	2.6±0.7	11.9±1.6
NVP 40(4)	1.62±0.38*	0.08±0.016	1.6±0.8*	10.2±1.3*
NVP 60(5)	2.21±0.28*	0.06±0.009	1.3±0.7*	10.1±2.0*
NVP 80(4)	2.59±0.46*	0.04±0.011*	0.6±0.2*	11.0±1.4
3TC 20(5)	0.85±0.16	0.10±0.005	3.5±0.8	13.2±1.3
3TC 40(5)	1.14±0.34	0.09±0.011	2.2±0.3	10.4±1.8*
3TC 60(4)	0.68±0.36	0.09±0.002	2.1±0.7	13.2±1.2
3TC 80(3)	0.76±0.42	0.09±0.013	1.6±0.4*	10.7±1.8
AZT 20(5)	0.92±0.33	0.10±0.010	2.7±1.3	12.3±1.5
AZT 40(5)	0.93±0.21	0.07±0.001	2.5±0.7	12.7±1.3
AZT 60(4)	1.24±0.27	0.08±0.016	1.8±1.3*	12.2±1.4
AZT 80(5)	2.03±0.15*	0.07±0.010	1.1±0.5*	9.2±1.9*

Values are expressed as Mean ± SD. The level of significance was set at *P<0.05 when compared with control at 0 mg/kg

In Fig.1, the liver of animals in the control group showed typical hepatolobular architecture while examination of the liver histological preparations obtained from all the experimental treated groups showed comparable changes as observed in Figs.2- 9. The hepatolobular architecture of the control animals shown in Fig.1 indicates

a normal histological appearance. The central vein (cv) in the center of the hepatic lobule is filled with blood (B). Hepatocytes, arranged in form of cords are rounded to polyhedral in shape and radiate peripherally. They also show nucleus (N) with clear nuclear membrane and nucleolus (NO). The cords are separated by sinusoids (S) with Kupffer cells (H&E stain, x 100).

The photomicrographs of rats administered 1000 mg/kg and 4000 mg/kg body weight of CMB as seen in Figs. 2 and 3 show severe distortion of the hepatic architecture. There is extensive ballooning of the hepatocytes (HB) and evidence of steatosis (S). Sinusoidal arrangement is also deranged (H&E stain, x 100). This distortion is more severe in the group administered 4000 mg/kg body weight of CMB as indicated in Fig.3 evidenced by necrotic areas and distorted areas in an overlapping interface (H&E stain, x 100).

The photomicrographs of rats administered 1000 mg/kg body weight of nevirapine as seen in Fig.4 show severe distortion of the hepatic architecture evidenced by extensive ballooning of the hepatocytes (H&E stain, x 200). The distortion is more severe in the rats administered 4000 mg/kg body weight of nevirapine evidenced by necrotic areas and distorted areas (H&E stain, x 100). Photomicrographs of the lamivudine administered animals show mild and moderate distortion of the hepatic architecture with observed steatosis (S). There are preserved areas within the hepatic architecture. Mild distortions were also observed in groups administered 1000 mg/kg body weight of zidovudine as seen in Fig. 8. It therefore appears that the pathological effect of the chronic administration of the antiretroviral drugs was dose-dependent.

Chronic administration of antiretroviral drugs indicated that the histological preparations of the kidney as shown in Figs.11 and 12 had normal architecture when compared to the control group shown in Fig.10. Section of the kidney shows normal proximal (PT) and distal tube (DT), glomerulus (G), blood vessels and interstitions. Podocytes (P) and corpuscular space (CS) appear within normal range (H&E stain, × 100).

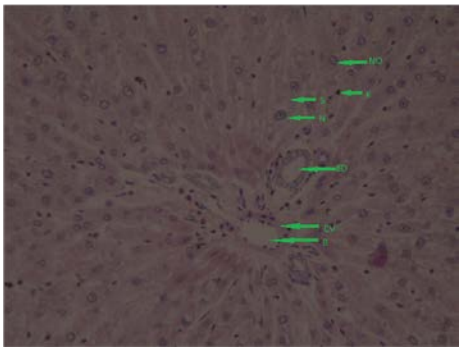

Fig. 1. Photomicrograph of liver section of rats from control group

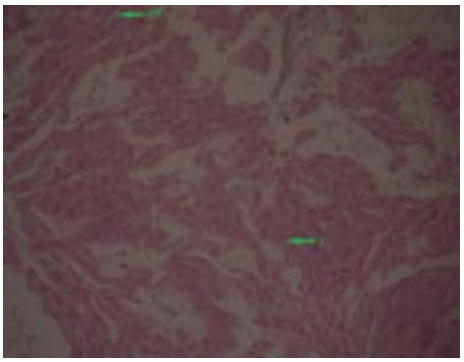

Fig. 2. Photomicrograph of liver section of rats administered 1000 mg/kg body weight

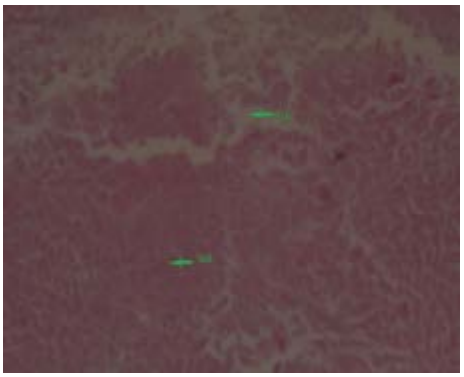

Fig. 3. Photomicrograph of liver section of rats administered 4000 mg/kg body weight of CMB

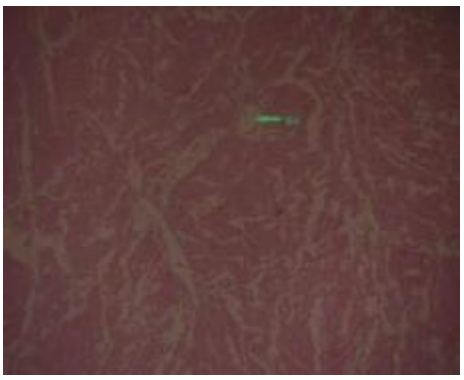

Fig. 4. Photomicrograph of liver section of rats administered 1000 mg/kg body weight of nevirapine

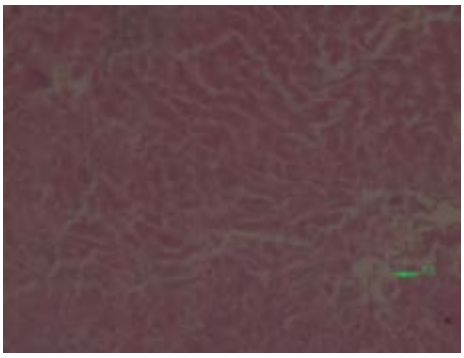

Fig. 5. Photomicrograph of liver section of rats administered 4000 mg/kg body weight of nevirapine

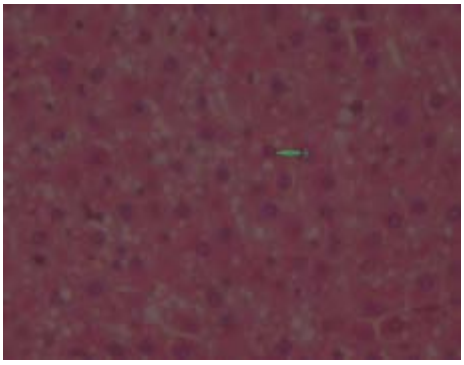

Fig. 6. Photomicrograph of liver section of rats administered 1000 mg/kg body weight of lamivudine

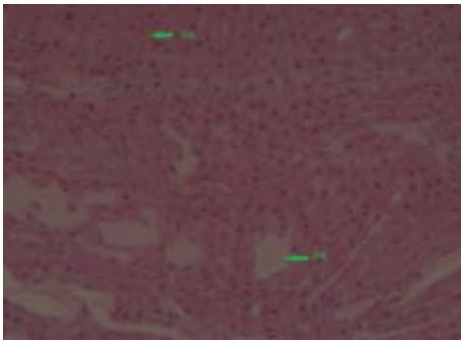

Fig. 7. Photomicrograph of liver section of rats administered 4000 mg/kg body weight of lamivudine

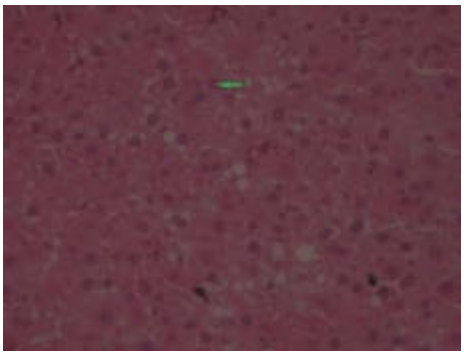

Fig. 8. Photomicrograph of liver section of rats administered 1000 mg/kg bodyweight of Zidovudine

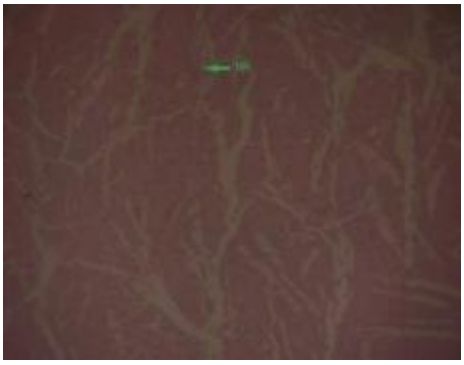

Fig. 9. Photomicrograph of liver section of rats administered 4000 mg/kg bodyweight of Zidovudine

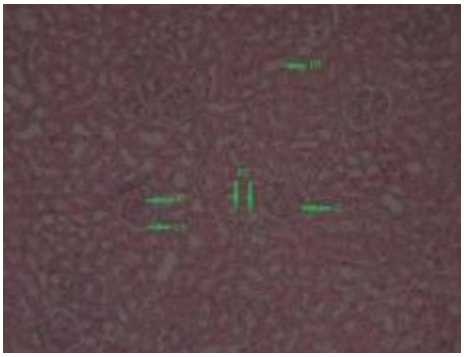

Fig. 10. Photomicrograph of kidney section of rats from control group

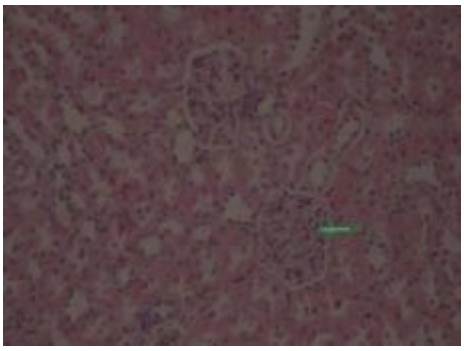

Fig. 11. Photomicrograph of kidney section of rats administered 4000 mg/kg body weight of CMB

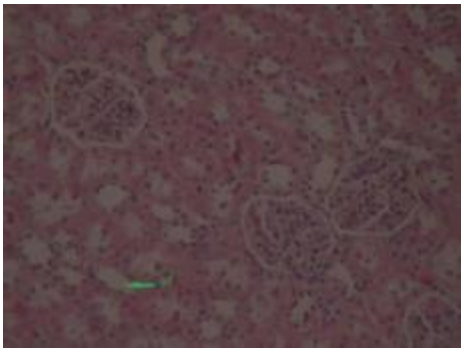

Fig.12. Photomicrograph of kidney section of rats administered 4000 mg/kg body weight of nevirapine

4. Discussion

In screening drugs, determination of LD_{50} is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. The 48 hours LD_{50} is defined as the oral dose at which fifty (50) percent of the exposed test animals died, usually within forty-eight (48) hours (OECD, 2001). Using the OECD limit test procedure the LD_{50} of nevirapine®, lamivudine® and zidovudine® exceeded 5000 mg/kg body weight respectively which corresponds to low toxicity substances suitable for *in vivo* studies though these values are greater than the reported LD_{50} of the active pharmaceutical ingredient in each drug. This may be due to factors such as the concentration of the formulation (dilute or concentrated), the type of formulation (wet or dry) and the presence of other ingredients other than the active pharmaceutical ingredients in the formulation with greater LD_{50} value [42].

Acute toxicity data are of limited clinical application since cumulative toxic effects do occur even at very low doses. Hence chronic toxicity studies are invaluable in evaluating the safety profile of drugs [43]. In the repeated dose 90 days oral toxicity study, administration of the antiretroviral regimens revealed an increase in percentage

weight gain of treated animals between week 0 and week 6. This increase was significant ($P<0.05$) in CMB-treated groups of 1000, 3000 and 4000 mg/kg body weight respectively when compared with the control group. Weight gain has been associated with the use of HAART at the initiation of therapy. This may be attributed to various metabolic complications of lipid/ glucose. These complications include diabetes, dyslipidemia and body fat changes.

However the long term effect of these drugs is usually a significant loss in weight. This was observed after 13 weeks of administration especially in the CMB-treated groups when compared to the control. The reason for this could be a modification of both fat metabolism and distribution by the NRTIs. AZT in particular has been reported to decrease the specific activities of cytochrome c oxidase and fatty acid synthase thus leading to a lowered cell lipid accumulation [44]. The presence of NRTIs especially in the CMB-treated group may be responsible for this weight loss.

Ratio of organs: body weight is normally investigated to determine whether the size of the organs has changed in relation to the weight of the whole animal. According to Moore and Dalley [45], an increase in organ/body weight ratio is an indication of inflammation while a reduction in the same parameter can be adduced to cell shape changes and tissue movements [46]. There was an increase in the liver/body weight ratio of some of the treated groups when compared with the control. This increase was significant in the groups treated with 3000 mg/kg body weight of NVP and 3TC.

Hepatomegally observed in treated groups may be attributed to the administration of these antiretroviral drugs hence they should be used with caution especially in individuals with liver related diseases. Furthermore there was an observed increase in the kidney/body weight ratio which was significant in some treated groups. This increase was significant in all CMB-treated rats. This could be attributed to the effects of the administered combined regimen causing a decrease in osmolality evidenced by the enlarged kidney since renal function indices were not affected rather than as a result of their toxicological effects.

Renal function indices are usually required to assess the normal functioning of different parts of the nephrons [47]. Kidney function is affected by a number of factors, which may ultimately result in its failure. Causes of kidney failure include destruction of the tubules in the kidney by drugs, including phytochemicals. As a result, the two main functions of the kidney: the glomerular filtration and tubular re-absorption and secretion may be affected [48]. Similarly, the serum concentrations of electrolytes could give an insight into the effect of drugs on the tubular and or glomerular part of the kidney. Therefore, the insignificant effect of the antiretroviral regimen at all the doses investigated on the concentration of electrolytes may suggest that the normal functioning of the nephrons at the tubular and glomerular levels were not affected by chronic administration of the drugs and their combination.

The plasma concentration of creatinine is relatively constant under normal circumstances, unless Glomerulus Filtration Rate (GFR) changes, as a result of defective renal function, plasma concentration of creatinine is a good index of measuring GFR [48]. In the current work, only the highest doses of NVP and AZT caused a significant difference in serum creatinine concentrations of the treated animals. Though plasma urea

concentration is less reliable than the creatinine as an index of GFR, by virtue of the fact that it diffuses back into the renal tubular cells and its plasma concentration is dependent on the state of the liver function and protein intake and oxidation [49]. Estimation of the serum creatinine and urea complement each other in evaluating this function of the kidney. This fact is supported by the observation that the serum urea concentrations of the treated animals were also not significantly ($p<0.05$) different except at administered doses of 3000 mg/kg and 4000 mg/kg body weight of nevirapine. However, this could be as a result of dehydration [50]. It can be suggested that administration of the antiretroviral drugs both singly and in combination did not have any toxicological effect on the glomerulus filtration rate of the treated animals.

One of the most common causes of hyperuricaemia is gout, in which there are either tophi or acute arthritis. Mild asymptomatic idiopathic hyperuricaemia has also been associated with hyperlipidaemia and coronary heart disease [49]. Hyperuricaemia as a result of chronic renal failure can be ascertained by correlating uric acid level with urea and creatinine. The general trend in the result of the chronic oral toxicity test in the current study suggests that the drugs has no effect on the serum level of uric acid except at an administered dose of 4000 mg/kg body weight of AZT which was significantly ($P<0.05$) different. This may have been due to over hydration from excessive water intake [50]. This assertion is informed from the fact that serum urea and creatinine levels, which are also expected to be raised if the problem were renal, are normal.

The liver is a vital organ involved in detoxification, protein synthesis and production of biochemicals essential for the process of digestion (e.g. bile). Albumin is the most abundant of the plasma proteins with the physiological role of maintenance of osmotic pressure, transportation of both endogenous and exogenous substances and serving as protein reserve. The ability of the liver to synthesize albumin is diminished if the synthetic function of the organ is affected [48]. Albumin synthesis is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure. The results of this study revealed a decrease in the serum albumin in the groups treated with CMB, NVP and AZT. This change was significant in the group treated with 4000 mg/kg body weight of NVP.

The assay of serum total protein alone may not portray the true picture of the metabolic state of the individual, since the concentration of the individual proteins do not rise or fall in parallel with one another [48]. Increased plasma total protein concentration may be due to dehydration, to increased plasma immunoglobulin concentration due to infection. Plasma concentration of proteins may decrease as a result of over hydration, impaired protein synthesis due to malnutrition and liver disease amongst other factors. In the current study, the total protein of groups treated with 3000 mg/kg and 4000 mg/kg body weight of CMB, NVP and AZT when compared to the control was significantly decreased. These results demonstrate the fact that the synthetic function of the liver of the animals exposed to higher doses of CMB, NVP and AZT was affected.

Bilirubin is an endogenous anion derived from hemoglobin degradation from the RBC. Bilirubin, a metabolic breakdown product of heme derived from senescent red blood cells, is also one of the most commonly used liver function tests [51]. From the results, the significant ($P<0.05$) increment in the total bilirubin level of groups treated with 3000 and 4000 mg/kg body weight of CMB, NVP and AZT could be adduced to impairment in the secretory function of these proteins amongst which are overproduction, defective conjugation in the hepatocyte

and presence of substances interfering with bilirubin-albumin binding sites. This may also adversely affect the functional activity of the liver.

The expression of toxicity of xenobiotics is usually determined biochemically by monitoring of some plasma enzymes and lipids. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are useful indices for identifying inflammation and necrosis of the liver [49,52]. ALT has its highest concentration in the liver with kidney and skeletal muscles having lesser activity of the enzyme. The activity of AST is located in the microsomal and mitochondrial portions of the liver cells as well as in the skin, skeletal and cardiac muscles, pancreas and kidney [49,48].

In the current study, all doses of NVP caused significant ($P<0.05$) changes in both AST and ALT levels when compared to the control and this increase was observed to be dose dependent. CMB also increased the level of AST and ALT significantly ($P<0.05$) except at a dose of 1000 mg/kg body weight. AZT also caused a significant increase ($P<0.05$) in the level of both enzymes. It may be inferred therefore that these changes may be due to the effect of the administered drugs. This suggests that chronic administration of antiretroviral regimen has hepatotoxic effects in rats. This is an indication that CMB, NVP and AZT can result in hepatic damage and can also lead to an increase in hepatotoxicity in patients with liver diseases.

ALP is a liver enzyme that is usually found in the walls of the intra- and extra-biliary ducts. Increased level of alkaline phosphatase has been attributed to the damaged structural integrity of hepatic cells because the enzyme alkaline phosphatase is located in the cytoplasm and is released into the circulation after cellular damage [49]. In the present study, the alkaline phosphatase activities of animals treated with chronic oral doses of NVP was significantly different ($P<0.05$). At administered doses of 4000 mg/kg body weight of AZT and CMB, ALP levels were significantly ($P<0.05$) higher when compared with the control. These results may have been due to disturbances in the secretory activity, or in the transport of metabolites or other hepatotoxic conditions [53]. However it was observed that lamivudine appears to be less hepatotoxic than the other drugs. This drug is preferred for HIV/AIDS patients with high risk of hepatotoxicity especially those with hepatitis B or hepatitis C coinfection [54].

5’-Nucleotidase (5’-NT) is an intrinsic membrane glycoprotein, present as an ectoenzyme in a wide variety of mammalian cells. 5’-NT hydrolyzes 5’-nucleotides to their corresponding nucleosides. The diagnostic value of 5’-NT has been shown to be superior to other liver enzymes, especially in liver metastasis [55]. This research revealed a significant increase ($P<0.05$) at all doses of NVP administered to the rats. CMB also caused a significant ($P<0.05$) increase in the level of this enzyme except at 1000 mg/kg body weight. However administration of 3TC did not cause any significant change in the level of 5’ NT.

Elevated activities of these liver enzymes indicate cell damage which might have resulted from several mechanisms; generation of toxic species, peroxidation of membranes etc. The exact mechanisms by which NVP and CMB causes adverse hepatic events have not been elucidated, but Lee [56] reports that drug-induced liver injury occurs via at least six mechanisms involving various intracellular organelles, with consequent disruption of intracellular calcium homeostasis, decrease in ATP levels and finally the hepatocyte get inflamed and rupture.

Several antiretrovirals, including AZT, have been reported to cause fatal acute hepatitis [57]. Mechanisms that have been suggested for AZT induced toxicity include alteration in liver mitochondrial DNA, alteration in oxidative phosphorylation coupling and changes in fine ultrastructure of liver mitochondrial [58]. The hepatotoxic effects of the antiretroviral regimens appeared to be buttressed by the histopathological findings from the liver tissues of the treated groups. Most of the liver slides viewed showed signs of derangement. Nevirapine appeared to be the most toxic while the least toxic was lamivudine. The presence of lamivudine in CMB may have reduced its toxicity. Most importantly, the toxicological effects of the antiretroviral drugs appeared to be dose-dependent.

A lipid profile is a measure of three components: total cholesterol (TC), triglycerides (TG) and lipoproteins (high and low density). TC comprises all the cholesterol found in various lipoproteins such as high density (HDL), low density (LDL) and very low density (VLDL). HDL-cholesterol is believed to play a key role in the process of reverse cholesterol transport that promotes the efflux of excess cholesterol from vessel wall to the liver for excretion. On the contrary, LDL cholesterol is responsible for cholesterol deposit on the wall of the artery[59].
TG is the neutral fat metabolite found in the tissue and blood and may contribute to the disorders relate d to coronary heart disease (CHD).

Changes in lipid profiles due to administration of the antiretroviral regimens are illustrated in Table 8. The results of this study indicate that there were no significant increases in the level of LDL cholesterol across all the groups. The increase in total cholesterol was significant in groups treated with 3000 and 4000 mg/kg body weight of NVP and AZT when compared with the control. However there was a significant decrease in the TG level of the rats treated with 2000 mg/kg, 3000 mg/kg and 4000 mg/kg body weight of NVP. These elevated levels of TC may be related with the peroxidation potential of the antiretroviral drugs or impairment in the biosynthesis of cholesterol. Increment in the levels of TC and reduction in TG may also be related to drug-induced lipid profile alteration and drug-induced protein metabolism [60,61]. These finding suggests that the antiretroviral drugs may have an effect on serum lipid profile.

The changes in lipids and lipoproteins differ between patients using an ART regimen containing either a PI or a NNRTI. Whereas many of the PI-based regimens are often associated with increased levels of TG, TC and LDL-cholesterol, NNRTI-based regimens are associated with marked increases of HDL-cholesterol level. In this research, there was an increase in the level of HDL-cholesterol in all NVP and CMB-treated groups. This increase was significant in the groups treated with 3000 mg/kg and 4000 mg/kg body weight of NVP. Based on these findings, HAART regimens containing NVP may be expected to result in a reduced risk of coronary heart disease. The presence of NVP in the CMB-treated group may have also caused a significant ($P<0.05$) increase in the HDL-cholesterol level. This is consistent with the findings of [62] which showed a high increase in HDL-cholesterol when NVP antiretroviral regimens were administered to HIV-infected individuals.

The drug induced alteration in lipid profile may be correlated with its lipid peroxidation phenomenon. Drug-induced lipid peroxidation and profile alteration can be correlated with drug-induced toxicity [60].The results of this research indicate an increase in the level of MDA produced in administered doses of 2000 mg/kg, 3000

mg/kg and 4000 mg/kg body weight of CMB and NVP respectively. This is in accordance with studies carried out by [63]which reported that HAART medications may increase oxidative stress levels above and beyond levels caused by the virus itself. It is therefore possible that the observed effect may be a consequence of this association. There was also an increase in AZT-treated rats in the MDA level which was significant in the group administered with 4000 mg/kg body weight of AZT. This may have been due to generation of reactive oxygen species (ROS), peroxide production and oxidative damage in mitochondria which have been reported to play vital roles in zidovudine-induced toxicity [64,58].

The presence of AZT and NVP in the combined therapy may have increased the effect of CMB on the level of non-enzymatic antioxidants in the treated rats. Laurent *et al* [65] also reported that the three commonly used fixed-dose combination therapy for HAART therapeutic regimes used on patients (lamivudine, stavudine and nevirapine) lowered plasma concentration of the antioxidant Vitamin C and increased thiobarbituric acid reactive substances (TBARs) (a marker for lipid peroxidation).

Oxidative stress (OS) in cells and tissues usually refers to increased generation of O^{2-} and H_2O_2 which can be achieved by raising oxygenconcentrations in tissues or adding certain toxins that increaseintracellular oxidant formation or activating a large number ofphagocytes [66]. Glutathione is the major endogenous antioxidant produced by the cell. HAART may reduce glutathione (GSH) synthesis, enhance GSH utilization, or limit intracellular reduction of its oxidized form (GSSG) consequent upon OS-mediated processes [67]. Though there was a decrease in the level of reduced gluthathione across the groups, it was only significant at 4000 mg/kg body weight of administered NVP.

Superoxide dismutase catalyzes the reaction of superoxide anion radicals (O_2^-) dismutation to hydrogen peroxide (H_2O_2), whereas catalase degrades H_2O_2 into a molecule of oxygen and water [68]. Superoxide ion and hydroxyl radicals are known to cause marked injuries to the surrounding tissues and organs. Therefore removing superoxide ion and hydroxyl radicals is probably one of the most effective defense mechanisms against a variety of diseases. Catalase protects cells from oxidative stress of hydrogen peroxide by its cleavage to water and oxygen [69]. In this study, there was an observed decrease in the level of SOD but this was only significant at the highest doses of CMB and NVP. This decrease in SOD activity suggests inactivation (or overutilization) of the enzyme probably caused by increased superoxide radical production, or an inhibition by the hydrogen peroxide as a result of corresponding decrease in the activity of CAT in NVP metabolism. This could also be responsible for the significant decrease in catalase activity as seen in higher doses of the administered regimens. It may be deduced therefore that NVP, CMB and AZT are associated with adverse effect on the level of enzymatic antioxidants.

Histopathology refers to the microscopic examination of tissue sections in order to study the manifestation of disease. The histopathology assessment in liver and kidney tissue was performed for all groups. Animals in the control group showed normal well defined histological structures without any signs of vascular, necrotic or inflammatory changes while those in the treated groups revealed signs of toxicity after administration of the antiretroviral regimen. These morphological changes were assessed semi-quantitatively and graded as follows: mild change, moderate change, severe change and severely severe.

The histopathological analysis of the hepatic tissue show varying degrees of changes. Lamivudine- treated animals had the least morphological change when compared with the control. The changes observed were mild and moderate. They were also less severe when compared to the NVP, CMB and AZT treated groups. Administration of 4000 mg/kg body weight AZT shows a greater severity of morphological changes such as distortion of the hepatic architecture and evidence of steatosis when compared with the lower administered doses. The morphological change due to the administration of AZT appears to be dose dependent.

Nevirapine and CMB treated animals show changes ranging from severe to severely severe. Severe distortion of the hepatic architecture can be observed in NVP and CMB treated animals. These morphological changes also appear to be dose dependent. It may be deduced that these drugs are hepatotoxic upon administration. However, the histopathological assessment of the kidney did not reveal any vascular, necrotic or imflamatory change in the treated groups. Hence it can be assumed that administration of the antiretroviral drugs was not nephrotoxic.

5. Conclusion

From this research, it can be observed that acute administration of the antiretroviral drugs may be toxic. These toxicological effects are more pronounced when the drugs were administered over a longer period and they appear to be dose-dependent. When administered as single doses, nevirapine appeared to be the most potent while lamivudine seemed to be the least toxic. The presence of nevirapine and zidovudine in the combined therapy may have contributed to its toxicity. However it was observed that even at high doses, the antiretroviral drugs used in this study were non-toxic to the kidney either as single doses or combination therapy.

Lipid profile alteration and oxidative stress were also associated with administration of these antiretroviral regimens. Nevirapine use as a single dose and in combined therapy was linked with increased MDA levels. This could have led to lipid peroxidation observed with the use of HAART. Interestingly however it was observed that the use of nevirapine led to an increase in HDL-cholesterol level. This may be beneficial in addressing the cardiovascular risks posed by the use of HAART and the HIV/AIDS disease.

Furthermore results from this study shows that a combination of lamivudine and zidovudine will be expectedly less toxic than a combination of lamivudine and nevirapine. A combination of zidovudine and nevirapine will expectedly be the most toxic amongst any of the possible 2-pill combinations. This research has shown that the antiretroviral drugs used in this study are hepatotoxic though within varying degrees. In conclusion, administration of these drugs should be done with utmost caution in HIV-infected individuals especially those who are co-infected with a liver-related disease.

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