Trop J Nat Prod Res, May 2024; 8(5): 7272-7277 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Pattern of Haematological Indices of Wistar Rats Administered Antiretroviral Therapy Singly or in Combination with Ascorbic Acid and α-Tocopherol

Kenneth Atoe[1]*, [1]Sylvester E. Idogun, Marcellinus U. Nwagu[2]

[1]Department of Chemical Pathology, Edo State University, Uzairue
[2]Department of Haematology and Blood Transfusion, Edo State University, Uzairue

ARTICLE INFO

Article history:
Received 20 October 2023
Revised 21 April 2024
Accepted 30 April 2024
Published online 01 June 2024

ABSTRACT

Antiretroviral therapy use in the treatment of HIV/AIDS, has previously been linked to anaemia or a decrease in haematologic performance. Given that HIV/AIDS has a significant impact on immune responses, using antioxidants in the treatment of these disease may improve immunological outcomes, as evidenced by haematologic indices. This investigation sought to ascertain the haematological response of Wistar arts treated orally with Lamivudine and Zidovudine along with Vitamins C and E. For 90 days, Wistar rats were given Zidovudine and Lamivodine orally along with vitamin C and E supplements. The rats were divided into groups: one group received Zidovudine and Lamivudine orally at a dose of 15 mg Zidovudine/kg/body and 7.5 mg Lamivudine/kg BW. Vitamin E was also administered at 25 IU/kg BW; the same dose was administered for Vitamin C. The other group received a combination of the antiretroviral drug (ARD) and an antioxidant. The last (or control) group received distilled water at 10ml/kg. At the end of the treatment , the Wistar rats were humanly sacrificed and whole blood collected into Ethylene Diamine tetraacetic acid containers for analysis of haematological indices. The effects of the antiretroviral therapy on red blood indices varied with the ARD administered; there was a significant increase in haematocrit levels in Lamivudine-treated Wistar rats ($p<0.05$). The results also revealed that lymphocyte concentration was more likely to be related with Lamivudine+Vitamin E treatment than with Lamivudine administration alone. Similarly, the combination of Zidovudine and Vitamin C was more likely to impact platelet count concentrations than Zidovudine alone.

***Keywords*:** Lamivudine, Zidovodin, ascorbic acid, α-tocopherol, haematology, platelets

Introduction

In medical practise, acceptable limits for haematological and immune system parameters are frequently employed to evaluate both wellness and disease circumstances. The benchmark limits may also serve as crucial indicators for monitoring the course of a disease or a treatment's effectiveness.[1] These variables vary based on factors such as genetic make-up, environmental factors, age, race, and gender. Clinically, haematological parameters have been deemed extremely important. They have capacity for cellular defence in addition to oxygenation and energy production. Elevated white blood cell counts is a sign of a number of illnesses, such as microbial infections, autoimmune diseases, inflammatory anomalies, injuries, and immune-related abnormalities. Reduction in red blood cell counts could indicate anaemia which results from a myriad of conditions. Apart from human physiological changes caused by disease or exposure to environmental changes, administration of drugs have been linked with changes in haematological parameters. [2,3,4] One of such medication which have been implicated in significant influence of haematological parameters is antiretroviral drug.

*Corresponding author. E mail: atoe.kenneth@edouniversity.edu.ng
Tel: +2348050624628

Citation: Atoe K, Idogun SE, Nwagu MU. Pattern of Haematological Indices of Wistar Rats Administered Antiretroviral Therapy Singly or in Combination with Ascorbic Acid and α-Tocophero. Trop J Nat Prod Res. 2024; 8(5): 7272-7277. https://doi.org/10.26538/tjnpr/v8i5.32

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Aside from the human papillomavirus (HPV), herpes simplex virus (HSV), hepatitis B and C, and human herpesvirus-8 (HHV-8), antiretroviral treatments or therapies (ART) are particularly crucial for HIV therapy.

In Nigeria, the Human Immunodeficiency Virus (HIV) first gained notoriety in the 1980s. The illness is now widespread and can be found in practically all nations. AIDS, a disease that can be extremely crippling, is brought on by HIV. Despite the extremely high morbidity of HIV/AIDS, there is currently no recognized cure for the condition. HIV/AIDS treatment has undergone a revolutionary change thanks to the development and usage of HAART, a combination of antiretroviral medications. Antiretroviral therapeutic medications have side effects and toxicities much like any other treatment. These medications are life-long for people living with HIV/AIDS. They must deal with these negative or harmful repercussions. Numerous hazardous effects, particularly those on metabolism, the kidney, liver, heart, reproductive system, and endocrine organs such the thyroid and adrenal glands are associated with this phenomenon. Since the majority of people living with HIV or AIDS are young, they frequently take antiretroviral medications for a very long time. Thus, the toxic/side effects of these pharmaceuticals greatly decreased the quality of their lives. Given that the disease burden of HIV/AIDS in Nigeria ranges from 3.2% to 8% of the total population, HIV concerns require a systematic and highly targeted intervention. This study sought to investigate the protective effects of oral antioxidant vitamins (C and E) on the organs and systems of Wistar rats that have received oral HAART therapy on a continuous basis. The study is therefore guided by two major research questions; does giving Wistar rats the antioxidant vitamins C and E lessen the harmful or undesirable effects of zidovudine and lamivudine? What impact would this have on the haematoloigal parameters of the test animals.

Apart from the determination of acute toxicity profile of orally administered lamivudine and zidovudine on Wistar rats, the aim of the study is to determine its impact on haematological parameters. Given that antioxidants are expected to enhance human physiological performance and improve resistance, it is part of the aim of the study determine whether augmenting the drugs with Vitamins C or E would improve haematological outcomes.

Materials and Methods

List of Reagents/Chemicals/Kits/Equipments

Potassium permanganate (Lot 1690; manufactured by TiannjinKermel chemical reagent company limited, china), distilled water (prepared in the laboratory) Na_2HPO_4(Lot: 20161113: Guangdong GuanghuaSei-Tech company, China), NaH_2PO_4 (Lot:20140702; Guangdong GuanghuaSci-Tech company limited, China). H_2SO_4 (code: 00289; LobaChemiePvt, Limited, India), Hydrogen peroxide (code: 00181; LobaChemiePvt, Limited, India) Na_2Co_3(code: 05809; LobaChemie Pvt. Limited, india), $NaHCO_3$ (code; 05894; LobachemiePvt limited, india). EDTA-Disodium (Code: 03730; LobaChemiePvt, Limited, India). Hydrochloric acid (Code; 0017; LobaChemiePvt, Limited, india), Adernaline (code; 06355; LobaChemiePvt limited, india), Pyrogallol(code; 00730; LobaChemie Pvt. Limted, India), Trichloroacertetic acid (Code; 06355; LobaChemiePvt limited, India) Adrenaline (code;06355; LobaChemie Pvt. Limited. India) Pyrogallol (code; 00730; LobaChemie Pvt. Limited. India) Trichloroacetic acid (code;06355; LobaChemiePvt limited, India. Urea kits by Randox, Creatinine kits by Randox, as well as kits for Liver function test, thyroid profile, as well as hormonal profile.

Equipments used included metabolic cages, microplate reader, electronic weighing balance (Kern and sohn GmbH, D-72336 Balingen, Germany) Centrifuge 412B (Techmel and Techmel, USA) spectrophotometer 20D (Techmel and Techmel, USA) rotary microtome (Bright B5143, Hutington, England) LABO trinocular microscope (labo microsystems GmbH, Germany) Omax 9.0 MP USB Digital Microscope camera (OMAX company limited, Korea).

Acute Toxicity Experiment (Lamivudine and Zidovudine)

One hundred Wistar rats (8 weeks old) were used and were randomly divided into ten groups using the methodology. [5] The treated group got Lamivudine at doses of 5, 10, 50, 100, 500, 1000, 1500, 2000, 2500, 3500 and 4000 mg/kg BW/day. For acute toxicity studies of Zidovudine (ZDV), ninety rats (8 weeks old) (10 per group) were administered ZDV. ZDV was suspended in 0.5% methylcellulose and administered at doses of 5, 10, 50, 100, 500, 1000, 1500, 2000, 2500, 3500 and 4000 mg/kg BW/day. Rats were given one treatment after a 7-day acclimatization period. Following the administration of drugs to the rats, number of mortalities were recorded at 14 days.

Ethical Approval

Study approval was obtained from the Faculty of Life Sciences Ethical Committee, University of Benin, Nigeria (Ref: LS231151, dated October 27th, 2020).

Acute toxicity study of vitamin E

Acute toxicity study was not conducted for Ascorbic acid, being a water-soluble vitamin. However, for Vitamin E, a fat-soluble vitamin, toxicity issues might arise due to storage. Acute toxicity study was therefore conducted for Vitamin E. Vitamin E was procured at Pirex Scientific Lts., Benin City, Nigeria. The method of Karber [6] was used in acute toxicity study. One group received each 10, 50, 100, 200, 800, 1200, and 1400 international units (IU)/kg body weight respectively via 2% ethanol, which served as a vehicle for vitamin E. The other group was administered with 10µL/kg of 2% ethanol in water. Total mortality was calculated after 14 days.

Methods

A total of 77 (Wistar rats) were administered antiretroviral drugs (Zidovudine and Lamivodine) with vitamin C and E (ascorbic acid and alpha-tocorpherol) orally for 90 days. The Wistar rats consisted of males and females and were grouped into 7 and sub-grouped into A, B and C. For Sub-group A, Zidovudine was administered to the rats orally at a dose of 15 mg/kg/body, and Lamivudine at a dose of 7.5 mg/kg for 90 days. Vitamin E was also administered at 25 IU/kg BW; the same dose was administered for Vitamin C. Rats in Sub-group B were given Zidovudine and Lamivudine along with antioxidant vitamins C and E and sub-group C received equal volume of distilled water (10 mL/kg) only, also for 90 days.

At the end of the study after the Wistar rats were humanly sacrificed and whole blood was obtained from the abdominal aorta using a sterile syringe, into Ethylene Diamine tetraacetic acid container. Following the methods of Muhammad *et al.*,[7] hamatological parameters were analyzed using the Hematology Analyzer model OS-7222.

Data Analysis

The IBM statistical software for social sciences, Version 23, were used to examine the data. Results were presented as mean and standard error of the mean (SEM). Analyses of variance (ANOVA) was used to compare the parameters for each group When the probability is less than 0.05 ($P<0.05$), or at a 95 % level of confidence, differences in the mean are deemed statistically significant. Graphs were presented for results using GraphPad Prism version 5.

Results and Discussion

The study investigated impact of augmentation of antiretroviral therapies on the improvement of haematolocal parameters. This became necessary due to association of reduced haematological functions with viral ailments and some antiviral therapies. [3,4] Importantly, no mortality of rats during Lamivudine acute toxicity study were observed below a dose of 50 mg/kg body weight (BW) (Figure 1). A dose of 1200 mg Zidovudine/kg BW caused less than 50% mortality (Figure 2). Vitamin E, caused 50% mortality at a dose of 675 IU/kg BW (Figure 3).

White cell counts and differentials of animal model administered antiretroviral therapy is presented in Table 1. Total white blood count (WBC) in control Wistar rats was 13.70 x 10^3. When rats were given zidovudine augmented with Vitamin E, WBC increased significantly to 16.4 x 10^3. Inclusion of Vitamins C and E with either Zidovudine or Lamivudine did not significantly impact on WBC. Lymphocyte (LYM) count was not affected by administration of antiretroviral drugs (ARD) augmented with Vitamins C and E (herein coded as A3RD). However, administration of LAM significantly reduced lymphocyte count by 30% (Table 1). Impact of antioxidants-augmented antiretroviral drug (A3RD) on Percentage granulocyte (GRA) was minimal ($p>0.05$). The general impact of ARD on the WBC differentials was non-significant ($p=0.2229$) (Table 2).

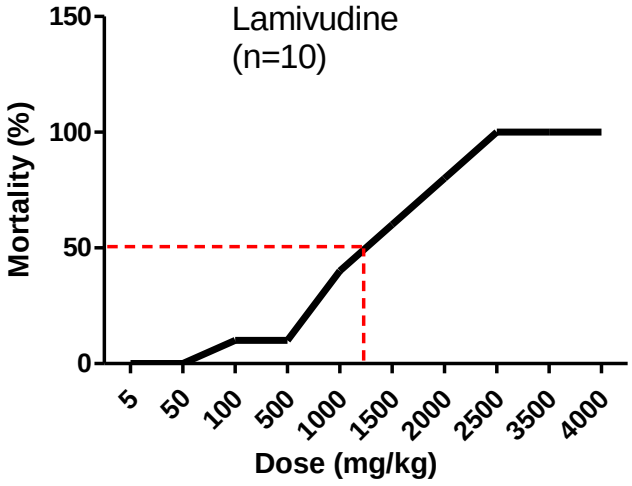

Figure 1: Rat mortality during acute toxicity study of lamivudine

Table 3 shows the red cell counts and indices of animal administered antiretroviral therapy. Results showed that antioxidant-augmented ARD had no significant effects on red blood cell counts (RBC) ($p>0.05$). Similarly, haemoglobin concentration was not affected by the ARD regimens; concentration of haemoglobin (HGB) ranged from 13.97 – 15.43 g/dl, while haematocrit concentration (HCT), or pack cell volume (PCV) ranged from 33.03 – 39.30% ($p>0.05$). Observably, there was a 18% increase in HCT due to administration of LAM only. Generally, there was no indicated anaemia due to administration of ARD or antioxidants-augmented antiretroviral drug (A3RD) (Table 3). Range of values for mean corpuscular volume (MCV) was 55.03 – 57.67 fl; mean corpuscular haemoglobin (MCH) ranged from 22.43 – 24.00 pg/cell. These indices as well as those of mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) were not affected by the ARD or A3RD administered to the Wistar rats. As with the WBC the treatments did not generally impact on observable minimal changes in red blood indices (Table 4).

No observable changes in platelet counts (PLT) were observed in the study (Table 5). Mean platelet volume (MPV) ranged from 6.97 – 7.17 fl ($p>0.05$). Plateletcrit (PCT) ranged from 0.44 – 0.60% ($p>0.05$). No observable changes in values of Platelet volume distribution width (PDW) and Platelet larger cell ratio (P-LCR) were not affected by administering either ARD or A3RD to the Wistar rats. No significant interaction between Antiritroviral treatment and platelet parameters were observed

Table 7 presents the correlation between white blood cell differentials and both red blood cell and platelet indices during antiretroviral therapy. Although significant negative bivariate correlation existed between total WBC and RBC; this was however very weak association ($R=-0.523$, $p=0.015$). Similar weak association existed between WBC and HCT; and between LYM and HGB respectively.

Anaemia has been linked to illness progression and poor clinical outcomes in people suffering one or both diseases. Low red blood cell counts, according to Belperio and Rhew, [8] decrease the clinical outcomes of HIV/AIDS patients. Calis and colleagues [9] have suggested that the incidence of severe anaemia among children with HIV-1 might be more than 25%. Kibaru et al.[10] discovered substantial anaemia related with the use of Zidovudine in HIV-1 affected children. Impact of natural products on haematological indices have also been reported.[11] Red cell aplacia have been described by John et al.[12] in red blood cells due to adiministration of Lamivudin.

It was important to establish association between haematological parameters and type of antiretroviral therapy (Figure 4). Results showed that LYM and P-LCR were more associated with Lamivudine+Vitamin E than with Lamivudine only. Similarly, the use of Zidovudine+Vitamin C was most likely to influence the concentrations of PCT and PLT than when Zidovudine was aumeneted with Vitamin E.

In this investigation, the use of lamivudine and zidovudine had no significant effect on haematological markers such as red blood cell counts and white blood cell counts. When compared to the control, the use of Lamivudine resulted in significantly higher haematocrit levels. The incorporation of Vitamins C and E had no effect on the haematological results.

Conclusion

The researchers hypnotized that Wistar Rats that are given antioxidant supplements should tolerate antiretroviral drugs more and should be generally look better and healthier, than the rats that receive the antiretroviral drugs without antioxidants. Similarly, it was originally expected that haematological functions of the animals with antioxidants supplements should be better and suffer less oxidative injuries. However the study showed that the antiretroviral drugs had no effect on haematological indices or differentials. However, haematocrit levels increased significantly in Lamivudine-treated Wistar rats. The findings also showed that lymphocyte concentration was more likely to be associated to Lamivudine+Vitamin E therapy than Lamivudine administration alone. Similarly, the combination of Zidovudine and Vitamin C was more likely than Zidovudine alone to affect platelet count concentrations.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The researchers are grateful to the Tertiary Education Trustfund (TETFund) for providing funding for this study. They are also grateful to Prof. Beckley Ikhajiagbe of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria, for his criticisms and also of providing assistance with data analyses

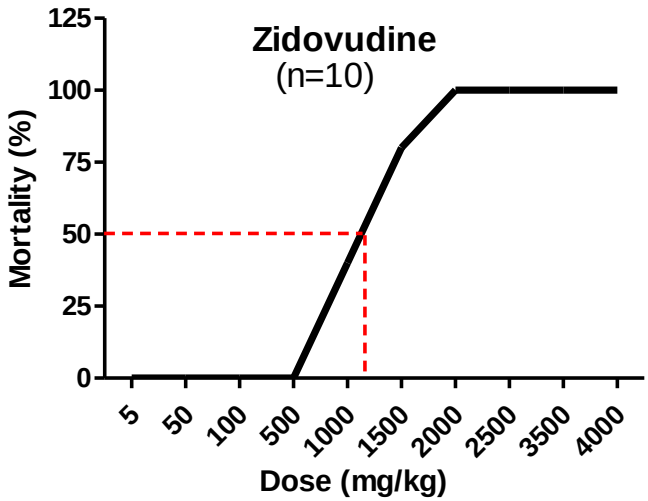

Figure 2: Acute oral toxicity of zidovudine on Wistar rat

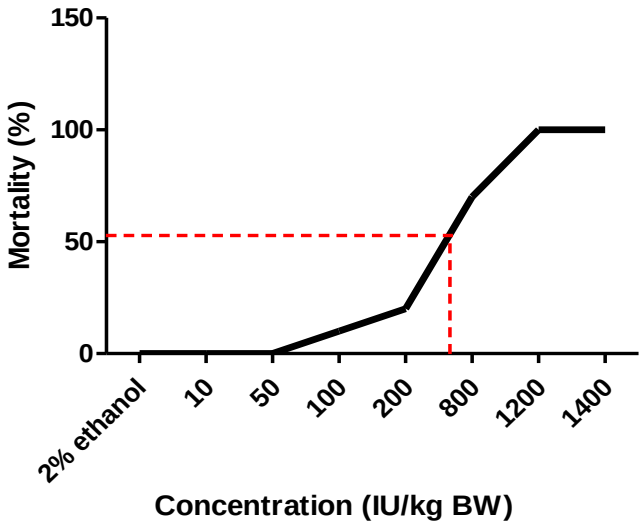

Figure 3: Acute oral toxicity of vitamin E on Wistar rat

Table 1: White cell counts and differentials of animal administered antiretroviral therapy

Treatment	Total WBC ($x 10^3$)	LYM count ($x 10^3$)	MON count ($x 10^3$)	GRAN count ($x 10^3$)	LYM (%)	MO (%)	GRA (%)
Normal	13.70^{ab}	10.13^{ab}	1.63^{ab}	1.93^{ab}	74.00^{a}	12.03^{a}	13.97^{ab}
Lam.	10.47^{a}	7.47^{a}	1.53^{ab}	1.43^{ab}	71.37^{a}	14.73^{a}	13.90^{ab}
Lam + Vit C	12.10^{ab}	8.90^{ab}	1.67^{a}	1.53^{ab}	73.27^{a}	14.13^{a}	12.60^{ab}
Lam + Vit E	11.20^{ab}	8.73^{ab}	1.33^{ab}	1.07^{a}	77.73^{a}	12.23^{a}	10.03^{a}
Zid.	12.40^{ab}	9.17^{ab}	1.53^{ab}	1.70^{ab}	73.13^{a}	12.17^{a}	14.10^{ab}
Zido + Vit C	13.50^{ab}	9.30^{ab}	2.13^{ab}	2.03^{b}	68.90^{a}	16.13^{a}	14.97^{b}
Zido + Vit E	16.40^{b}	12.47^{b}	2.20^{b}	2.00^{ab}	74.97^{a}	13.23^{a}	11.80^{ab}
F-statistics	1.327	1.265	1.660	1.613	1.087	0.884	1.715
p-value	0.309	0.334	0.203	0.216	0.416	0.532	0.190

Table 2: Summary ANOVA Table for results of white cell indices during antiretroviral therapy

Source of Variation	df	Sum of Squares	Mean square	% of total variation	P value
Antiritroviral treatments	6	41.8	6.967	0.05	0.4345
WBC differentials	6	78510	13080	98.7	< 0.0001
Interaction	36	307.4	8.54	0.39	0.2229
Residual (error)	98	687.7	7.017		
Total	146	79540			

Table 3: Red cell counts and indices of animal administered antiretroviral therapy

Treatment	RBC ($x 10^6$)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg/cell)	MCHC (g/dl)	RDW (%)	
							CV	SD
Normal	6.04^{a}	14.50^{a}	33.03^{b}	56.90^{a}	24.00^{a}	42.23^{a}	14.40^{a}	30.23^{a}
Lam.	6.97^{a}	15.43^{a}	39.30^{a}	56.30^{a}	22.73^{a}	40.33^{a}	15.53^{a}	31.30^{a}
Lam + Vit C	6.70^{a}	15.30^{a}	36.93^{ab}	55.17^{a}	22.83^{a}	41.43^{a}	15.13^{a}	30.83^{a}
Lam + Vit E	6.22^{a}	13.97^{a}	34.17^{ab}	55.03^{a}	22.43^{a}	40.83^{a}	15.23^{a}	30.87^{a}
Zid.	6.61^{a}	15.53^{a}	36.87^{ab}	55.73^{a}	23.47^{a}	42.13^{a}	14.53^{a}	30.17^{a}
Zido + Vit C	6.16^{a}	14.73^{a}	35.53^{ab}	57.67^{a}	23.90^{a}	41.47^{a}	14.93^{a}	32.27^{a}
Zido + Vit E	5.86^{a}	13.83^{a}	34.37^{ab}	56.93^{a}	23.60^{a}	41.57^{a}	15.13^{a}	31.90^{a}
F-statistics	1.572	0.883	1.025	0.819	1.668	1.347	0.675	0.501
p-value	0.227	0.532	0.449	0.573	0.202	0.301	0.672	0.797

Table 4: Summary ANOVA Table for results of red blood cell indices during antiretroviral therapy

Source of Variation	Degrees of Freedom	Sum of Squares	Mean square	% of total variation	P value
Antiritroviral treatments	6	17.99	2.999	0.05	0.3434
Red blood cell parameters	7	39530	5647	98.97	< 0.0001
Interaction	42	97.58	2.323	0.24	0.669
Residual (error)	112	294.4	2.628		
Total	167	39940			

Table 5: Platelet counts and indices of animal model exposed to antiretroviral therapy

Treatment	PLT (x10^3)	MPV (fl)	PCT (%)	PDW (%)	P-LCR (%)
Normal	735.00^a	7.07^a	0.52^a	15.57^a	6.77^a
Lam.	722.67^a	6.90^a	0.50^a	15.93^a	6.13^a
Lam + Vit C	849.00^a	7.07^a	0.60^a	16.10^a	6.60^a
Lam + Vit E	639.00^a	6.97^a	0.44^a	15.87^a	6.47^a
Zid.	757.00^a	7.03^a	0.53^a	15.80^a	6.30^a
Zido + Vit C	808.00^a	7.17^a	0.56^a	15.40^a	5.90^a
Zido + Vit E	733.33^a	7.03^a	0.52^a	15.40^a	6.43^a
F-statistics	0.567	0.189	0.584	0.449	0.177
p-value	0.750	0.975	0.738	0.834	0.979

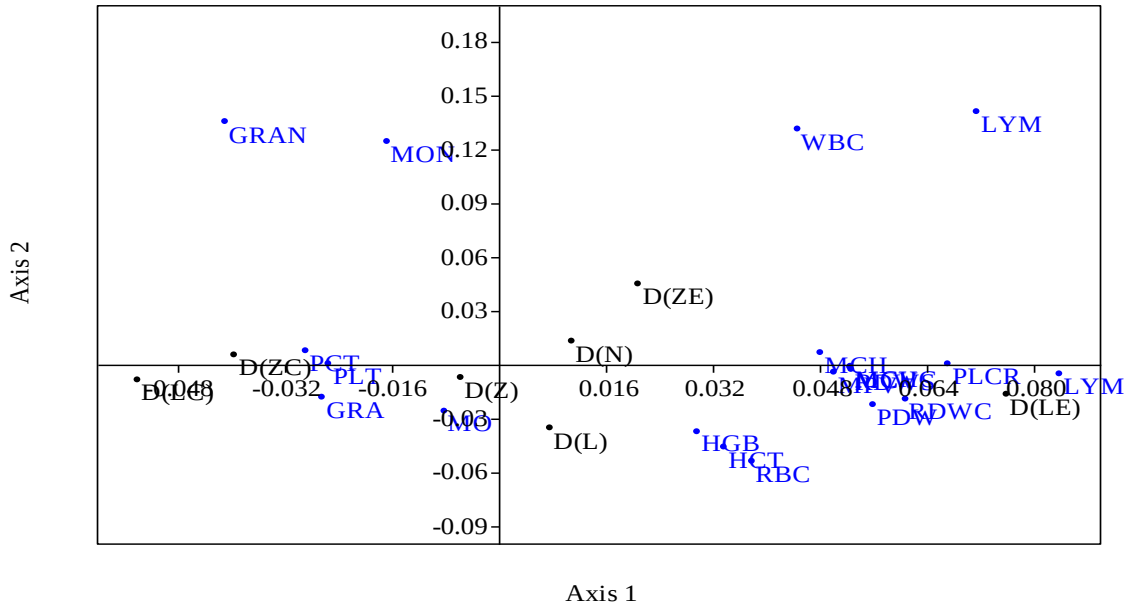

Figure 4: Correspondence analysis biplot showing association between haematological parameters and type of antiretroviral therapy

Table 6: Summary ANOVA Table for results of platelet counts and indices during antiretroviral therapy

Source of Variation	Df	Sum of Squares	Mean square	% of total variation	P value
Antiritroviral treatment	6	16100	2684	0.17	0.7544
Platelet parameters	4	9245000	2311000	95.74	< 0.0001
Interaction	24	64240	2677	0.67	0.9397
Residual (error)	70	330800	4725		
Total	104	9656000			

Table 7: Correlation between white blood cell differentials and both red blood cell and platelet indices during antiretroviral therapy

		Total WBC	LYM Count	MON Count	GRAN Count	LYM	MO	GRA
RBC	R	***-0.523****	***-0.527****	-0.370	-0.293	-0.258	0.196	0.278
	p-value	0.015	0.014	0.099	0.198	0.258	0.394	0.222
HGB	R	-0.420	***-0.443****	-0.282	-0.125	-0.317	0.181	0.412
	p-value	0.058	0.044	0.216	0.590	0.161	0.433	0.063
HCT	R	***-0.436****	***-0.441****	-0.308	-0.190	-0.265	0.172	0.324
	p-value	0.048	0.045	0.174	0.409	0.246	0.456	0.151
MCV	R	0.222	0.216	0.156	0.275	-0.028	-0.061	0.141
	p-value	0.334	0.346	0.501	0.228	0.904	0.793	0.542
MCH	R	0.216	0.166	0.200	0.386	-0.164	-0.011	0.335
	p-value	0.348	0.473	0.385	0.084	0.477	0.961	0.138
MCHC	R	0.062	-0.005	0.112	0.241	-0.199	0.051	0.313
	p-value	0.791	0.984	0.629	0.293	0.386	0.826	0.168
RDWC	R	-0.063	-0.013	-0.132	-0.178	0.149	-0.111	-0.200
	p-value	0.787	0.956	0.569	0.441	0.521	0.631	0.384
RDWS	R	0.066	0.120	-0.029	-0.046	0.171	-0.162	-0.163
	p-value	0.776	0.605	0.901	0.842	0.459	0.482	0.481
PLT	R	0.300	0.273	0.143	0.348	-0.033	-0.109	0.174
	p-value	0.187	0.231	0.535	0.122	0.888	0.639	0.451
MPV	R	0.247	0.239	0.129	0.299	0.036	-0.155	0.087
	p-value	0.280	0.297	0.578	0.188	0.876	0.501	0.706
PCT	R	0.342	0.328	0.131	0.366	0.028	-0.183	0.138
	p-value	0.129	0.146	0.571	0.102	0.904	0.427	0.551
PDW	R	-0.187	-0.274	0.034	0.086	-0.336	0.261	0.355
	p-value	0.418	0.230	0.883	0.709	0.137	0.253	0.114
PLCR	R	0.225	0.255	0.039	0.179	0.186	-0.243	-0.090
	p-value	0.327	0.264	0.868	0.438	0.419	0.288	0.699

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

References

1. Dadkhah M, Matin S, Safarzadeh E, Rezaei N, Negaresh M, Salehzadeh H, Matin S, Sharifiazar A, Abazari M. Hematological Parameters as Diagnostic Factors: Correlation with Severity of COVID-19. Acta Biomed. 2022;93(2):e2022061. doi: 10.23750/abm.v93i2.12320.
2. Takami A. Molecular Immunology in Hematological Disorders. Int J Mol Sci. 2022;23(17):9584. doi: 10.3390/ijms23179584.
3. McLean E, Cogswell M, Egli I, Wojdyla D, De Benoist B. Worldwide prevalence of anaemia WHO vitamin and mineral nutrition information system 1993–2005. Public Health Nutr 2009; 12(4):444–454
4. Assefa M, Abegaz WE, Shewamare A, Medhin G, Belay M. Prevalence and correlates of anemia among HIV infected patients on highly active anti-retroviral therapy at Zewditu Memorial Hospital Ethiopia. BMC Hematol 2015; 15(1):6
5. Wang W, Dong Z, Zhang J, Zhou X, Wei X, Cheng F, Li B, Zhang J. Acute and Subacute Toxicity Assessment of Oxyclozanide in Wistar Rats. Front Vet Sci 2019; 6:294 doi: 103389/fvets201900294
6. Karber G. Reihenversucbe. Arch Exper Pathol Pharmacol 1991; 162: 480-483.
7. Muhammad NO, Akolade JO, Usman LA, Oloyede OB. Haematological parameters of alloxan-induced diabetic rats treated with leaf essential oil of *Hoslundia opposita* (Vahl). EXCLI J. 2012;11:670-6.
8. Belperio PS, Rhew DC. Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature. Am J Med 2004; 116(7):27–43.
9. Calis JC, van Hensbroek MB, de Haan RJ, Moons P, Brabian BJ, Bates I. HIV-associated anemia in children: systemic review from global perspective. AIDS 2008; 22(10):1099–1112 doi: 101097/QAD0b013e3282fa759f
10. Kibaru EG, Nduati R, Wamalwa D, Kariuki N. Impact of highly active antiretroviral therapy on hematological indices among HIV-1 infected children at Kenyatta National Hospital-Kenya: retrospective study. AIDS Research and Therapy 2015; 12 26 https://doiorg/101186/s12981-015-0069-4
11. Ogeyemhe BE, Amaechi RA, Ekpruke CD, Airiagbonbu BO, Odigie EB. comparative benefits of cocos nucifera l. husk, milk and shell extracts on body weight changes and haematological indices in male Rats: Trop J Nat Prod Res 2020; 21: 4(8):455-62. doi.org/10.26538/tjnpr/v4i8.22.
12. John MA, Rhemtula YA, Menezes CN, Grobusch MP. Lamivudine-induced red cell aplasia. J. of Med Micro 2008; 57(Pt 8) 1032–1035 https://doiorg/101099/jmm047782-0