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Research Article

Hypoxis hemerocallidea Changes Hematological and Biochemical Indices in Spontaneously Hypertensive Rats under Highly Active Antiretroviral Therapy

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Abstract

Context: While highly active antiretroviral therapy (HAART) is very effective in treatment of HIV-infected patients, its deleterious effects on hematological and biochemical indices in hypertensive conditions are not well understood. Concomitant *Hypoxis hemerocallidea* Fisch. and C.A Mey (*Hypoxideceae*)-herbal therapy with HAART is a common practice in HIV management in South Africa, however, there is scant scientific evidence for this adjuvant therapy.

Objective: Evaluating effects of HAART and H. hemerocallidea on hematological and biochemical indices in hypertension.

Material and Methods: Thirty SHR and five SD rats (adult males) randomly divided into seven groups were used. HAART combinations at their standard recommended doses and HH were administered orally daily for 56 days. In the morning of 57th days after overnight fasting, blood samples were collected and analyzed for hematological and biochemical indices.

Results: Neutrophil-lymphocyte ratio and Platelet revealed statistically significant increases ($p<0.01$) in HAART and HAART + HH (100 mg/kg) groups. Biochemical indices showed a statistically significant ($p<0.05$) increase in their values in the HAART alone group and a significant reduction ($p<0.03$) in groups with adjuvant treatment with HAART.

Discussion: Previous reports suggested an increasing frequency of hypertension and other non-communicable diseases among HIV positive patients. Some authors believe this is due to the side effects of HAART, others have argued that these effects are because of the aging of the HIV infected population due to their increased survival in the post-HAART era. Our study show that HAART can exacerbate hypertension, which plant extract with antihypertensive property can mitigate under HAART insult.

Keywords: HAART; *Hypoxis hemerocallidea*; Hematology Indices; Biochemical Parameters

Introduction

Highly active antiretroviral therapy (HAART) has been of great importance in the treatment of HIV-infected patients resulting in reduced morbidity, mortality and impairment of hematological complications [1]. However, the use of HAART has been accompanied by an increase in undesirable co-morbid side effects such as metabolic complications, disposition to diabetes and cardiovascular diseases (CVDs) [2]. Hypertension is an important risk factor for CVDs and it is a multifactorial disease arising from the combined action of many environmental, behavioral and genetic variants especially in HIV positive patients, as well as oxidative stress and inflammation which may result from drug use [3,4].

Reports [5,6] suggests increasing frequency of hypertension among HIV- infected patients on HAART especially in combinations which includes protease inhibitors (PI); whether these trends are due to direct effect of HAART or to aging of the HIV population due to increasing survival rate of the patient in the post HAART era is still debatable [7]. Indeed, in developed countries with unlimited access to HAART, CVDs has become one of the major causes of

death in HIV infected patients. In developing countries, however, with a delayed roll-out of antiretroviral therapy pericardial disease (often related to TB), HIV-associated cardiomyopathy, and HIV-associated pulmonary hypertension are the most common cardiac manifestations in HIV infected patients [8]. The pathogenesis of hypertension is complex, and probably results from the interaction of multiple modulating genes with environmental factors. The physiopathology includes increase cytokines secretion which induces dysregulation of endothelial and vascular smooth muscle cell growth and imbalance of endogenous vasodilators and constrictors [9].

Of great importance in understanding progression of hypertension are hematological indices, particularly red cell distribution width (RDW), neutrophil-lymphocyte ratio (NLR), and mean platelet volume (MPV) which are established markers of systemic inflammation and vascular pathology [10-14]. Correlation between these hematological indices and hypertension are relevant in predicting the severity of hypertension and end organ damage [15-18]. Also, various biochemical parameters such as serum electrolyte (Na, K and Cl), lipid profiles (Total cholesterol (TCh), High density lipoproteins (HDL), Low density lipoproteins (LDL), Total glyceride (TG), Total proteins (TP), and TCh: HDL ratio), kidney and liver function tests are of importance in assessing progression of hypertension.

The co-morbid conditions potentially associated with HAART has resulted in a more holistic approach to the management of HIV, especially in Sub-Saharan Africa (SSA), where co-administration of herbal based alternative traditional medicine (ATM) is a common practice. An estimated 80% of Africans are said to rely on ATM for the treatment of various pathological conditions [19]. However, not all of these substances have been researched scientifically to validate their efficacy and efficiency. One of such highly consumed herb especially in the treatment of HIV/AIDS is *Hypoxis hemerocallidea* Fisch. and C.A Mey. (HH) [19,20]. It has also been used for a wide range of treatments such as infertility and treatment of various sexual related disorders [21]. Reports have also been documented of its antidiarrheal, antimicrobial and antioxidant properties [22-25].

Understanding the effect of HAART and HH on hypertension is crucial, as there is scarcely any documented report regarding the effect of the co-administration of HAART and HH in hypertensive state. This study, therefore, documents the effect of HAART on progression of hypertension and whether the co-administration of HH would exacerbate or mitigate the blood pressure elevation using hematological and biochemical indices in characterized rat model.

Materials and Methods

Ethics approval

The University of KwaZulu Natal Animal Research Ethics Committee approved the experiment procedures and assigned Reference Number: 008/15/ANIMAL based on the National Centre for the Replacement, Refinement and Reduction of Animal in Research (NC3Rs) Guideline. The study was conducted at the Biomedical Resource Unit animal housing of the University. The animals received humane care in accordance with the principle of Laboratory Animal Care of the National Medical Research Council and the Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health Publication no. 80-23, revised 1978).

Chemicals/drugs

The antiretroviral drugs [Lamivudine (3TC), Stavudine and Nevirapine (Aspen)] were purchased from Pharmacare Ltd, Port Elizabeth, South Africa.

Extraction of *Hypoxis hemerocallidea*

The *Hypoxis hemerocallidea* (HH) fresh corms were bought from a local herb shop in Umbilo Road, Durban, KwaZulu Natal, between June and July 2014. The corm was authenticated by Mr. Khathi Edward at the Department of Life Science, Westville Campus of University of KwaZulu- Natal. Durban, South Africa and a voucher specimen kept in the department herbarium. HH fresh corms were extracted as described by Ojewole [26]. The corms were washed with water, cut into smaller pieces, air-dried at room temperature (25-28˚C) and grounded into powdered form in a commercial blender. The milled corm was soaked in hot distilled water and extracted twice, using on each occasion with 2.5 L of hot distilled water (at 90-100˚C) for 12 h. The combined extracts were concentrated to dryness in a rotary evaporator at 70 ± 1˚C and the resulting crude aqueous extract was freeze-dried. Without any further purification, aliquot portions of the extract were weighed and dissolved in distilled water at room temperature for use on each day of the experiment.

Animal management and experimental design

We used thirty Spontaneous Hypertensive Rats (SHR) and five Sprague-Dawley (SD), all adult male, 16 weeks old, weighing between 242.0 ± 0.71 and 286.0 ± 0.70 g for the study. The rats were selected from the colony maintained under regulated animal house conditions of temperature, humidity and 12 h day/night cycle in the Animal Holding Facility, Biomedical Resource Unit of the University of KwaZulu Natal, Westville Campus, South Africa. All rats were housed in plastic cages (5 rats/cage) having dimension of 36 x 24 x 15 cm and soft wood shavings employed as bedding in the cages and allowed free access to food and drinking water.

The animals were divided into seven groups of five rats per group as shown in Table 1.

Groups	Treatment
A	(SD Rats) Normotensive –ve control
B	(SHR) Hypertensive –ve control
C	(SHR) HAART (a cocktail of Lamivudine, Stavudine and Nevirapine at 300, 600 and 400 mg/kg respectively) administered as a daily dose.
D	(SHR) HAART + HH extract (100 mg/kg)
E	(SHR) HAART + HH extract (200 mg/kg)
F	(SHR) HH extract alone (100 mg/kg)
G	(SHR) HH extract alone (200 mg/kg)

Table 1: Grouping of the rats into various experimental protocol.

All drugs and extracts were administered by oral intubation once daily and distilled water served as the vehicle which was also administered to the control groups as placebo.

Anthropometric measurements and blood pressure

For each of the groups basic anthropometric measurements such as body weights (BW), mid-body circumference (MBC), body length (BL) and BMI of the rats were recorded on the first day before the commencement of the experiment, thereafter weekly and on the last day shortly before animal sacrifice. On weekly basis, the blood pressure was non-invasively measured using Tail Cuff machine for blood pressure (IITC Life Science MRBPsystem, USA) which takes three readings for the Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Mean Arterial Pressure (MAP), and heart rate (HR). The average of the three readings was used as the representative examination value. These measurements were performed under controlled conditions in a quiet room. In this study, the SHR animals were defined hypertensive at diastolic blood pressure (DBP) of ≥100 mmHg or systolic blood pressure (SBP) of ≥140 mmHg taken from an average of three readings.

Collection of samples for hematological and biochemical analyses

The experiment lasted for 56 days; all the animals were weighed and sacrificed 24 h after the last treatment by Halothane® inhalation for 3 min via a gas anesthetic chamber (100 mg\kg). Blood samples were aseptically collected by cardiac puncture after overnight fasting separately into two tubes: one to determine blood count (hematological assessments) into a 3 mL anticoagulated bottle (1 g/L K2 EDTA) and the other for biochemical analysis of serum into a 5 mL BD Vacutainer® SST™ II Advance bottle. The hematological analyses such as WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, NE, LY, MO, EO and BA were carried out using automated blood analyzer (Beckham Coulter® Ac. T™ 5diff OV). Blood samples collected into serum bottle were allowed to stand for 30 min; centrifuged at 3000rpm for 15 min in a Beckman bench centrifuge and the serum was decanted into Eppendorf tubes. Serum total bilirubin (T.Bil), total protein (TP), Albumin (ALB), total cholesterol (TCh), triglycerides (TG), High density lipoprotein- cholesterol (HDL) and Low density lipoprotein-cholesterol (LDL) were determined by colorimetric methods. The obtained hematological and biochemical values were supplemented by calculation of Neutrophil-lymphocyte ratio (NLR), TCh: HDL, LDL: HDL and TG: TCh ratios.

Statistical analysis

Data are presented as mean ± SEM and were analyzed using either a one-way or a two-way ANOVA followed by a Bonferroni post-hoc test for differences between groups. Changes in body weight and blood pressure by group and time point were analyzed using a repeated-measures two-way ANOVA. Statistical significance was set at $p < 0.05$ using Graph Pad Prism® version 5.2.

Result

Anthropometric measurements and blood pressure

Table 2 showed that the percentage body weight difference in all the hypertensive groups (B-G) were statistically significantly higher ($p < 0.001$) compared to the non-hypertensive group A. However, no statistically significant difference was recorded for body weight differences when comparing the hypertensive groups only. Other anthropometric measurements such as MBC, BL and BMI percentage differences also followed a similar pattern when

the non-hypertensive animals were compared with the hypertensive groups. However, there were statistically significant differences in values of these parameters between the hypertensive groups. A significant decrease ($p < 0.030$) was recorded between group B (SHR -ve control) when compared with groups C, E, F and G (HAART alone, HAART + HH 200 mg/kg, HH 100 mg/kg and HH 200 mg/kg respectively) and between group B compared to D (HAART + HH 100 mg/kg) and group C compared to D, in percentage MBC difference (both with a p-value of 0.001).

Concerning percentage BL difference, a statistically significant decrease ($p < 0.030$) was recorded between the values of group B and C while a moderately high significant decrease was recorded when group C was compared with groups D, E and F.

The observed values for BMI showed a statistically significant ($p < 0.010$) decrease when group B was compared with groups C, whereas a statistically significant elevation ($p < 0.001$) was recorded when group C was compared with groups D, E, F and G and a non-significant difference in comparison between group B and F (Table 2).

	Initial BW (g)	Final BW (g)	BW % Diff	Initial MBC (cm)	Final MBC (cm)	MBC % Diff	Initial BL (cm)	Final BL (cm)	BL % Diff	BMI
Group A	255.40 ± 8.18	365.00 ± 11.62	42.90	13.60 ± 0.44	18.68 ± 0.41	37.35	18.22 ± 0.52	21.80 ± 0.50	19.65	11.1
Group B	250.60 ± 1.91	300.40 ± 4.02	19.87	12.76 ± 0.54	17.46 ± 0.27	36.94	19.40 ± 0.25	21.40 ± 0.24	10.31 [a]	18.69
Group C	257.20 ± 4.85	305.00 ± 9.76	18.58[a]	14.14 ± 0.06	18.10 ± 0.40	28.00[b]	20.06 ± 0.15	21.62 ± 0.50	7. 78 [a]	30.69 [b,c]
Group D	286.80 ± 5.31	336.40 ± 10.07	17.29[a]	15.74 ± 0.39	18.28 ± 0.26	16.14[c]	19.68 ± 0.61	23.30 ± 0.35	18.39 [ab]	5.11 [b,c]
Group E	273.00 ± 2.91	330.60 ± 5,09	21.09[a]	14.60 ± 0.19	17.96 ± 0.24	23.01 [b]	19.16 ± 0.36	22.54 ± 0.45	17.64 [ab]	6.78 [b,c]
Group F	242.60 ± 8.78	301.20 ± 7.14	24.16 [a]	14.18 ± 0,42	17.84 ± 0.35	25,81[b]	18.94 ± 0.44	21.92 ± 0.33	15.73 [ab]	9.76 [b,c]
Group G	244.80 ± 10.10	290.20 ± 19.06	18.55 [a]	13.66 ± 0.50	17.10 ± 0.89	25.18[b]	19.86 ± 0.45	21.92 ± 0.52	10.37 [ab]	17.24 [b,c]

Table 2: Anthropometric parameters of different experimental groups**.**

Values with the same superscripts are statistically significant for values across groups for a particular parameter

a- compared to Group A; b- compared to Group B; c- compared to Group C

BW- Body weight MBC- Mean Body circumference BL- Body Length

BMI- Body Mass Index % Diff- Percentage Difference between Final value and Initial value

The comparison of the blood pressure readings in Table 3 showed that the Systolic as well as diastolic blood pressure were higher in all the hypertensive groups from B to G (170.00 ± 2.59, 241.20 ± 10.97, 229.00 ± 2.35, 236.60 ± 3.20, 188.80 ± 4.40 and 186.60 ± 0.60 respectively) than in the non-hypertensive group A (123.80 ± 0.58). The mean SBP % difference showed highly significant increase in groups C (29.12%) and E (20.41%) ($p<0.0001$) and a moderate significant increase in groups D (15.54%) and F (13.60%) when all are compared to the group B (-1.24%).

	Initial SBP (mm/Hg)	Final SBP (mm/Hg)	SBP % Diff	Initial DBP (mm/Hg)	Final DBP (mm/Hg)	DBP % Diff
Group A	126.20 ± 5.30	123.80 ± 0.58	-1.90	96.60 ± 6.40	96.20 ± 1.02	-0.40
Group B	178.20 ± 5.85	176.00 ± 2.59	-1.24	128.20 ± 2.80	125.80 ± 4.79	-1.87
Group C	186.80 ± 12.72	241.20 ± 10.97	29.12[ab]	169.80 ± 12.70	171.40 ± 1.63	1.78
Group D	198.20 ± 1.88	229.00 ± 2.35	15.54 [abc]	168.40 ± 9.72	181.80 ± 1.99	7.96[cd]
Group E	196.00 ± 2.55	236.60 ± 3.20	20.41 [abd]	144.60 ± 8.56	172.80 ± 1.72	19.59 [cd]
Group F	166.20 ±12.69	188.80 ± 4.40	13.60 [abcd]	138.80 ± 11.63	140.00 ± 1.76	0.86
Group G	175.80 ± 2.33	186.60 ± 0.60	6.14 [abcd]	129.40 ± 1.63	134.40 ± 2.10	3.86

Table 3: Systolic (SBP) and Diastolic (DBP) Blood Pressure in groups

Values with the same superscripts are statistically significant for values across groups for a particular parameter a- compared to Group A; b- compared to Group B; c- compared to Group C; d- compared to Group D.

SBP- Systolic Blood Pressure

DBP- Diastolic Blood Pressure

% Diff- Percentage Difference between Final value and Initial value

Hematological parameters

The results of the hematological parameters revealed that there were no statistically significant differences in the values of most of the parameters except for the platelet (PLT) count when comparison was made between all the groups using a Two-Way ANOVA followed by Bonferroni post-test (Table 4). PLT count mean value recorded a highly statistically significant decrease ($p < 0.001$) in group C (SHR + HAART) and group G (SHR + HH 200 mg/kg) compared with group A (Normotensive control). Also, a highly statistically significant decrease ($p < 0.001$) was recorded in the PLT value of group C compared with group B (SHR +ve control). However, comparison between the group C with either groups D or group E (SHR + HAART + HH 100 mg/kg and SHR + HAART + HH 200 mg/kg respectively) showed a moderately statistically significant decrease ($p < 0.01$), while the comparison of group C with either groups F or group G (SHR + HH 100 mg/kg and SHR + HH 200 mg/kg respectively) showed a highly statistically significant decrease in PLT count ($p < 0.001$). Another hematological parameter of prognostic importance is the Neutrophils - Lymphocyte Ratio (NLR) which also revealed some level of statistically significant increases in groups C and D when compared with the control groups A and B ($p < 0.01$).

	Group A	Group B	Group C	Group D	Group E	Group F	Group G
WBC (10^3/L)	5.94 ± 0.92	3.30 ± 0.27	3.02 ± 0.70	3.18 ± 0.06	3.22 ± 0.40	2.94 ± 0.18	4.76 ± 0.72
RBC (10^3/L)	7.64 ± 0.31	8.67 ± 0.09	8.37 ± 0.40	8.99 ± 0.014	8.714 ± 0.12	8.60 ± 0.12	8.534 ± 0.17
HGB (g/dL)	13.52 ± 0.56	13.68 ± 0.17	12.86 ± 0.67	13.98 ± 0.31	13.84 ± 0.27	13.56 ± 0.33	13.28 ± 0.33
HCT (%)	42.40 ± 1.87	43.32 ± 0.46	41.28 ± 2.09	44.80 ± 0.97	44.10 ± 0.85	43.18 ± 0.97	42.50 ± 1.09
MCV (fL)	55.60 ± 0.40	49.80 ± 0.20	49.40 ± 0.24	50.00 ± 0.71	50.60 ± 0.51	50.00 ± 0.55	48.00 ± 2.03
MCH (Þg)	17.70 ± 0.09	15.72 ± 0.10	15.38 ± 0.12	15.58 ± 0.21	15.90 ± 0.16	15.76 ± 0.17	15.58 ± 0.14
MCHC (g/dL)	31.92 ± 0.23	31.52 ± 0.09	31.16 ± 0.13	31.26 ± 0.06	31.38 ± 0.07	31.36 ± 0.40	31.32 ± 0.11
RDW (%)	11.08 ± 0.14	11.66 ± 0.20	12.62 ± 0.10	12.48 ± 0.10	12.48 ± 0.26	12.26 ± 0.21	12.34 ± 0.22
PLT (10^3/L)	713.80 ± 43.56^{ab}	704.00 ± 33.93^{cd}	597.60 ± 118.55^{acefg}	666.20 ± 36.55^{e}	692.40 ± 15.79^{f}	687.20 ± 11.43^{g}	635.60 ± 24.76^{bd}
MPV (fL)	5.84 ±0.01	5.84 ± 0.02	6.02 ± 0.09	5.88 ±0.04	5.68 ±0.10	5.70 ± 0.09	5.94 ± 0.14
NE (%)	0.64 ± 0.09	0.39 ± 0.06	0.52± 0.15	0.51± 0.03	0.40 ±0.03	0.38 ± 0.03	0.60 ± 0.14
LY (%)	4.72 ± 0.66	2.53 ± 0.21	2.06 ± 0.45	2.13 ±0.06	2.50 ±0.35	2.21 ± 0.13	3.60± 0.54
MO (%)	0.522±0.21	0.342±0.06	0.39 ± 0.10	0.52 ±0.02	0.27 ±0.05	0.34 ± 0.05	0.52 ± 0.09
EO (%)	0.04 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.00 ±0.00	0.00 ±0.00	0.00 ± 0.00	0.02 ± 0.00
BA (%)	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ±0.00	0.01 ±0.00	0.01 ± 0.00	0.016 ± 0.00
NLR (%)	13.56^{a}	15.42^{b}	25.24^{ab}	23.94^{ab}	16.0	17.20	16.67

Table 4: Haematological parameters in groups.

Values with the same superscripts are statistically significant for values across groups for a particular parameter

There were no statistically significant differences in the values of white blood cell count (WBC), Red blood cell count (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean cell volume (MCV), Mean cell hemoglobin (MCH), Red cell distribution width (RDW), Mean platelet volume (MPV), Neutrophil (NE), Lymphocyte (LY), Monocyte (MO), Eosinophil (EO) and Basophil (BA).

Biochemical parameters

Various biochemical parameters such as serum electrolyte (Na, K and Cl), lipid profile (TCh, HDL-Ch, LDL- Ch, TG, TP, and TCh: HDL, kidney and liver function tests analyzed were recorded in Table 4. Two-way ANOVA followed by Bonferroni post-test of Na and Cl values show varying level of statistically significant differences when all the hypertensive groups were compared with the normotensive group as well as comparison between the SHR -ve control and the SHR treated groups. A significant decrease ($p < 0.01$, 0.001) was seen when group A was compared with group B for Na and Cl respectively. Whereas comparison between group B (SHR +ve control) and groups C, D, E, F, and G showed varied degrees of statistically significant increase for Na ($p < 0.05$, 0.01, 0.01, 0.01 and 0.05) and ($p < 0.01$, 0.01, 0.001, 0.05 and 0.01) for Cl respectively. Analyses of serum creatinine and blood urea nitrogen revealed no statistically significant differences in the post-test comparison of all groups (Table 5). Likewise, while there are no statistical changes in the value of GGT and ALT, there are many interesting changes in the values of ALP and AST. Group A showed a significant decrease in ALP value compared to group B, C, D, E, F, and G ($p < 0.001$, 0.01, 0.001, 0.001, 0.001 and 0.001 respectively). The value of ALP for all the treated hypertensive animals, group D and E were significantly higher compared to group B ($p < 0.01$, 0.001), and further more comparison group C to group D, group E and group F showed a highly significant increases ($p < 0.001$, 0.001, 0.001 respectively) in the value of ALP (Table 5).

	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Na^+ (mmol/L)	142.80 ± 1.93[a]	136.60± 1.36 [abc]	141.80 ± 1.83 [b]	142.60 ± 1.75 [c]	143.00 ± 0.63 [c]	142.00 ± 0.55 [c]	141.60 ± 0.93 [b]
K^+ (mmol/L)	4.66 ± 0.15	4.98 ± 0.19	5.08 ± 0.19	5.22 ± 0.29	5.58 ± 0.10	4.98 ± 0.15	5.32 ± 0.29
Cl^- (mmol/L)	105.0 ± 2.21 [a]	97.20 ± 0.97 [ab]	103.0 ± 2.39 [b]	103.20 ± 1.83 [b]	104.8 ± 1.36 [b]	102.20 ± 0.58 [b]	101.60 ± 0.75 [b]
BUN (mmol/L)	5.96 ± 0.27	6.82 ± 0.14	6.74 ± 0.51	5.50 ± 0.90	5.84 ± 0.65	7.56 ± 0.18	8.02 ± 0.37
CRS (µmol/L)	33.00 ± 2.83	30.40 ± 1.75	32.00 ± 2.00	30.20 ± 3.26	29.40 ± -.75	28.40 ± 2.12	32.40 ± 1.44
ALB (g/L)	16.00 ± 0.70	17.60 ± 0.40	17.40 ± 0.51	17.40 ± 0.40	18.20 ± 0.20	18.40 ± 0.40	17.40 ± 0.87
ALP (IU/L)	173.60± 7.57[a]	252.60± 7.88[ab]	222.80 ± 20.28 [ac]	300.40 ± 31.82 [abcd]	410.00 ± 78.86 [abcde]	278.60 ± 13.36 [ace]	249.20 ± 18.95 [ade]
ALT (IU/L)	59.00 ± 5.27	54.60 ± 1.33	67.20 ± 5.04	68.80 ± 4.91	70.80 ± 5.53	56.60 ± 1.08	60.40 ± 4.50
AST (IU/L)	107.60± 3.09[a]	167.60± 1.42[ab]	123.80 ± 9.95 [b]	121.80 ± 13.10 [b]	124.60 ± 11.70 [b]	142.40 ± 5.61	152.20 ± 13.72 [a]
GGT (IU/L)	3.20 ± 1.02	1.60 ± 0.25	1.60 ± 0.25	2.40 ± 0.51	1.80 ± 0.37	2.20 ± 0.49	1.60 ± 0.24
TCh (mmol/L)	0.94 ± 0.09	0.94 ± 0.04	1.02 ± 0.06	1.14 ± 0.08	1.14 ± 0.08	1.08 ± 0.07	0.96 ± 0.07
HDL (mmol/L)	0.59 ± 0.04	0.60 ± 0.02	0.64 ± 0.05	0.72 ± 0.07	0.74 ± 0.06	0.62 ± 0.02	0.54 ± 0.03
LDL (mmol/L)	0.03 ± 0.08	0.01 ± 0.05	0.04 ± 0.09	0.11 ± 0.08	0.09 ± 0.08	0.14 ± 0.05	0.12 ± 0.07
T. Bill (µmol/L)	5.40 ± 0.75	3.14 ± 0.84	5.20 ± 0.73	4.60 ± 1.21	4.20 ± 1.07	4.40 ± 1.25	5.14 ± 1.29
TG (mmol/L)	0.70 ± 0.19	0.71 ± 0.06	0.75 ± 0.12	0.68 ± 0.10	0.67 ± 0.07	0.70 ± 0.09	0.63 ± 0.07
TP (g/L)	59.40 ± 1.29	59.60 ± 0.98	60.80 ± 1.11	58.00 ± 1.30	58.60 ± 1.29	62.40 ± 1.17	62.00 ± 0.89
TCh: HDL (%)	159	156	167	154	154	174	177
TG: TCh (%)	74.4[a]	75.5[b]	73.5[c]	59.6[abc]	58.8 [abc]	64.8	65.6

Table 5: Biochemical parameters in experimental groups.

Values with the same superscripts are statistically significant between the indicated groups.

Discussion

Previous reports have suggested an increasing frequency of hypertension and other non-communicable diseases among HIV infected patients [27,28]. While some researchers believe this is due to the side effects of HAART [29], others [30,31] have argued that these effects are as a result of the aging of the HIV population due to increased survival of the patients in the post HAART era. The findings from our study show that HAART can predispose to hypertension, which is a risk factor for the development of cardiovascular diseases.

A significant lower body weight of all hypertensive groups compared to that of normotensive group was observed. This may be attributed to reduced appetite in the hypertensive animal as previously reported that there is a negative correlation between hypertension and adiponectin level that results in an effect on appetite [33-34]. As observed in other studies [30,35], we found a correlation between hypertension and BMI. BMI levels were higher in the hypertensive animals that were not exposed to any treatment and much higher in the hypertensive animals treated with HAART and those treated with a higher dose of *Hypoxis hemerocallidea* alone. This showed that the administration of HAART exacerbates the progression of hypertension. Two of the most debilitating complications of obesity, especially centrally located obesity, responsible for the high morbidity and mortality are hypertension and heart disease [36,37]. It is known that high BMI/obesity is a risk factor for hypertension especially in the HIV patients and in the general population [31,38]. However, the adjuvant administration of *Hypoxis hemerocallidea* extract (100 mg/kg and 200 mg/kg) was seen to mitigate the increase in BMI in an inverse dose dependent manner, whereas the administration of the extract alone showed a weaker mitigating effect in a similar inverse dose dependent manner. This finding shows that adjuvant administration of the *Hypoxis hemerocallidea* may have a beneficial antihypertensive effect and this is in consonance with the report of Ojewole., *et al*. [39], who reported antihypertensive property of *Hypoxis hemerocallidea*. The significant increase in the systolic as well as diastolic blood pressure in the HAART administered hypertensive group and HAART + *Hypoxis hemerocallidea* exposed groups showed that there is progression in the hypertension, which was mitigated at lower dose of adjuvant administration of *Hypoxis hemerocallidea*.

Hematological indices such as red cell distribution width, main platelet volume, neutrophil- lymphocyte ratio are established markers of vascular pathologies that have also been proposed as a prognostic tool for prediction of severity of hypertension and end organ damage [17,18,40-42]. In this study, a non-statistically significant increase recorded in group C may have resulted from ineffective erythropoiesis due to chronic inflammation. Inflammatory cytokines have been found to suppress the maturation of erythrocytes, which enable juvenile red cells to enter into the circulation and increase heterogeneity in size [43]. Another likely mechanism for this observation is the oxidative stress. Red blood cells have powerful antioxidant capacity and serve as a primary oxidative sink, which makes them prone to oxidative damage and reduces the cell survival. This enhances the release of juvenile erythrocytes into circulation; hence the reduced RDW in the groups administered with *Hypoxis hemerocallidea* which has been established to have antioxidative property [43]. Although there is no statistically significant difference in the values of MPV, a slight increase seen in the value of HAART alone administered group suggests a stepwise progression in the severity of hypertension in this group. NRL is an independent factor for mortality and major adverse events in acute and chronic ischemic heart diseases [14] and increased NRL may indicate hypertensive end-organ damage. In this study, the values of NLR were highly significantly higher in groups HAART alone and HAART + *Hypoxis hemerocallidea* (100 mg/kg but lower and non-significant in HAART + *Hypoxis hemerocallidea* (200 mg/kg), and *Hypoxis hemerocallidea* alone (100 and 200 mg/kg) groups. NLR does not only indicate hypertensive end-organ damage but may also give prognostic clues about the activity of disease and response to therapy. Our observed values show that the progression of hypertension is higher in the HARRT administered group and a low dose of *Hypoxis hemerocallidea* mitigate this progression; and demonstrate the likely antihypertensive property of the plant extract.

The biochemical indices result further support the possibility of HAART predisposition to elevating the progression of hypertension and the mitigating ability of the adjuvant extract. Statistically significant increase in the Na^+ level recorded in HAART alone group and statistical significant decreases in all the *Hypoxis hemerocallidea* administered groups established an increase progression of hypertension under HAART insult and a reduction in the progression of hypertension progression in the *Hypoxis hemerocallidea* treated groups, showing an inverse correlation between blood pressure and Na^+ level. The association of TCh : HDL and TG; TCh ratios in hypertension has been reported in previous studies [7,31]. Our findings showed a statistically significant increase in these values in the HAART alone group when compared with all other treated groups and a significant reduction in groups where the adjuvants were co- administered with HAART demonstrating the positive effect of co-administration of the of the extract.

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This is the first study, to our knowledge, to document the relationship between HAART, *Hypoxis hemerocallidea* and hypertension in an experimental animal model using hematological and biochemical indices.

Conclusion

Hematological and biochemical indices are good markers of progression of hypertensive disease with a stepwise relationship between hypertension and these indices. This work demonstrates that HAART has the potential to exacerbate the progression of hypertension. The plant-based adjuvant with strong antioxidant and anti-hypertensive properties may mitigate hypertensive effect when used at appropriate doses. Although crude extract of *Hypoxis hemerocallidea* was used in this study, a follow up study using specific isolate of the bioactive component of the plant would be necessary to confirm the observations.

Authors Contribution and Acknowledgement

OO Azu, AI Jegede conceived and designed the experiment: AI Jegede, IO Onanuga, U Offor and OO Ogedengbe performed the experiment: AI Jegede-Analyzed the data and wrote the manuscript, OO Azu proofread and edited the manuscript.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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