Original Research Article

Body weight and platelet count changes in zidovudine administered Wistar albino rats treated with ethanolic extracts of *Annona muricata* and *Fagara zanthoxyloide*.

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ABSTRACT

Zidovudine administration either in single or fixed-dose combination usually results in a decrease in body weight and in some cases thrombocytopenia. The present study investigated the body weight and platelet count changes observed in zidovudine pre-administered Wistar albino rats on treatment with ethanolic extracts of the leaves *Annona muricata (AM)* and roots of *Fagara zanthoxyloide (FZ)*. Plants samples were collected from Alakahia community, Rivers state and Opoo community, Ogun state; while Wistar albino rats were grouped into normal control, negative control (receiving zidovudine at100g/ml/ Kg bw), *AM* and *FZ* extract treatment (at 4.5 and 3.8 g/ml/Kg B.W respectively) groups with analysis performed bi-weekly. All tests were performed using standard procedures with all reagents of analytic grade. Phytochemical screening of the extracts showed significantly high amounts of alkaloids (10.47- 21.15mg/100g), phenols (10.60-15.22 mg/100g) and tannins (14.23- 50.19mg/100g). The investigation into their proximate compositions also showed high fat (5.78 ± 0.02) in *FZ*, moisture (10.47 ± 0.05) in *AM* and fibre (7.37 ± 0.03) in *FZ*. The amino acid phenylalanine (5.10-6.01g/100g), isoleucine (4.44-5.20g/100g), lysine (4.03-5.31g/100g) was observed to be available in the extracts. In the normal control group body weight increased by 20.75g at week 6 while administration of Zidovudine, resulted in a decreased in bodyweight by 1.14g in week 2 and 3.32g in week 6. Extract treatment caused a significant increase ($p \leq 0.05$) in body weight by 17.95g (*AM*) and 18.23g (*FZ*) at week 6. Platelet count was also observed to significantly decrease ($p \leq 0.05$) by 33.42% in the negative control group when compared to the normal control. This was observed to significant increases in extract treatment by 49.56% (AM) and 51.32% (FZ). The results thus suggest a possible beneficial effect of the extracts of *AM* and *FZ* in checkmating the weight and platelet loss observed as a side effect of zidovudine therapy as well as the possible use in haemorrhagic conditions to reduce bleeding without thromboembolism.

Keywords: *Annona muricata*, *Fagara zanthoxyloide*, platelets, weight loss, coagulation assay

INTRODUCTION

All living organisms require food for energy which is essential for growth and general body maintenance. Energy homeostasis thus plays a vital role in the survival of living organisms and has been closely linked with the innate expression of appetite (Satya, 1997, Satya *et al*., 1999).

Appetite expression is important for keeping the body weight about an expected setpoint; as an increase in body weight may invariably be the result of an increase in appetite and weight loss as a result of a loss of appetite (anorexia).

The management of HIV/AIDs in Nigeria involves the use of a combination of drugs (fixed-dose combination therapy) one of which is zidovudine to delay the progression of HIV infection and offer better protection against infections (Hammer *et al*., 1997; Staszewski *et al*., 1998; Barry *et al*., 1999). Recent publications by the National AIDS Control Organization (NACO) stipulates that fixed-dose combination of two nucleoside reverse transcriptase inhibitors (NRTI) (such as zidovudine or stavudine and lamivudine) and one none nucleoside reverse transcriptase inhibitor (such as nevirapine or efavirenz) is the highly recommended highly active antiretroviral therapy (HAART) and the first line in treatment of HIV/AIDs (Kaibalya *et al*., 2014). Among them, zidovudine has proven to be highly effective and the preferred NRTI in NACO sponsored antiretroviral therapy (ART), but however, does not cure AIDS or completely kill the virus, but prevents further damage by slowing the production of new viruses.

Prolonged administration of zidovudine has been linked to the development of anaemic condition in about 10% of patients while others may display signs of leucopenia (resulting in a higher risk of bacterial infection) and loss of appetite (resulting in decreased body weight). Although rare, thrombocytopenia has been implicated in some patients undergoing fixed-dose combination therapy involving this drug. Platelets are important in the circulatory system due to their ability to aggregate and initiate coagulation. A decrease in platelet count may prove detrimental as seen in haemorrhagic conditions, but increased platelet count may be linked to cardiovascular diseases resulting from clotting without vascular damage (vascular thrombosis) which may lead to thromboembolism. Hence the need to monitor the platelet counts and coagulation effects with drugs which cause alterations in the level of platelets.

Coagulation effects may be assayed by a measure of the bleeding time as well as prothrombin and activated partial proplastin time which measures the content and activity of coagulation factors (Li *et al*., 2013). While activated partial proplastin time measures the sensitivity to the level of factor VIII, IX, XI and XII, prothrombin time measures the integrity of the coagulation protein factor VII (Koch and Biber, 2007; Hoffman and Monroe, 2005). Prolonged prothrombin time (higher than the normal 28-36 seconds) would thus indicate a deficiency in coagulation

factors V, VII and XI (Davison *et al*., 2012) while prolonged APPT represents a deficiency of factors VIII, IX, XI and XII (Laffan and Bradshaw, 1995).

Plants have been known to possess an exorbitant array of chemicals within them as a result of their adaptability to predators, parasites, environmental and stressful conditions. These biomolecules have been observed to elicit certain physiological characteristics when consumed by animals. *Annona muricata* and *Fagara zanthoxyloide* extracts has been implicated in folkloric medicine for the treatment of blood related disorders and had necessitated the burden of research into the coagulation effect of these plant extracts in rat models with zidovudine as this would aid in establishing cost-effective and readily available measures for coping with some of the side effects of fixed-dose combination therapy.

MATERIALS AND METHOD

Plant materials

The leaves of *Annona muricata* (AM) was obtained for Alakahia community (3.92°N, 7.80°E) in Obio/Akpor Local government area of Rivers state; while roots of *Fagara zanthoxyloide* (FZ) was obtained from Opoo community (8.28°N, 3.67°E) in Itesiwaju Local Government Area of Oyo State. The plant materials alongside pictures were identified by the Chief Technologist Department of Plant Science and Biotechnology Herbarium, University of Port-Harcourt and University of Ibadan Herbarium (UIH) with a voucher copy placed in the herbarium for reference.

Preparation of Ethanol Extracts

The leaves of *Annona muricata* and the roots *Fagara zanthoxyloide* were washed and air dried at room temperature (25˚C) and was milled separately with a mechanical grinder (M3383L40 Thomas-Willey) until a uniform coarse powder was achieved. Three hundred grams of each of the dried powdered samples were placed in a conical flask and microwave-assisted extraction was performed using 3 litres of absolute ethanol. The mixture was agitated at intervals on a rotary shaker and the extracts were centrifuged twice at 1500rpm for 15 min in an LCM-3000 centrifuge (Wilten-bioteknika Microspin-12). Filtration of the contents of the conical flask was performed with Whatman No 1 filter paper and evaporated to dryness at 40°C with a rotary evaporator. This was lyophilized to recover the residue as sticky pastes which were stored at 4°C

in a refrigerator. The powdered extracts were rehydrated and diluted immediately before the experimental treatment.

Acute toxicity study

The toxicity study was carried out using Wistar albino rats (30-37g) divided into six groups of four rats each (one control group and 5 treatment group). They were acclimatized for seven days while on standard feed and water ad libithum. The treatment group were administered leaf extract of *Annona muricata* and *Fagara zanthoxyloide* at 2,4,6,8 and 10 g/ml/Kg BW while the control group was administered only distilled water (2.5ml/kg orally). Observation for signs of acute toxicity (respiratory distress, salivation, lacrimation, yellowing of fur etc) and mortality was performed during the critical first four hours and afterwards daily. The number of deaths (caused by the extract) within this period of time was noted. A graph of dose to experimental response was constructed for each extract from which the LD50 of the various extract was determined.

Animal handling

Healthy albino rats were purchased from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers state, Nigeria and divided into groups (of 5 rats each) and housed in Griffin and George modular cage system. Each group was kept in a well-aerated room at a temperature of 28-31˚C and humidity of 50-55%. They were allowed to acclimatize to new housing conditions for a period of 14 days prior to experimentations and were fed with standard food pellets (from Top feeds) and water *ad libitum*.

Group 1 served as the normal control group which received no zidovudine or extract treatment

Group 2 (Negative control) received 100mgZDV/Kg bw

Group 3 received 4.5g/ml/Kg bw of *Annona muricata* alongside 100mgZDV/Kg bw.

Group 4 received 3.8g/ml/Kg bw of *Fagara zanthoxyloide* alongside 100mgZDV/Kg bw.

All animals were treated in a manner that complied with the National Institute of Health (NIG) guidelines for the care and use of laboratory animals (NIH, 1985). Treatment with extracts was performed for a period of 6 weeks with analysis and observation performed bi-weekly.

Analysis of extract

Phytochemical screening was performed by the methods described by Sofowara (1993), Trease and Evans (1989) and Harbone (1973). The proximate analysis was performed by the method described in the Association of Official Analytical Chemists (AOAC, 2010). The amino acid determination would be performed using an amino acid analyzer by the method of Prasad, (2017).

Animal treatment analysis

Twenty four hours after termination of experimentation, the animals were weighed and anaesthetized by placing them in an airtight container containing cotton balls soaked with chloroform. Blood samples were collected by severing the jugular vein and placed in labelled heparinised bottles for assay. The blood samples were centrifuged at 500 rpm using an LCM-3000 centrifuge (Wilten-bioteknika Microspin-12) for 12 minutes to obtain the serum. The spleen and heart were also excised after dissection of the abdominal cavity of each animal.

The changes in body and organ weights were determined by the method described by Akinnawo *et al*., (2005) using a digital balance. The excised organs were fixed with 10% formalin for 24hours and then dehydrated in graded concentrations of xylene. They were embedded in molten paraffin wax and sectioned into 5 microns slices. The sectioned slices were fixed on glass slides and stained with haematoxylin and eosin (H&E) for examination with a microscope fitted with a camera unit. Photomicrography of sections of the tissues was taken and processed in a photo laboratory.

Platelet count was estimated using the automated Haematology Analyser K-X-21 (manufactured by Symex, Kobe, Japan) while following standard procedures stipulated by the manufacturer. Bleeding time was determined by the method of Tschopp and Zucker, (1972) while Prothrombin time and activated partial proplastin time were estimated by the Quick's one stage method as described in Elderbi *et al*., (2010).

Average daily food and water intake were estimated by the method described by Oyeyipo *et al*., (2010).

STATISTICAL ANALYSIS

Results were expressed as Mean ± Standard error of mean with analysis of variance performed using SPSS software version 20 for Windows (SPSS Inc. USA) and Student t-test. The significant level during the test would be set at $p \leq 0.05$.

RESULTS

Phytochemistry

Phytochemical investigations into the ethanolic extracts of the leaves of *Annona muricata* and roots of *Fagara zanthoxyloide* revealed the presence of alkaloids, flavonoid, tannins, phenols and saponin in both samples as shown in Table 1. Quantitative phytochemical examination of the extracts revealed that the roots extract of *Fagara zanthoxyloide* contained a significantly higher ($p \leq 0.05$) concentration of alkaloid and tannins than leaves extract of *Annona muricata*, while the leaves extract of *Annona muricata* was observed to be richer in flavonid and phenols when compared with the root extracts of *Fagara zanthoxyloide* (Table 2). Terpenoid was observed to be absent in the extracts of *Annona muricata* while steroids were absent in the root extracts of *Fagara zanthoxyloide*.

Table 1. Qualitative phytochemical analysis of leaf extract of *Annona muricata* and root extract of *Fagara zanthoxyloide*

Phytochemical	Leaf extract of *Annona muricata*	Root extract of *Fagara zanthoxyloide*
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+
Tannins	+	+
Terpernoids	-	+
Steroids	+	-
Saponins	+	+
Phenols	+	+

Present: +, **Absent**: -

Table 2. Quantitative phytochemical analysis of leaf extract of *Annona muricata* and root extract of *Fagara zanthoxyloide*

Phytochemical (mg/100g)	Leaf extract of	Root extract of *Fagara*

	Annona muricata	*zanthoxyloide*
Alkalkoids	10.47 ±0.09*	21.15 ± 0.67**
Flavonoid	17.53 ± 0.80**	5.06 ± 0.10*
Glycosides	4.01 ± 0.11**	2.52 ± 0.08**
Tannins	14.23 ± 0.26***	50.19 ± 0.07**
Terpenoid	NIL	31.33 ± 0.78
Saponins	6.32 ± 0.04*	8.53 ± 0.07*
Steroids	3.60 ± 0.18	NIL
Phenols	15.22±0.18*	10.60±0.62**

Result expressed as Mean ± Standard error of Mean of triplicate determinants. Values with different superscript are statistically significant ($p \leq 0.05$).

Proximate analysis

Table 3 shows the result of the proximate analysis of the leaves of *Annona muricata* and roots of *Fagara zanthoxyloide*. *Annona muricata* leaves were observed to possess a significantly high ($p \leq 0.05$) carbohydrate, crude fat and moisture when compared with the root extracts of *Fagara zanthoxyloide.* The crude fibre, ash and protein were observed to be significantly higher ($p \leq 0.05$) in the roots of *Annona muricata.*

Table 3. Proximate analysis of leaf of *Anonna muricata* and roots of *Fagara zanthoxyloide*

Proximate parameter	Leaf extract of *Anonna muricata*	Root extract of *Fagara zanthoxyloide*
Crude protein (mg/g)	2.13 ± 0.03*	5.78 ± 0.02**
Crude fat (mg/g)	6.37 ± 0.04**	3.48 ± 0.19*
Crude ash (mg/g)	0.77 ± 0.02*	6.11 ± 0.06**
Crude fiber (mg/g)	4.55 ± 0.11*	7.37 ± 0.03*
Moisture (mg/g)	10.47 ± 0.05**	8.27 ± 0.11**
Carbohydrate (%)	52.59 ± 0.73	45.25 ±0.23*

Result expressed as Mean ± Standard error of Mean of triplicate determinants. Values with different superscript are statistically significant ($p \leq 0.05$).

The result of the amino acid analysis of the extracts are shown in table 4 with *Annona muricata* and *Fagara zanthoxyloide* observed to both contain the amino acids lysine, phenylalanine, arginine, leucine and isoleucine in significant high ($p \leq 0.05$) concentration. Phenylalanine was

observed to be the amino acid with the highest concentration in extracts of *Fagara zanthoxyloide* while lysine concentration was highest in *Annona muricata.*

Table 4. Quantitative amino acid profile (g/100g) of leaf extracts of *Anonna muricata* and root extract of *Fagara zanthoxyloide*

Amino acid (g/100g)	Leaf extract of *Annona muricata*	Root extract of *Fagara zanthoxyloide*
Lysine	$5.31\pm0.207^{***}$	4.03 ± 0.155
Phenylalanine	$5.10\pm0.051^{*}$	$6.01\pm0.012^{*}$
Arginine	$4.19\pm0.355^{**}$	$3.60\pm0.090^{*}$
Glycine	2.51 ± 0.251	1.13 ± 0.071
Leucine	$4.03\pm0.151^{*}$	$2.72\pm0.030^{***}$
Isoleucine	$4.44\pm0.170^{*}$	$5.20\pm0.05^{**}$
Valine	$3.63\pm0.204^{**}$	$4.45\pm0.127^{*}$
Glutamate	$4.95\pm0.104^{*}$	$3.17\pm0.028^{*}$

Result expressed as Mean ± Standard error of Mean of triplicate determinants. Values with different superscript are statistically significant ($p\leq0.05$).

Acute toxicity (LD_{50})

Result of the acute toxicity study showed on the leaf extract of *Annona muricata* and *Fagara zanthoxyloide* are illustrated in Tables 5 and 6. The results revealed that administration as from 8g/ml/Kg bw for *Annona muricata* and 6 g/ml/Kg bw for *Fagara zanthoxyloide* resulted in signs of toxicity and oral administration below this levels was well tolerated in mice even beyond 7 days. This result for the toxicity of *Annona muricata* was however slightly higher than the findings by Abdul-Wahab *et al*., 2018 in which he observed kidney toxicity above 5g/Kg bw and Bertin *et al*, (2017) who observed 100% mortality at 5g/Kg bw when *Annona muricata* from Benin was investigated.

Fagara zanthoxyloide acute toxicity test result showed signs of toxicity as from 6g/Kg bw with animals witnessing weight loss, lacrimation and reduced irritability (Table 6). The median acute toxicity value (LD_{50}) which was estimated at 3.8g/ml/Kg bw which suggests that the extract possessed no short time toxicity (Ghosh *et al*., 1984). This value was also similar with what was observed for *Fagara zanthoxyloide* of Ugandan origin by Ogwal-Okeng *et al*., (2003) at 5g/ml/Kg bw however the reduced irritability at dosages higher than the LD_{50} may possibly

culminate in the findings of Ogwal-Okeng *et al*., (2003), suggesting a direct effect of extracts nervous system.

Table 5. Acute toxicity test on *Annona muricata*

Group	Dosages (g/ml/Kg BW)	No. of rats used	No. of mortality	Remarks
Control	-	4	0	
1	2	4	0	
2	4	4	0	
3	6	4	0	
4	8	4	1	Salivation, weight loss.
5	10	4	2	Lacrimation, reduced irritability

Table 6. Acute toxicity test on *Fagara zanthoxyloide*

Group	Dosages (g/ml/Kg BW)	No. of rats used	No. of mortality	Remarks
Control	-	4	0	
1	2	4	0	
2	4	4	0	
3	6	4	1	Reduced irritability, fur coat changes observed
4	8	4	2	Muscle paralysis, weight loss.
5	10	4	2	Weakness and salivation

Table 7. Changes in body, spleen and heart weights in zidovudine pre-administered albino rats treated with the leaf extract of *Annona muricata* and root extract of *Fagara zanthoxyloide*

GRP	Body weight (g)			Spleen (g)			Heart weight (g)		
	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
Group 1	235.18±0.18^{a,c}	247.66±0.19^{c}	255.93±0.07^{a}	0.32±0.08^{b}	0.33±0.69^{b}	0.35±0.28^{a}	0.64±0.28^{a}	0.64±0.55^{a}	0.66±0.96^{b,c}
Group 2	231.86±0.29^{a}	227.74±0.31^{a}	221.40±0.25^{a,b}	0.21±0.14^{a}	0.39±0.52^{d}	0.36±0.23^{a,d}	0.55±0.05^{a,c}	0.52±0.30^{a,b}	0.66±0.03^{c}
Group 3	233.32±0.02^{c,d}	235.16±0.08^{d}	239.35±0.01^{a,d}	0.52±0.18^{a}	0.53±0.11^{a}	0.62±0.81^{a,d}	0.79±0.28^{a}	0.73±0.05^{a}	0.69±0.11^{c}
Group 4	239.97±0.11^{a,c}	244.87±0.21^{b,c}	250.09±0.18^{a,b}	0.55±0.82^{a}	0.51±0.58^{b}	0.41±0.39^{a}	0.72±0.52^{a,b}	0.72±0.28^{b}	0.76±0.81^{c,d}

Result expressed as Mean ± Standard error of Mean of triplicate determinants.

^{a} compares group 1, ^{b} compares group 2, ^{c} compares group 3 and ^{d} compares group 4 with other groups. Values with same superscript are statistically significant ($p \leq 0.05$).

Body and organ weight changes

The results of the changes in the body weights of the animals and their selected organs are depicted in table 7 above. The normal control group showed significant increases ($p \leq 0.05$) in the body weight from week 2 through to 6. Administration of zidovudine in the negative control group resulted in a decrease in the body weight at weeks 2 which was observed to significantly increase ($p \leq 0.05$) at weeks 4 and 6 when compared to the normal control group. Treatment with extracts of *Annona muricata* resulted in an increase in the body weight within the group as time progress. These values were however observed to be significantly higher ($p \leq 0.05$) than the values for the negative control group but lower than that of the normal control. A similar trend was observed for *Fagara zanthoxyloide* treatment in group 4 but in comparison with the *Annona muricata* treatment (group 3), the weight changes were significantly higher ($p \leq 0.05$) than that observed in group 3.

Changes in the weights of the spleen and heart are depicted in table 5. No significant changes were observed in the weights of the spleen and hearts of the normal control group as time progressed in weeks 2 and 4. A slight significant increase ($p \leq 0.05$) in the weights of the spleen and heart was however observed after 6 weeks in the normal control group. The result for the negative control group revealed a decrease in the weights of the spleen and heart as the duration of administration of ZDV increased and when compared to the normal control groups. Treatment with the extract of the leaves of *Annona muricata* resulted in increases in the weights of the spleen and heart at week 2 when compared with the normal and negative control groups. The weight of the heart in the *Annona muricata* treatment group was observed to decrease at week 4 and 6 but with values higher than that observed for the negative and normal control. *Fagara zanthoxyloide* administration resulted in a significant increase in the spleen and heart weights at week 2 when compared to the positive and negative control group but reduced at weeks 4 and 6.

Platelet count

The result for the platelet count depicted in table 8, showed that the administration of zidovudine (negative control group) resulted in a significant decrease ($p \leq 0.05$) in the platelet count at week 2 when compared with the normal control group while a significant decrease was further observed in weeks 4 and 6. Treatment with leaf extracts of *Annona muricata* resulted in a significant

increase ($p \leq 0.05$) in the platelet count at week 2 when compared to the negative control which was observed to increase till week 6.

Table 8. Platelet counts in zidovudine pre-administered albino rats treated with the leaf extract of *Annona muricata* and root extract of *Fagara zanthoxyloide*

PLATELETS (x 10^6µ/L)			
GRP	**Week 2**	**Week 4**	**Week 6**
Group 1	**7.45±0.02^{a}**	**7.46±0.16^{c,d}**	**7.45±0.42^{a,d}**
Group 2	**4.96±0.12^{b,d}**	**4.80±0.01^{c,d}**	**4.56±0.04^{a,c}**
Group 3	**7.23±0.15^{a,d}**	**7.08±0.40^{d}**	**6.82±0.45^{c}**
Group 4	**7.10±0.11^{c}**	**7.04±0.04^{a,b}**	**6.90±0.04^{b,c}**

Result expressed as Mean ± Standard error of Mean of triplicate determinants.
[a] compares group 1, [b] compares group 2, [c] compares group 3 and [d] compares group 4 with other groups.
Values with same superscript are statistically significant ($p \leq 0.05$).

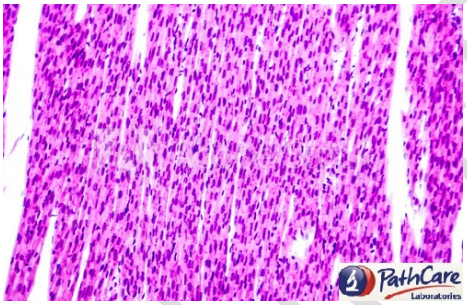


Plate 1. Heart of normal control group showing a clearly regular pattern of cardiac myofibres

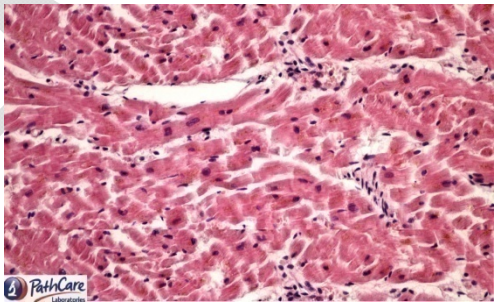


Plate 2. Heart of negative control group at week 2 showing disarray in the pattern of myofibres

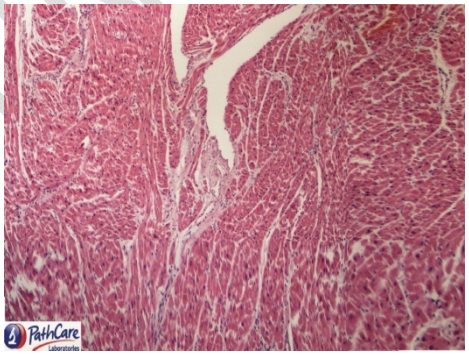


Plate 3. Heart of negative control group at week 4 showing disarray in the pattern of myofibres

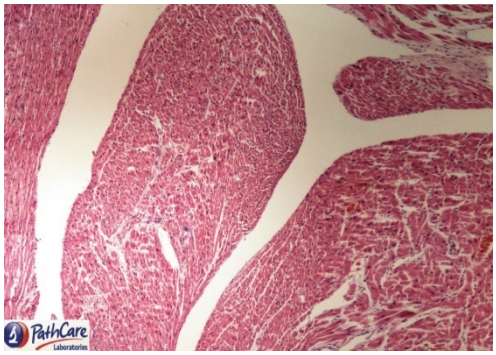
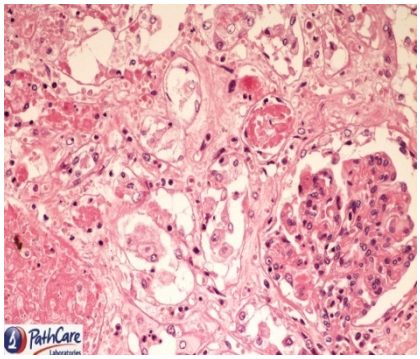
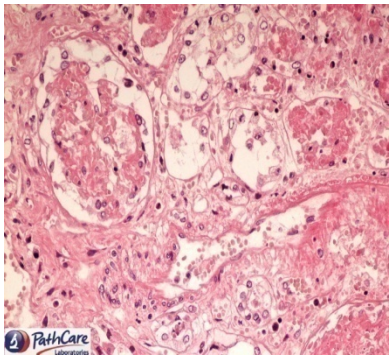


Plate 4. Heart of negative control group at week 6 showing disarray in the pattern of myofibres





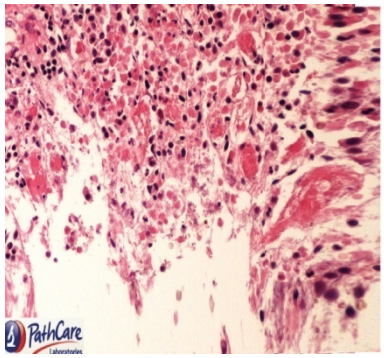
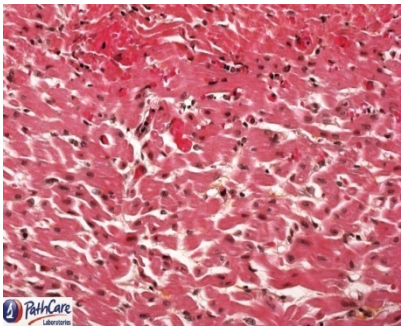
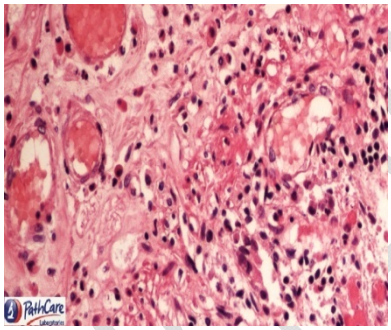


Plate 5. Heart of *AM* + ZDV at week 2 **Plate 6.** Heart of *AM* + ZDV at week 4 **Plate 7.** Heart of *AM* + ZDV at week 6
All showing cardiac architecture to be normal with the edematous intermuscular spaces of normal range





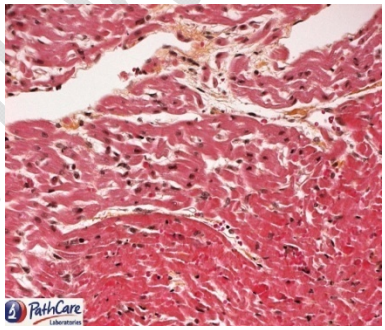


Plate 8. Heart of *FZ* + ZDV at week 2 **Plate 9.** Heart of *FZ* + ZDV at week 4 **Plate 10.** Heart of *FZ* + ZDV at week 6
All showing normal architecture with nuclei of cardiomyocytes and myofibres clearly seen

Coagulation assay

Table 9 shows the results of the study on bleeding time and several other coagulation parameters. The bleeding time was observed to be significantly increased at week 2 in the negative control group when compared with the normal control group at week 2 and this was observed to increase also in week 4 and 6. Treatment with extract of *Annona muricata* leaves, however, resulted in a decrease in the bleeding time at week 2 when compared with the negative control group but the values were significantly higher than that observed for the normal control. The bleeding time further reduced at weeks 4 and week 6 when compared to the negative control group. The decreases in bleeding time were observed to be significantly pronounced ($p \leq 0.05$) in the extracts of *Fagara zanthoxyloide* than in the extracts of *Annona muricata*.

Table 9. Bleeding, prothrombin and activated partial proplastin time in zidovudine pre-administered albino rats treated with the leaf extract of *Annona muricata* and root extract of *Fagara zanthoxyloide*

	Bleeding time (s)			Prothrombin time (s)			Activated partial proplastin time (s)		
GRP	**Week 2**	**Week 4**	**Week 6**	**Week 2**	**Week 4**	**Week 6**	**Week 2**	**Week 4**	**Week 6**
Group 1	**4.07±0.18^{a}**	**4.07±0.21^{a,c}**	**4.07±0.08^{a,d}**	**25.47±0.43^{a}**	**25.17±0.52^{b,c}**	**25.47±0.13^{a,b}**	**57.96±0.36^{a,b}**	**57.96±0.36^{a,c}**	**57.96±0.36^{b}**
Group 2	**4.59±0.02^{a,c}**	**4.73±0.93^{b,c}**	**4.87±0.32^{a,b,d}**	**28.93±0.02^{a,c}**	**28.37±0.93^{a,d}**	**29.08±0.32^{c,d}**	**58.19±0.33^{b,d}**	**58.73±0.12^{a,d}**	**58.87±0.42^{a,b}**
Group 3	**4.21±0.83^{a,c}**	**4.18±0.19^{c}**	**4.12±0.18^{b,c}**	**24.93±0.29^{a,d}**	**25.09±0.48^{a,c}**	**26.42±0.74^{c,d}**	**56.01±0.24^{a,b}**	**57.67±0.19^{a,c}**	**58.96±0.12^{b}**
Group 4	**4.16±0.08^{a,d}**	**4.14±0.63^{d}**	**4.09±0.28^{d}**	**25.73±0.08^{c}**	**26.04±0.63^{a,b}**	**26.18±0.28^{b,c}**	**57.63±0.25^{a}**	**57.84±0.42^{a}**	**56.99±0.74^{d}**

Result expressed as Mean ± Standard error of Mean of triplicate determinants.
[a] compares group 1, [b] compares group 2, [c] compares group 3 and [d] compares group 4 with other groups. Values with same superscript are statistically significant ($p \leq 0.05$).

Table 10. Daily sleep time, estimated food and water intake in zidovudine pre-administered albino rats treated with the leaf extract of *Annona muricata* and root extract of *Fagara zanthoxyloide*

	Sleep time (hours)			Food intake (g/day)			Water intake (ml/day)		
GRP	**Week 2**	**Week 4**	**Week 6**	**Week 2**	**Week 4**	**Week 6**	**Week 2**	**Week 4**	**Week 6**
Group 1	**8.44±0.41 ^{a}**	**7.36±0.87 ^{a,c}**	**7.72±0.28 ^{b,c}**	**35.48±1.31 ^{a}**	**35.79±0.36^{b,d}**	**34.63±1.19 ^{a,d}**	**18.07±1.46 ^{a,d}**	**17.09±0.73 ^{a,c}**	**17.11±0.59 ^{c,d}**
Group 2	**9.32±0.47 ^{a}**	**8.25±0.59 ^{a,b}**	**8.61±0.19 ^{b,c}**	**30.58±0.90 ^{a,d}**	**30.23±1.88^{c,}**	**30.03±3.64 ^{a,c,e}**	**18.78±0.55 ^{a}**	**20.20±1.14 ^{c}**	**17.68±2.94 ^{a}**
Group 3	**7.57±0.08 ^{b}**	**5.64±0.22 ^{b,c}**	**7.39±0.02 ^{c,d}**	**32.40±0.66 ^{a,d}**	**32.50±0.74^{c,d}**	**32.50±0.47 ^{b}**	**16.50±0.16 ^{a}**	**14.38±0.44 ^{a,d}**	**17.59±0.82 ^{c}**
Group 4	**7.27±0.30 ^{b,c}**	**5.75±0.53 ^{a,c}**	**6.53±0.76 ^{c}**	**28.34±2.26 ^{a,c}**	**31.02±0.81 ^{c}**	**30.51±0.68 ^{c,e}**	**17.13±1.98 ^{a,b}**	**18.19±0.54 ^{a}**	**17.22±0.11 ^{c,d}**

Result expressed as Mean ± Standard error of Mean of triplicate determinants.
[a] compares group 1, [b] compares group 2, [c] compares group 3 and [d] compares group 4 with other groups. Values with same superscript are statistically significant ($p \leq 0.05$).

The prothrombin time was observed to significantly increase at week 2 following administration of zidovudine when compared with the negative control group. However, this value was observed to decrease at weeks 4 and 6 when but at significantly higher ($p \leq 0.05$) proportion than what was observed for the normal control group. Treatment with extract of *Annona muricata* and *Fagara zanthoxyloide*, however, revealed a significant decrease in prothrombin time when compared with the normal and negative control groups. The prothrombin time was observed to significantly increase ($p \leq 0.05$) at weeks 4 and 6 when compared with the value obtained at week 2. A similar trend as observed for prothrombin time was also observed for the result of the activated partial proplastin time. However, the administration of *Fagara zanthoxyloide* after 6 weeks resulted in a significant decrease ($p \leq 0.05$) in activated partial proplastin time when compared to that after 4 weeks of treatment. The value was however significantly lower ($p \leq 0.05$) than that for the normal and negative control groups.

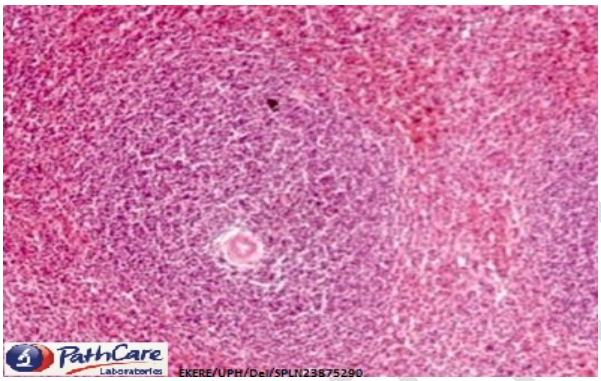


Plate 11. Spleen of normal control group showing evenly distributed red and white pulp regions with no pathological lesions. **H&E X800**

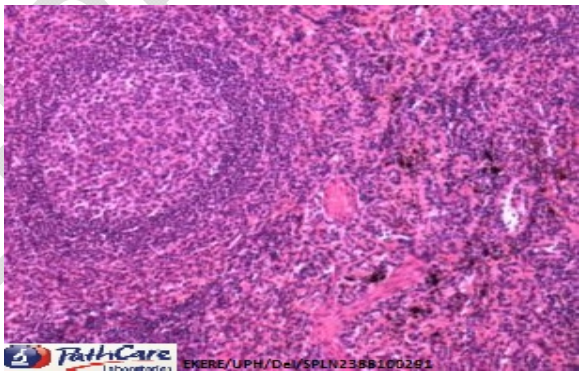


Plate 12. Spleen of Neg. control group at week 2 showing red and white pulp regions with parenchyma cells. **H&E X800**

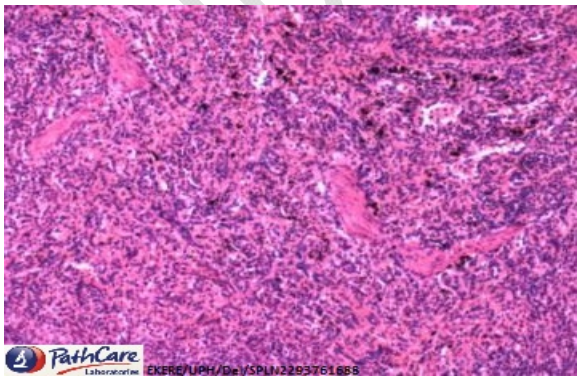


Plate 13. Spleen of Neg. control group at week 4 showing red and while pulp regions with infiltration of neutrophils and lymphocytes. **H&E X800**

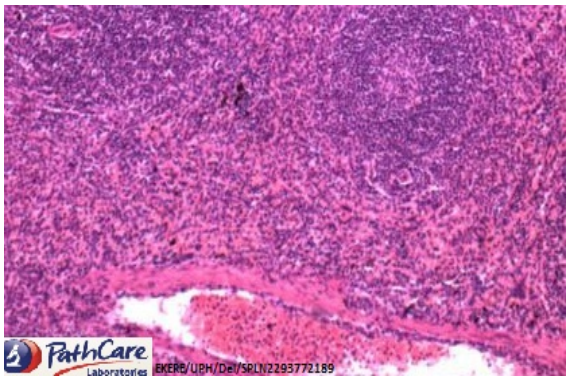


Plate 14. Spleen of Neg. control group at week 6 showing a normal central artery constrictions in red pulp with cyanocilic fibrosis and necrotic stroma. **H&E X800**

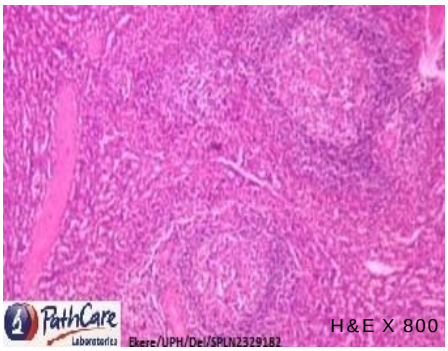


Plate 15. Spleen of *AM* + ZDV at week 2 showing white pulp with stream of myeloblast

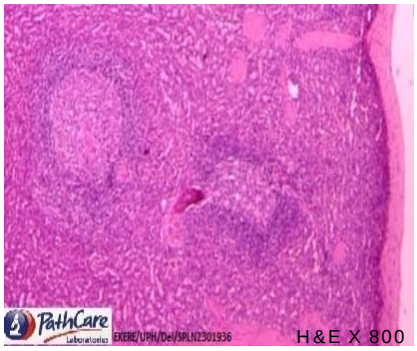


Plate 16. Spleen of *AM* + ZDV at week 4 showing splenic vacoulation and sinusoidal space.

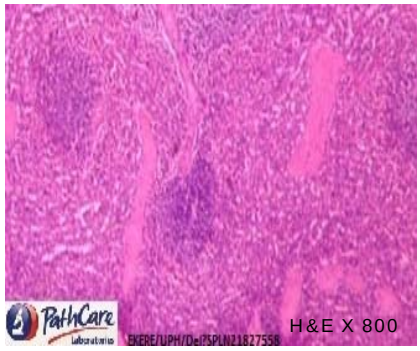


Plate 17. Spleen of *AM* + ZDV at week 6 showing lymphoid sheath and malpighian follicles

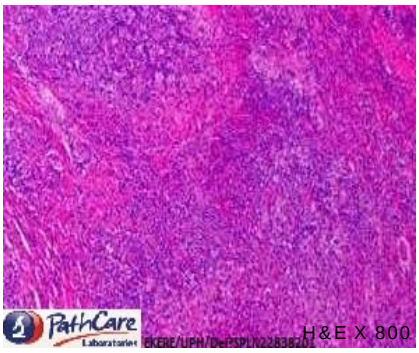


Plate 18. Spleen of *FZ* + ZDV at week 2 showing red blood sinusoids

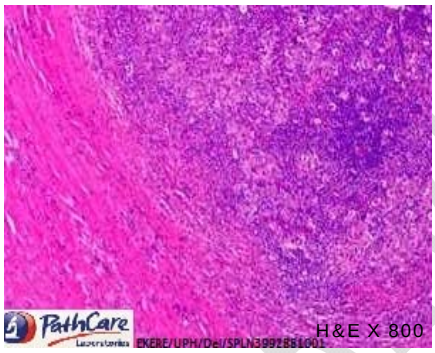


Plate 19. Spleen of *FZ* + ZDV at week 4 showing region of β-lymphocyte aggregation and lymph nodes

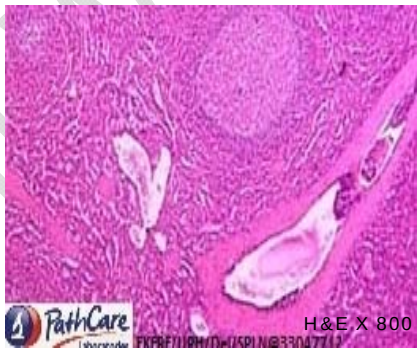


Plate 20. Spleen of *FZ* + ZDV at week 6 showing a lymphocyte around the splenic capsule

Average daily sleep time, estimated daily food and water intake

The result for the average daily sleep time, estimated food and water intakes are depicted in table 10 above. There was an observed decrease in the sleep time observed in the normal control group at weeks 4 and 6 when compared with the average daily sleep time at week 2 while the negative control group showed an increase in the average daily sleep time at week 2 when compared with the normal control group. This value was, however, observed to significantly decrease at week 4 but increased at week 6 while administration of extracts, resulted in a reduction in sleep time when compared with the negative control group at week 2. This was observed to significantly reduce at week 4 when compared to the value in week 2 but increased at week 6. The same trend as observed wit *Annona muricata* was also observed for *Fagara zanthoxyloide* but to a significantly ($p \leq 0.05$) lesser degree.

The result of the estimated average food intake showed that administration of zidovudine resulted in a significant decrease ($p \leq 0.05$) in the estimated average daily food intake in the negative control group which also decreased as the experimentation progressed till week 6. Treatment with extracts, on the other hand, resulted in an increase in the estimated average daily food when compared with the negative control group. The value was however significantly

different from that observed for the normal control group with the impact significantly higher in the *Annona muricata* treatment group.

Histological examination

The result of the splenic histopathology showed a reduction in the pore size of the splenic sinusoids seen in the negative control group as well as atrophy. Despite the increase spleen weights observed from the result of organ weight changes in the treatment groups, the histopathological results showed no case of splenomegaly.

Histopathology of the heart of the negative control group showed disarray of myofibers at weeks 4 and 6. However, no severe architectural change in the intercalated discs, conductive and connective tissues, as well as blood vessels, was observed in both the negative control and treatment groups.

DISCUSSION

Phytochemistry and proximate analysis

The potency of plants extracts has been linked to the presence of chemicals in the plants which can elicit a number of physiological changes on various systems of the body. The results of the phytochemical and proximate analysis revealed that the extract possessed a distinct array of phytochemicals which separately and synergistically may be the reason for the acclaimed folkloric use of these plants in the treatment of several ailment.

Amino acid analysis

The amino acid analysis showed a vast array of amino acids which are known to serve as building blocks of several proteins thus enhancing anabolism and building a favourable body composition (Ohtani *et al*., 2001). They are necessary components of various body systems and necessary for the growth and development of muscles. The presence of this array of amino acids may aid in the bodybuilding process in treatment groups and thus elicit an increased weight gain when compared to the negative control group. They have also been implicated by Okonkwo *et al*., (2013) to be harnessed by anaemic rats in building and restoring their blood cells. Thus these plants may possess haematopoietic potentials when administered in such conditions.

Body weight changes

The results of the body weight changes on the administration of zidovudine may culminate the findings that the drug, in inducing loss of appetite, may elicit certain factors which may lead to a subtle or progressive derangement of the neurochemical signalling for appetite in the hypothalamus of the brain. The end result as observed in the food intake study (table 10) was a possible termination of neurotransmitters/neuromodulators involved in appetite stimulation leading to reduced food intake which may have resulted in body weight loss as seen in Table 7.

The administration of the extract, however, may have cushioned or reversed the neurochemical signal derangement effects of zidovudine thereby regulating food intake and invariably the body weight changes about values obtained for the normal control group (Table 10). It may be suggested that the observed result may be due to certain biochemical components the extracts of these plants. Alkaloids, found in relative proportion in these extracts have been implicated to cause increased feed intake and thus may elicit body weight gain. The nutraceutical and bioactive components of the extracts may possibly have lead to an increased release of hormones such as cholecystokinin (CCK), peptide YY, and glucagon-like peptide-1 (Tucci, 2009) which in synergy, may result in a decrease in the gastric emptying time (thus increasing the absorption of nutrients), inhibit upper gut motility (slowing intestinal transit and emptying) which would improve body metabolism. Fiber also present in significant proportion in both plants, has also been linked with an increase in nutrient absorption (especially trace elements) and water retention thus enabling proper food passage through the gastrointestinal tract.

The result of the phytochemistry, proximate and body weight analysis thus may suggest that extracts of these plants may be used to boost food intake and nutrient absorption thus alleviating the anorexic effects which accompany most prescription medication.

Organ weight changes

Organ weights have been judged as one of the most sensitive indicators to drug toxicity even in the absence of any morphological changes (Yung *et al*., 2013). However, the decrease in organ weights observed in the negative control group when compared to the normal control may partially be due to the decrease in body weights observed during the study (Nirogi *et al*., 2013). Subsequent increase at week 4 and decrease at week 6 in the weight of the spleen although unexplained, may be linked to an underlying pathological condition unexplored in this study as

may be implied from the alterations in the architecture of the spleen on histopathological examination. The increases in organ weights observed on administration of extract treatment may be related to the increased body weight observed in this group (Miyauchi *et al*., 2013; Mandal *et al*., 2012) as this was not be linked to any sign of splenomegaly or cardiac hypertrophy from histopathological findings.

Histological examination

The spleen is an important organ of the haematopoietic system is involved in performing quality control as blood passes through its maze. It detects any old or damaged red cells as it filters the blood, synthesis of immunoglobulin G as well as performs an immunosurveillance function (Chapman and Azevedo, 2018). Studies have shown that the spleen is also involved in clearing out old platelets as well as act as a storage location for platelets. The reduction in the pore size of the splenic sinusoids seen in the negative control group may possibly result in increased destruction of cellular components of blood with compromised membranes which transverse the red pulp. Splenic atrophy also observed in the negative control group may be the result of prolonged administration of drugs which occurs usually secondary to body weight loss (Suttie, 2006). Despite the increase spleen weights observed from the result of organ weight changes in extract treatment group, the histology results showed no case of pathological splenomegaly and as such would rule out the possibility of platelet sequestration as is the case with thrombocytopenia caused by hepatic cirrhosis or congestive splenomegaly (Koduri and Nathan, 2006; Zaorsky *et al*., 2017).

The result of the histology of the heart indicated no significant damage to the myofibers of cardiac muscles and as such implies the absence of cardiovascular disorders both on the administration of zidovudine and treatment with extracts. Thus the drug, as well as the treatment procedure with extracts at the dosage used in this study, did not elicit any cardiovascular damage

Platelet count

The platelet count decrease observed in the negative control group despite being lower than what was observed for the normal control, was however within the normal range, as the case of thrombocytopenia which has been observed to be a conflicting side effect of zidovudine therapy was not established during the duration of experimentation. Although the mechanism of platelet count reduction was not justified from the result of the study, some studies have hypothesized

that zidovudine on administration may result in increased oxidation of the membranes of cellular components of the blood thus making them susceptible to destruction by mechanical abrasion. This coupled with the splenic histological examination, which revealed a reduction in the pores of the splenic sinusoids, may indicate a possibility for the destruction of cellular components of the blood as they pass through the sinusoid pores. The increase in platelet count on the administration of extracts may be linked to the antisickling and blood boosting potential acclaimed to these plants in folkloric medicine (Gadhwal *et al.,* 2016) and also the preservation of splenic architecture and functionality as the duration of administration increased. This may be linked the presence of several bioactive components such as phenols, phenylalanine, leucine, lysine which have been implicated in increasing the concentration of cellular components of the blood (Oboh and Akindahunsi 2004; Nwaoguikpe and Ejele, 2010; Osuagwu, 2010; Igwe *et al*., 2012).

Coagulation assay

The activated partial proplastin time (APTT) and prothrombin time (PT) are used to investigate coagulopathies and also monitor drugs (Armando *et al.,* 2015). Studies by Allison *et al*., (2006) and Bordia *et al*., (1996) have indicated the effect of plant phytochemicals (saponins) in inhibiting calcium metabolism and the steps of the arachidonic pathway in platelets thus resulting in a decrease in the coagulation effects of extracts and also to prevent cardiovascular disease.

According to Gadag *et al*., (2010) an increase in the bleeding time as seen in the negative control group may result in a drop in the systolic blood pressure which invariantly leads to circulatory collapse, shock and possibly cardiac arrest; as such these extracts may also play a role in cases of cardiovascular-related complications as seen with the results of the coagulation study and histology of the heart on treatment with extracts.

The increased prothrombin time observed in the negative control group may indicate inhibition of the tissue factor pathway which requires factor VII or a deficiency of coagulation factors V and X (Davison *et al*., 2012). Reduction of the prothrombin time and activated partial proplastin time on treatment with extracts may be due to the effect of extracts in increasing the synthesis of fibrinogen (Loizou, *et al.,* 2018; Cook and Ubben, 1990) or in some cases increase in plasma velocity.

The observed regulation in the platelet count and coagulation assay by extracts may suggest the use of these extracts as anticoagulants in the prevention or treatment of thromboembolic disorders associated with diseased conditions (Hirsh *et al*., 2004, 2007). This may be as a result of the tannins and flavonoid phytochemicals present in extracts which according to (Kamarudin *et al.,* 2016) reported a correlation between these phytochemicals and coagulation times. The thromboembolic potential of these extracts was further reiterated by the reduction in bleeding time in the treatment groups when compared to the negative group.

Sleep time and estimated food intake

The decrease in sleep time seen in the negative control group might be correlated with the side effects of zidovudine in resulting in fatigue and general body weakness. Administration of extracts however as seen in the result of extract treatment group may have tackled the possible stress-induced insomnia caused by the drug. The reduced average daily sleep time may thus increase the activity and agility of animals in the treatment groups under similar environmental conditions as the negative control group. This result may suggest a possible beneficial effect of these extracts with regards to increased energy generation for daily activity and tackling stress-induced insomnia (Cano *et al*., 2008).

Loss of appetite, food intake and changes in body composition are three inter-related factors which influence the well-begin of animals. The decrease in food intake in the negative control group may be used as a measure of appetite (Mohammed *et al*., 2010) as the administration of zidovudine has been linked with loss of appetite as one of its side effect. The resultant effect would be changes in body composition as evident in the decrease in body weight. The administration of extracts may have reversed the loss of appetite or resulted in an increased appetite as seen in the increased food intake and thus body weight gain.

CONCLUSION

The extracts of the plants used in the study were observed to initiate body weight increases possibly due to the nutraceutical potentials these extracts posses or the physiological changes elicited by the components of the extracts. The increase in organ weight observed in relation to body weight did not signify any architectural abnormalities with the organs investigated as the histology of the heart muscles and spleen showed no signs of an abnormal mass, contusions or

tumours. The histology of the heart showed no physiological or architectural defects while the histology of the spleen only showed a decrease in the red pulp sinusoids.

Although the decreased platelet count on the administration of zidovudine was not at a critical or life-threatening level, the platelet counts increase on the administration of extracts along with results of the coagulation assay may signify the possible application of extracts of the plants in thromboembolic related disorders.

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