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Some Haematological Parameters in Rats treated with Phenyl Hydrazine and *Acalypha wilkesiana* Leaf Extracts

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ABSTRACT

Medicinal plants prevent and ameliorate various diseases. In Nigeria, different species of *Acalypha* has demonstrated antidiabetic, antimicrobial, and antioxidant properties. This study investigated some haematological parameters in phenyl hydrazine induced-anaemic Wistar rats treated with aqueous and methanol leaf extracts of *A. wilkesiana*. Anaemia was induced subcutaneously with phenyl hydrazine at 40 mg/kg body weight, and treated with both extracts. Rats were divided into nine groups of five each. Groups A, B and C were normal control, anaemic control and vitamin E control respectively. Groups D, E, F and G, H, I was given 200, 400 and 600 mg/kg body weight of both leaf extracts, respectively. After 14 days post-treatment, Group B showed significant reduction ($p < 0.05$) of some haematological parameters when compared with control. Groups treated with vitamin E and *A. wilkesiana* showed significant reverse and restoration of some haematological parameters towards and above control values in a relatively non-dose dependent and non-significant manner ($p > 0.05$), except lymphocytes, haemoglobin, mean corpuscular volume and haematocrit which recorded significant increase ($p < 0.05$). However, platelets and platelet crit recorded significant decrease ($p < 0.05$). The study revealed that the leaf extracts may be relatively anti-anaemic with better hematopoietic potentials than Vitamin E**.**

Keywords: Wistar rats, induced-anaemia, *Acalypha wilkesiana,* haematological parameters

INTRODUCTION

Anaemia is a public health disease affecting both the rich and poor, totaling about one third of the world's population (Chaparro and Suchdev, 2019). It is a condition in which the haemoglobin (HGB) concentration, haematocrit (HCT), or red blood cell (RBC) count and/or quantity of circulating RBC numbers (and their oxygen-carrying capacity) are lower than normal and insufficient to meet physiological needs (Ahmed *et al*, 2013; Schreir, 2018; Chaparro and Suchdev, 2019). Its incidence may result from parasitic infections, malaria and nutritional deficiencies of irons. Anaemia might lead to increased morbidity and mortality, decreased work productivity, and impaired neurological development (Ahmed *et al*, 2013; Chaparro and Suchdev, 2019). Iron deficiency is the most common cause of anaemia, although some cases may be inherited. It primarily affects women of childbearing age, children, and the elderly and could result in high-output heart failure and mortality. It might also be caused by inadequate RBC production, excessive RBC destruction, or blood loss (Ahmed *et al*, 2013; Schreir, 2018; Chaparro and Suchdev, 2019). Treatment of anemia depends on the form and underlying conditions with acute and/or severe cases requiring transfusion of packed red blood cells.

Traditional medicine and the use of plants to treat several ailments has a long history and is still being used as a primary health care system in different communities, States, Countries and the World. Any part of the plants such as, the leaf, bark, stem, root, etc., can be used to alleviate, treat, rejuvenate, or prevent disease conditions (Mintah *et al*, 2019).

Most of these plants have been shown to possess phytochemicals, otherwise called secondary metabolites. These secondary metabolites have biological properties such as antioxidant activity, antimicrobial effect, antitumor properties; etc, which provides health benefits other than those provided by macronutrients and micronutrients (Saxena *et al*, 2013). The secondary metabolites are said to protect plants from disease and damage and contribute to the colour, aroma and flavor of the plant. General, the chemicals that protect the plant cells from environmental hazards like pollution, stress, drought, UV exposure and pathogenic attack are referred to as phytochemicals. So, it is well established that plants produce the secondary metabolites to protect themselves, but recent studies have revealed that many phytochemicals can also protect human against diseases (Saxena *et al*, 2013). Most modern medicine today uses active compounds isolated from plants and more than 70% of these active compounds indicate a positive correlation between their modern therapeutic use and their traditional uses (Mintah *et al*, 2019).

Acalypha wilkesiana is an important ornamental and herbal plant whose leaves are used in the treatment of various infections like fungi infections, bacterial infection etc. *A. wilkesiana* belongs to the family Euphorbiaceae (spurge family). It has different names because of its unique colour. Among the names its commonly called is copperleaf and Joseph's coat (Ifeanacho *et al*, 2017). The locals in the Northern part of Nigeria call it "Jiwene" and "Jinwinini", while those in the West call it "aworoso" (Ifeanacho *et al*, 2017).

Earlier studies indicate that *A. wilkesiana* (*godseffiana*) have different phytochemicals, antimicrobial, antioxidant and anti-inflammatory properties (Olubodun *et al*, 2021a). This study therefore investigated the haematologic potential of aqueous and methanol leaf extracts of *A. wilkesiana* in phenyl hydrazine induced anaemic Wistar rats.

MATERIALS AND METHODS

a) Study area

The experiment was carried out at the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria (Latitude 6° 23' 44"; Longitude 5° 36' 49"; Altitude 360 feet).

b) Plant Materials

Fresh leaves of *A. wilkesiana* were obtained from the Ugbowo campus of the University of Benin, Benin City, Nigeria. The plant was identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria and the voucher specimen (UBH-A549) was prepared and deposited in the herbarium of the same Department.

c) Preparation of Plant Extracts

The leaves of *A. wilkesiana* were air-dried at room temperature for a period until a constant weight was obtained. After showing brittle properties, the leaves were crushed and reduced into fine powder using a mechanical blender. The powdered sample (1000g) was soxhlet extracted with 2000ml methanol, concentrated with a rotary evaporator at 40^{o}C. The weight of the concentrates was taken and the percentage yield calculated. The concentrated extract was stored in a refrigerator at -4^{o}C until required for further studies. Another quantity of the fresh leaves of *A. wilkesiana* was macerated with distilled water (250ml) and allowed to soak for 48hours. The mixture was filtered using a piece of clean, sterile Muslin cloth to remove debris and then filtered on Whatman filter paper. The filtrate was concentrated to dryness with a rotary evaporator in vacuo. The dried powdery sample was stored at -4^{o}C until required for use.

d) Experimental Rats

A total of thirty male Wistar albino rats with mean weight of 179 ± 5 g were purchased from the Animal House, University of Benin, Benin City, Nigeria and they were maintained in the Laboratory Animal Unit of the Department of Biochemistry, Faculty of Life Sciences, University of Benin. They were fed with standard diet and water ad libitum, and acclimatized two weeks before commencement of treatment. The experiment was approved by the University of Benin ethical committee and the rats were handled in accordance with the guidelines on the care and wellbeing of research animals.

e) Acute Toxicity Study

Acute toxicity (LD_{50}) study was carried out by the method described by Lorke (1983) with slight modifications. Thirty-five rats were divided into seven groups of five rats each. The aqueous extracts of *A. wilkesiana* were administered orally to the rats in groups B - G in doses of 10, 100, 1,000, 1,600, 2900, and 5,000 mg/kg body weight. Control /Normal Group (A) received water. The rats were allowed to feed *ad libitum*, and observed regularly for two days (48 hours) for any changes that may occur physically or in behaviour, in-coordination and/or mortality. The aqueous extracts were safe up to the dose of 5,000 mg/kg body weight and did not cause mortality in the rats during the period of study.

f) Induction of anaemia

Anaemia was induced by the method described by Harris and Kugler (1971) and modified by Sanni *et al*. (2005). The rats were subjected to subcutaneous administration of phenyl hydrazine (Fisher Scientific Company, New Jersey, USA), at a dose of 40 mg/kg body

weight, for three (3) days, within the duration of the experiment.

g) Treatment of phenyl hydrazine-induced anaemia with leaf extracts

Groups D, E, F, G, H and I rats were treated by daily oral administration of aqueous and methanol leaf extracts, after diagnosis of anaemia, that is, three(3) days after phenyl hydrazine injection, at a dose of 40 mg/kg body weight for 14 days. The plant crude extracts were reconstituted with distilled water and given to the rats orally.

h) Experimental Design

Thirty male Wistar rats were randomly divided into six groups of five rats each (n=5). The baseline values were determined before the rats were induced with anaemia. Then, calculated amount of the leaf extracts of *A. wilkesiana* were constituted in distilled water from the stock solution according to the individual weights to give doses of 200, 400 and 600 mg/kg body weight and administered orally to the rats in groups D - I for the aqueous and methanol extracts respectively, for a period of 14 days as illustrated: Group A, Normal control, received distilled water only; Group B, Anaemic control, received phenyl hydrazine injection only; Group C, received phenyl hydrazine injection and Vitamin E (500mg/kg body weight); Groups D, E and F and Groups G, H and I, received phenyl hydrazine injection and 200, 400 and 600 mg/kg body weight of *A. wilkesiana* for the aqueous and methanol leaf extracts respectively. Administration of the leaf extracts of *A. wilkesiana* was performed orally once daily after induction of anaemia, by gavage for fourteen (14) days. On the fifteenth (15) day, the blood of the rats was recovered for analyses. Water was provided *ad libitium* to all groups.

i) Collection of Blood

The initial and final body weight changes were monitored at the beginning and end of the experiment. On the 15th day, after an overnight fast, the rats were anaesthetized in chloroform and blood samples were collected by cardiac puncture into labeled bottles for haematological analyses.

j) Haematological Analysis

Haemoglobin(HGB), red blood cell (RBC) count, white blood cell (WBC), lymphocytes (LYM), platelets (PLT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and other haematological parameters were analyzed using an automated analyzer (SYSMEX K-21N: SYSMEX CORPORATION, JAPAN).

k) Data Analysis

The data from the results were expressed as mean ± standard error of mean for the number of observations. The results were analyzed using one-way analysis of variance (ANOVA). Inter-group comparisons were done by the Duncan's post hoc test. IBM SPSS Statistics, version 26 (IBM Corp., Armonk, N.Y., USA) was used for the analysis. Differences between groups were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Acute toxicity study

The results of the acute toxicity study presented a median lethal dose (LD_{50}) value greater than 5,000 mg/kg body weight. The toxicity studies of the aqueous and methanol leaf extracts in the rats showed that the LD_{50} was greater than 5000 mg/kg. The study agrees with the report of other authors who recorded LD_{50} of greater than 5000 mg/kg

for the same plant species (Sule *et al*, 2012) and other plant species (Iniaghe *et al*, 2013; Onu *et al*, 2013; Okpo and Amoo, 2016; Olubodun *et al*, 2021b). The rats did not show any visible signs of toxicity such as shivering, hair erection, reduced feeding, water intake, breathing, and movement, when administered the extracts. Their behaviour, physical characteristics, and activities remained relatively unchanged as they moved about in the cages. The plant may therefore be considered non-toxic since the study did not observe any deleterious effects that may have been accompanied or preceded by clinical signs of toxicity of physical or/and behavioural changes in the rats (Iniaghe *et al*, 2013; Onu *et al*, 2013; Okpo and Amoo, 2016).

Body weight and percentage change

Changes in body weight are generally indicative and often used as indicators of chemical toxicology that may induce changes in organisms (Onu *et al*, 2013; Olubodun *et al*, 2021b). Table 1 show the effects of aqueous and methanol leaf extracts of *A. wilkesiana* on body weight of phenyl hydrazine-induced anaemic rats. Significant ($p < 0.05$) decrease in weight was observed in Group B rats (i.e. anaemic control rats), in the first week and relatively non-significant decrease in the second week of the study (Table 1). Rats in Groups C to I recorded no significant difference ($p > 0.05$) in their body weight when compared with control in the first week but recorded significantly increased body weight gain in the second week of the study which corresponded to control values and in some doses higher weight gain when compared with normal control values.

Body weight loss has been reported to be an indication of abnormal condition and may arise from decreased food intake due to the immobility accompanying stress, or reduced absorption of food (Okpo and Amoo, 2016; Olubodun *et al*, 2021b). Weight losses were also recorded in other studies with various treatment and induction of anaemia with phenyl-hydrazine (Sanni *et al*, 2005; Ogbe *et al*, 2010; Olubodun *et al*, 2021b) and relative increase when treated with other plant extracts (Olubodun *et al*, 2021b). The relatively non-significant increase in body weight observed in the leaf extract groups when compared to untreated anaemic rats may be attributed to the leaf having the ability to inhibit the loss in body weight by increasing food intake through attenuation of abnormal condition of stress or improving the absorption capacity of the intestine (Okpo and Amoo, 2016; Olubodun *et al*, 2021b).

Table 1: Effects of aqueous and methanol leaf extracts of *Acalypha wilkesiana* on body weights of phenyl hydrazine-induced anaemic rats

Assays	Groups	Mean body weights of rats (g)		
		Day 1	Day 7	Day 14
Assay one:				
Control	A	183.00±2.63^{a}	190.40±4.20^{a}	203.65±9.10^{a}
Anaemic control	B	184.46±4.10^{b}	176.30±3.74^{b}	184.16±8.40^{b}
Vitamin E	C	179.20±7.90^{a}	182.20±6.60^{a}	187.50±3.50^{c}
200mg/kg AE *Acalypha wilkesiana*	D	174.02±7.63^{c}	174.96±3.40^{a}	176.60±6.70^{d}
400mg/kg AE *Acalypha wilkesiana*	E	183.80±4.61^{a}	188.80±6.70^{b}	196.50±2.60^{a}
600mg/kg AE *Acalypha wilkesiana*	F	177.26±4.14^{a}	178.04±2.72^{a}	179.80±3.42^{bd}
Assay two:				
Control	A	183.00±2.63^{a}	190.40±4.20^{a}	203.65±9.10^{a}
Anaemic control	B	184.46±4.10^{b}	176.30±3.74^{b}	184.16±8.40^{b}

Vitamin E	C	179.20±7.90[a]	182.20±6.60[a]	187.50±3.50[c]
200mg/kg ME *Acalypha wilkesiana*	G	187.50±3.50[c]	185.00±8.00[a]	192.50±7.63[d]
400mg/kg ME *Acalypha wilkesiana*	H	184.06±8.69[a]	186.80±6.78[b]	191.25±10.00[b]
600mg/kg ME *Acalypha wilkesiana*	I	183.20±7.27[a]	184.40±8.00[a]	185.80±9.40[d]

AE: Aqueous leaf extract, ME: Methanol leaf extract, Body weights of rats are expressed as means ± SEM of five replicates Means of same column with different superscripts are significantly different ($p < 0.05$).

Table 2 shows the effects of aqueous and methanol leaf extracts of *A. wilkesiana* on percentage change in body weight of phenyl hydrazine-induced anaemic rats. While the rats in Groups B significantly decreased in the percentage change in body weight in the first seven (7) days after treatment with phenyl-hydrazine injection, there was significant increase in percentage body weight when compared with the weight after 7 days but none significant decrease was recorded when compared with the initial weight showing improvement in the body weight. The rats in Groups C to I (40 mg/kg phenyl-hydrazine injection + vitamin E, 200 mg/kg *A. wilkesiana*, 400 mg/kg *A. wilkesiana*, and 600 mg/kg *A. wilkesiana* respectively), recorded significant ($p > 0.05$) increase in percentage change in body weight when compared with the anaemic control (Groups B). Groups B rats recorded -4.2% decreases in percentage change in body weight in the first 7 days, it recorded -0.2% decrease after 14 days. Groups C, D, E, F, G, H and I recorded an increase of 4.6, 1.5, 6.9, 1.4, 6.9, 3.9 and 1.4 % respectively with control having the highest percentage increase of 11.3%.

The percentage change in body weight observed in the rats induced with anaemia and simultaneously treated with vitamin E and graded doses of the leaf extracts when compared with normal controls may be due to abnormal condition as a result of anaemia, decreased food intake, and/or reduced absorption of food (Okpo and Amoo, 2016; Olubodun et al, 2021b).

Table 2: Percentage change in body weight in phenyl-hydrazine induced anaemic rats treated with aqueous and methanol leaf extracts of *Acalypha wilkesiana*

Assay	***Group***	***Initial body weight* (g)**	***Final body weight* (g)**	***Change in body weight* (%)**
Control	A	183.00±2.63	203.65±9.10	11.3
PHZ only	B	184.46±4.10	184.16±8,40	- 0.2
PHZ+ Vitamin E	C	179.20±7.9	187.50±3.50	4.6
PHZ+200mg/kg AE *A. wilkesiana*	D	174.02±7.63	176.60±6.70	1.5
PHZ+400mg/kg AE *A. wilkesiana*	E	183.80±4.61	196.50±2.60	6.9
PHZ+600mg/kg AE *A. wilkesiana*	F	177.26±4.14	179.80±3.42	1.4
PHZ+200mg/kg ME *A. wilkesiana*	G	187.50±3.50	192.50±7.63	6.9
PHZ+400mg/kg ME *A. wilkesiana*	H	184.06±8.69	191.25±10.00	3.9
PHZ+400mg/kg ME *A. wilkesiana*	1	183.20±7.27	185.80±9.40	1.4

PHZ: Phenyl hydrazine, AE: Aqueous leaf extract, ME: Methanol leaf extract, Initial and Final body weights of rats are expressed as means ± SEM of five replicates.

Haematological Parameters in Phenyl-hydrazine induced Anaemic Rats treated with Aqueous and Methanol Leaf Extracts of *A. wilkesiana*

The investigation of haematological parameters may be used to determine the deleterious effects of substances on the blood constituents of an animal or to explain blood relating functions of chemical compounds in living systems (Iniaghe et al, 2013). The haematological parameters also provide information about haemolysis and bone marrow activities. Haemoglobin (HB), Packed cell volume (PCV) and red blood cells (RBC) are associated with the total population of red blood cells while mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) relates to individual red blood cells (Pluncevic-Gligoroska et al, 2019).

Tables 3 and 4 shows the effects of aqueous and methanol leaf extracts of *A. wilkesiana* on haematological parameters in phenyl hydrazine-induced anaemic rats. The induction of anemia significantly elevated the concentrations of HCT, MCV and MCH and reduced the concentrations of PLT and PCT relative to the normal control ($p < 0.05$). WBC and LYM were not significantly affected with induction of anemia as well as graded dose treatment with the aqueous extracts relative to the normal control ($p > 0.05$). MON and GRA concentrations were not significantly altered between the groups relative to the normal control and anaemic control ($p > 0.05$), However, the concentrations of MON and GRA were significantly reduced by treatment with 200 mg/kg aqueous extracts ($p < 0.05$) (Table 3). Haemoglobin concentration was significantly elevated with 200 mg/kg aqueous extracts ($p < 0.05$), but was not significantly affected by the induction of anemia ($p > 0.05$) relative to the normal control (Table 3).

Obinna *et al*, (2018) reported that an alteration in any one parameter may alter other haematological parameters since there is direct relationship between RBC, PCV and HB concentration. The PCV and RBC counts were significantly decreased on induction of anaemia with phenyl hydrazine in all the rats, indicating that the chemical could effectively induce anaemia at the given dosage after 3 days. Earlier report also recorded induction of anaemia with phenyl hydrazine hydrochloride in rats (Ogbe *et al*, 2010) and rabbits (Obinna *et al*, 2018) at various time intervals. The group B rats (anaemic control) which were not treated with the supplement vitamin E and aqueous and methanol extracts, seems to recover from anaemia after two week of treatment when compared with control and the extracts treated groups. This may be due to the decreasing effect of phenyl hydrazine. Ogbe *et al*. (2010) reported that phenyl hydrazine induces development of Henz bodies on RBC membranes after six days of exposure, which may likely protect the rats against further destruction by the chemical (Ogbe *et al.*, 2010).

The study did not record any significant differences in most of the haematological parameters studied after induction of anaemia and treatment with the aqueous or methanol leaf extracts of *A. wilkesiana*, except the mean corpuscular volume (MCV) (Tables 3 and 4). Significant increases in MCV was observed at all doses of the extract when compared with normal control.

The non-significant effect of the extracts on RBCs might be indicative of the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis). Platelet count reduction has been reported to indicate adverse effect on the oxygen carrying capacity of the blood as well as thrombopoietin (Iniaghe *et al*, 2013). This study also recorded non-significant platelet count signifying that the oxygen carrying capacity of the blood was unaffected when the aqueous or methanol

leaf extracts were administered at graded doses to the Wistar rats.

The MCV is an index which reflects how small or large the RBCs are relative to normal. The significant increase recorded in MCV at all doses of the aqueous and methanol leaf extracts, the anaemic group and vitamin E group when compared to the normal control, suggests that the extracts may interfere with iron uptake into haemoglobin. The increase in MCV observed in this study is in agreement with the report of Ikewuchi *et al.* (2011) who reported increase in MCV on alloxan induced diabetic rats; but is contrary to the observation of Iniaghe *et al.* (2013).

Failure to produce haemoglobin results in cells smaller than normal cells. This occurs in many diseases, including iron deficiency anemia, thalassemia (an inherited disease in which globin chain production is deficient), and anemia associated with chronic infection or disease. Iron is an essential component of many enzymes in cells and is also part of the haem group in haemoglobin (which consists of a porphyria ring containing iron). Much of the body's iron stores are within red blood cells where iron is critical for haemoglobin synthesis. Iron deficiency could be due to inadequate intake or absorption of iron, excessive loss with external hemorrhage, or interference with iron metabolism (Iniaghe *et al*, 2013).

Table 3: Effect of aqueous leaf extracts of *Acalypha wilkesiana* on haematological parameters in phenyl hydrazine-induced anaemic rats

Heamatological parameters	Group					
	A	B	C	D	E	F
WBC ($x10^4\mu l$)	1.39±0.07	1.59±0.10	1.60±0.17	1.30±0.20	1.24±0.15	1.53±0.11
LYM ($x10^4\mu l$)	1.10±0.06	1.36±0.12	1.36±0.16	1.05±0.14	1.02±0.13	1.29±0.14
MON ($x10^3\mu l$)	1.86±0.13	1.52±0.09	1.55±0.10	1.33±0.30	1.40±0.37	1.54±0.25
GRA ($x10^3\mu l$)	1.08±0.13	0.78±0.14	0.93±0.06	1.10±0.47	0.80±0.31	0.78±0.18
RBC ($x10^6\mu l$)	$6.71±0.18^a$	$5.61±0.30^b$	$5.90±0.18^{ab}$	$6.24±0.27^a$	$6.39±0.18^a$	$6.25±0.32^a$
HGB (g/10ml)	$1.48±0.32^a$	$1.37±0.61^b$	$1.53±0.52^a$	$1.61±0.67^c$	$1.64±0.45^c$	$1.60±0.24^c$
HCT (x10%)	$3.89±0.61^a$	$3.77±1.18^{ab}$	$4.21±1.37^c$	$4.31±2.03^{cd}$	$4.42±0.74^{cd}$	$4.27±0.61^{cd}$
MCV ($x10\mu m^3$)	$5.81±1.39^a$	$6.49±5.53^{ab}$	$6.79±2.71^b$	$7.69±0.82^c$	$6.93±1.73^{ab}$	$6.91±3.61^{ab}$
MCH (x10Pg)	$2.20±0.30^a$	$2.46±1.05^b$	$2.54±1.57^a$	$2.88±0.39^{bc}$	$2.57±0.56^{ab}$	$2.58±1.23^{ab}$
MCHC(x10g/dL)	$3.80±0.45^a$	$3.62±0.13^b$	$3.63±0.60^{ab}$	3.75±0.59	$3.71±0.46^a$	$3.74±0.33^a$
PLT ($x10^7/\mu L$)	$7.52±2.93^a$	$3.62±1.92^b$	$3.63±9.36^a$	$3.75±3.40^a$	$3.71±3.21^a$	$3.74±3.41^a$
MPV (μm^3)	6.78±0.17	7.24±0.24	7.28±0.32	7.33±0.14	7.08±0.11	7.28±0.16
PCT (%)	$0.51±0.02^a$	$0.35±0.02^b$	$0.40±0.06^b$	$0.34±0.02^b$	$0.38±0.02^b$	$0.35±0.02^b$
PDW (x10%)	1.52±0.52	1.61±0.60	1.59±1.00	1.42±0.34	1.46±0.13	1.48±0.49

WBC: White blood cell, LYM: Lymphocyte, MON: Monocyte, GRA: Granulocyte, RBC: Red blood cell, HGB: Haemoglobin. HCT: Haemotocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RDW: Red blood cell distribution width, PLT: Platelet count, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet diffusion width, Group A: normal control, B: anaemic control, C: 500 mg/ kg body weight standard vitamin E treatment. D: 200 mg/kg aqueous extract treatment, E: 400 mg/kg aqueous extract treatment, F: 600 mg/kg aqueous extract treatment. Results are expressed as means ± SEM of four (4) replicates. Values with different superscripts are significantly different in the rows ($p < 0.05$).

In Table 4, haemoglobin concentration was significantly elevated when 600 mg/kg body weight methanol leaf extracts of *A. wilkesiana* was administered to the anaemic rats ($p < 0.05$), when compared with the normal and anaemic control. The same effect was not observed among anaemic rats given 200 mg/kg and 400 mg/kg methanol

leaf extracts of *A. wilkesiana* relative to the normal and anaemic control. Oral treatment with 600 mg/kg methanol leaf extracts significantly elevated the concentration of HCT relative to the normal control, anaemic control and vitamin E supplement group ($p < 0.05$). Though treatment of anaemic rats with 200 mg/kg and 400 mg/kg body weights saw an elevation of HCT, it was not statistically significant relative to anaemic control and vitamin E supplement group ($p > 0.05$). The concentration of MCV was elevated across the graded doses of *A. wilkesiana* when compared with normal control and anaemic control.

The observed elevation in haemoglobin (HGB) and haematocrit (HCT) concentration following treatment with *A. wilkesiana* leaf extracts at different doses implies that the plant extract may contain erythropoeitic properties that enhanced erythropoiesis. This is similar to the results of Obeagu *et al.* (2014) and Egba *et al.* (2013) who used the leaf extracts of *Telfairia*. There were no significant differences in WBC, LYM, GRA, MON, between the anaemic control and the normal control. There were no significant changes in the levels WBC, LYM, GRA, MON, MCHC, PLT and PCT in the various treatment groups relative to the anaemic group.

There was significant elevation in the concentration of MCV when treated with the 200 mg/kg and 600 mg/kg when compared with the anaemic control ($p < 0.05$). There was progressive elevation of MCH concentration across the graded dose of the methanol extracts, but only 600 mg/kg body weight was significantly elevated ($p < 0.05$) when compared to the anaemic control.

Table 4: Effect of methanol leaf extracts of *Acalypha wilkesiana* on haematological parameters in phenyl hydrazine-induced anaemic rats

Heamatological parameters	Group					
	A	B	C	D	E	F
WBC ($x10^4\mu l$)	1.39 ± 0.07^a	1.59 ± 0.10^b	1.60 ± 0.17^b	1.33 ± 0.05^a	1.52 ± 0.17^{ab}	1.18 ± 0.10^c
LYM ($x10^4\mu l$)	1.10 ± 0.06^a	1.36 ± 0.12^c	1.36 ± 0.16^c	1.10 ± 0.04^a	1.21 ± 0.11^{ac}	0.88 ± 0.10^b
MON ($x10^3\mu l$)	1.86 ± 0.13	1.52 ± 0.09	1.55 ± 0.10	1.30 ± 0.04^a	1.83 ± 0.38^a	1.42 ± 0.10^a
GRA ($x10^3\mu l$)	1.08 ± 0.13	0.78 ± 0.14	0.93 ± 0.06	1.00 ± 0.15^a	1.28 ± 0.45^a	1.58 ± 0.52^b
RBC ($x10^6\mu l$)	6.71 ± 0.18^a	5.61 ± 0.30^b	5.90 ± 0.18^{ab}	5.80 ± 0.17^a	5.95 ± 0.12^a	6.27 ± 0.27^a
HGB (g/10ml)	1.48 ± 0.32^a	1.37 ± 0.61^b	1.53 ± 0.52^a	1.50 ± 0.03^a	1.48 ± 0.06^a	1.64 ± 0.06^c
HCT (x10%)	3.89 ± 0.61^a	3.77 ± 1.18^{ab}	4.21 ± 1.37^c	4.07 ± 0.05^{ac}	3.93 ± 0.12^{ab}	4.36 ± 0.11^c
MCV ($x10\mu m^3$)	5.81 ± 1.39^a	6.49 ± 5.53^{ab}	6.79 ± 2.71^b	7.04 ± 0.16^b	6.63 ± 0.31^b	6.99 ± 0.29^b
MCH (x10Pg)	2.20 ± 0.30^a	2.46 ± 1.05^b	2.54 ± 1.57^a	2.59 ± 0.07^a	2.48 ± 0.12^a	2.62 ± 0.10^a
MCHC(x10g/dL)	3.80 ± 0.45^a	3.62 ± 0.13^b	3.63 ± 0.60^{ab}	3.67 ± 0.04^{ab}	3.75 ± 0.06^{ab}	3.79 ± 0.02^a
PLT ($x10^7/\mu L$)	7.52 ± 2.93^a	3.62 ± 1.92^b	3.63 ± 9.36^a	5.18 ± 0.50^b	7.18 ± 0.49^a	4.85 ± 0.33^b
MPV (μm^3)	6.78 ± 0.17	7.24 ± 0.24	7.28 ± 0.32	7.13 ± 0.26^a	7.15 ± 0.19^a	7.14 ± 0.20^a
PCT (%)	0.51 ± 0.02^a	0.35 ± 0.02^b	0.40 ± 0.06^b	0.37 ± 0.03^b	0.51 ± 0.03^a	0.35 ± 0.02^b
PDW (x10%)	1.52 ± 0.52	1.61 ± 0.60	1.59 ± 1.00	1.43 ± 0.06^a	1.57 ± 0.08^a	1.57 ± 0.09^a

WBC: White blood cell, LYM: Lymphocyte, MON: Monocyte, GRA: Granulocyte, RBC: Red blood cell, HGB: Haemoglobin. HCT: Haemotocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RDW: Red blood cell distribution width, PLT: Platelet count, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet diffusion width, Group A: normal control, B: anaemic control, C: 500 mg/ kg body weight standard vitamin E treatment. G: 200 mg/kg

methanol extracts treatment, Group H: 400 mg/kg methanol extract treatment, I: 600 mg/kg methanol extract treatment. Results are expressed as means ± SEM of four (4) replicates. Values with different superscripts are significantly different in the rows ($p < 0.05$).

Induction of anaemia significantly reduced the concentrations of RBC, MCHC, PLT and PCT relative to the normal control ($p < 0.05$). WBC and LYM were elevated by Vitamin E treatment and 400 mg/kg body weight methanol extract treatment relative to the normal control ($p < 0.05$), but was not significantly affected when compared with the anaemic control ($p > 0.05$). MON and GRA concentrations were not significantly altered between the groups relative to the normal control and anaemic control ($p > 0.05$). However, significant reduction was shown in groups treated with 200 mg/kg methanol leaf extracts of A. wilkesiana. Concentrations of RBCs were significantly increased ($p < 0.05$) with methanol leaf extracts of 600 mg/kg A. wilkesiana when compared with anaemic control. Haemoglobin concentration was not significantly reduced on induction of anaemia relative to normal control, but there was significant elevation of HGB on treatment with vitamin E and graded dose of methanol leaf extracts of A. wilkesiana. Furthermore, treatment with methanol leaf extracts of A. wilkesiana significantly reduced the concentration of HGB relative to the anaemic control ($p < 0.05$).

Platelets are fragment of cells that participates in blood clotting, they initiate repair of blood vessels walls and are also considered an acute phase reactant to infection or inflammation; Plateletcrit (PCT) showcase the precise method of determining the degree of acute blood loss while mean platelet volume (MPV) is used when investigating the ability of a drug to enhance blood clotting (Ganong, 1999). Platelets are dynamic blood particles whose primary function, along with the coagulation factors, is haemostasis, or the prevention of bleeding. The significant reduction in thrombocytic parameters - Platelet count and Platecrit on induction of anemia could translate the anti-thrombopoetin potency of Phenyl hydrazine and that the blood clotting mechanism of the animals would be inadequate with consequents effects of high loss of blood in case of injury (Lawal *et al*, 2015). However, treatment with different doses of *A. wilkesiana* extracts along with vitamin E supplement, resulted in non-significant increase in Platelet and Plateletcrit concentrations when compared to the anaemic group. This indicates that the administered doses of the aqueous and methanol leaf extracts did not ameliorate the thrombocytopenic effects of phenyl hydrazine in the anaemic rats. This could be due to the dosage of the extracts and the period of administration. This result agrees with the report recorded by Obiandu *et al.* (2022) on the actions of *Persea americana.*

CONCLUSION

The results suggest that the aqueous and methanol leaf extracts of *A. wilkesiana* and vitamin E, increased the percentage change in body weight in the rats. The study revealed that the extracts may have a positive effect on the haemopoietic system. The study indicates that *A. wilkesiana* leaf extracts may be relatively anti-anaemic with better hematopoietic potentials than Vitamin E**.**

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