OSMOREGULATORY AND HAEMATOPOIETICEFFECT OF AQUEOUS EXTRACTS OF *Terminaliacatappa* LEAF FOLLOWING PHENYL HYDRAZINE INDUCED HAEMOLYTIC ANAEMIA IN WISTAR RATS

ABSTRACT

Anemia is an extremely common condition, affecting up to a third of the world's population. In many cases, it is mild and asymptomatic, requiring no treatment. Prevalence increases with age, and is more frequent in women of childbearing age, pregnant women and the elderly. The aim of this study was to investigate the effect of aqueous Terminaliacattapa extract on hematological indices in phenyl hydrazine-induced anemic Wistar rats. The methodology adopted was that of randomly selecting 25 male Wistar rats divided into 5 groups (A-E). Hemolytic anemia was induced by intraperitoneal injection of phenyl hydrazine at 10mg/kg body weight for 7 days. Treatment was carried out orally using aqueous extracts of *Terminaliacattapa* leaves at 100mg/kg body weight and 200mg/kg body weight, once daily for 14 days. The results revealed a significant increase ($p<0.05$) in the number of red blood cells, white blood cells, haemoglobin and plasma cells, but no significant difference ($p<0.05$) in the number of blood cells, platelets and plasma cells, compared with the norm and control. The results of this study revealed that the aqueous extract of *Terminaliacatappa* at different concentrations can synergistically interfere with the osmoregulatory and hematopoietic system of the blood, and could be a panacea in the management of anemia.

Keysword:

Introduction

Anemia is also known as a reduction in the absolute number of circulating red blood cells [1]. But also, their inability to carry oxygen to meet physiological needs. It is usually diagnosed by a low hemoglobin concentration or a low hematocrit level [1]. Anemia can also be diagnosed by red blood cell count, mean corpuscular volume, reticulocyte count, blood film analysis or hemoglobin electrophoresis [2]. Anemia is associated with increased morbidity and mortality in women and children [3]. It begins at birth [4,5] and is associated with reduced work productivity

in adults [6] and impaired cognitive and behavioural development in children [7] (sentence too long). Preschool children (PSC) (Define acronym) and women of childbearing age (WRA) (Define acronym) are particularly affected. Phenyl hydrazine (PHZ), a non-immunogenic drug, causes alterations in the membrane of red blood cells, leading to oxidative denaturation of hemoglobin. This shortens the lifespan of erythrocytes [8], which are eradicated by the spleen and liver, resulting in compensated hemolytic anemia (sentence too long).

At this point, the authors should first present the medicinal plants as a whole, i.e. their presence, the metabolites they possess, their importance and their contribution, and then mention the effects of these medicinal plants.

The effect of medicinal plants in disease management has taken center stage in investigations and research by various scientists has shown that these plants have little or no side effects and relatively low cost [9]. *Terminaliacatappa* L. is a large tree widely distributed in the tropics, particularly in coastal areas, whose kernels are edible. *Terminaliacatappa* belongs to the Combretaceae family (Combretum family).It has been found to be rich in a range of phytochemicals, and various studies have demonstrated the plant's anti-inflammatory, hepatoprotective, antidiabetic, wound-healing, anticancer, hypocholesterolemic, antioxidant and radical-scavenging effects [10,11,12,13,14,15].

A study by Aimola I A ,Ihuwa H M, Nok A.J, Mamman A I [16] isolated a new fetal hemoglobin-inducing compound (active fraction of *Terminaliacatappa* distilled water) from *Terminaliacatappa* leaf, which acts synergistically and recommends a dual modulatory effect on inherent erythropoiesis.

The main haematological parameters are useful markers for verifying the adverse effect of herbal extracts or drugs on blood constituents. They are good indicators of the physiological and biochemical state of animals [17], and researchers have shown that ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters [18].

2. MATERIALS AND METHODS

2.1 Collection and preparation of plant material

Fresh leaves of T. catappawere collected in the environment of UNICROSS, Okuku, Cross River State, Nigeria (specify period and date of harvest). Identification and authentication were carried out at the botany department of the University of Calabar, with a reference number of 206 for future reference to the department's herbarium. Leaves were then air-dried (specify sun or shade) at room temperature for 21 days to a constant weight.

2.2 Extraction of T. catappa

Dried leaves were ground to powder using a blender (make and model?) and sieved (make and size?). Next, 400 g of pulverized plant material(*T. catappa*) were dissolved in 1,200 mL of 70% petroleum ether for 72 hours. This was followed by vacuum filtration, and the extracts were concentrated using a water bath evaporator (make and model?) at 40°C to obtain a solvent-free extract, and stored in a refrigerator at 4°C.

2.3 Animal management

Twenty-five (25) male Wistar rats were obtained from the breeding unit of the Department of Medical Biochemistry, Cross River University of Technology. The animals were acclimatized for a period of 7 days, in a well-ventilated room at room temperature and relative humidity of 29°C and 70% respectively, with a 12-hour natural light/dark cycle. They were fed and watered ad libitum. Good hygiene was maintained by daily cleaning and removal of faeces and splashes from their cages.

2.4 Induction of hemolytic anemia

Hemolytic anemia was induced by intraperitoneal (I.P.) injection of phenylhydrazine (PHZ) at 10 mg/kg for 7 days. Anemia was considered induced by comparing the PCV (Define abbreviation) of PHZ-induced animals with that of normal control (non-induced) animals after 24 h of the last induction.

2.5 Experimental design

Experimental rats were randomly divided into five (5) groups, with five animals per group, and treated for a period of fourteen (14) days.

Group A: normal control (non-anemic control)

Group B: anemic rats (phenyhdrazine-induced) without treatment (anemic control)

Group C: Feroton-treated anemic rats (standard control)

Group D: anemic rats treated with 100mg/kg body weight of aqueous extract of T. catappa leaves (ALETC1)

Group E: Anaemic rats treated with 200mg/kg body weight aqueous extract of T. catappa leaves (ALETC2).

Treatment was administered orally via an oropharyngeal cannula once daily for 14 days.

2.6 Sample collection

After sacrifice, blood samples were collected by cardiac puncture into labelled EDTA vials and flat sterile tubes and stored at room temperature until processing, which took place within 30 min of collection.

2.7 Determination of hematological parameters

Total red blood cell (RBC) count, white blood cell (WBC) count, differentials, platelets, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were analyzed using a hematology analyzer from Sysmex, Kobe, Japan.

2.8 Statistical analysis

Statistical Package for Scientific Solutions (SPSS) version 20.0 was used for analysis. The data obtained were analyzed using a one-way analysis of variance (ANOVA) followed by a post hoc test at $P<0.05$.

3. RESULTS

The results obtained indicate that the effects of T. catappa leaf aqueous extract on the haematological profile of phenylhydrazine-induced anaemic rats after administration produced a significant (P<0.05) increase in RBC, Hb and PCV compared with normal, standard and anaemic control (fig.1-3).

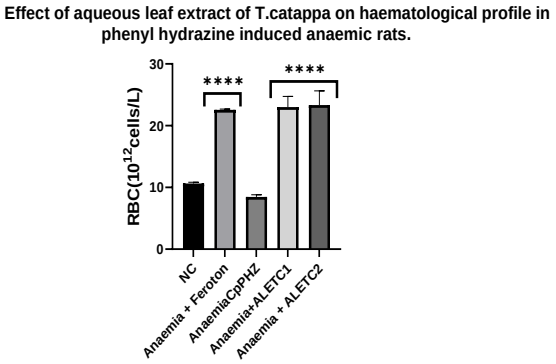

Fig1: Effect of aqueous extract of Terminaliacatappa leaf on red blood cells following phenyl hydrazine-induced anemia in Wistar rats.

Results were expressed as mean ± SD (n=5) *** significant at P<0. 05 vs. control NC: Normal control, Standard: Anemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ ; Anemic rats control, induced with phenylhydrazine, Anemia + AETC1: Anemic rats + aqueous extract of Terminaliacatappa (100mg/Kgbwt) , Anemic rats + AETC2: Anemic rats + aqueous extract of Terminaliacatappa (200mg/Kgbwt).

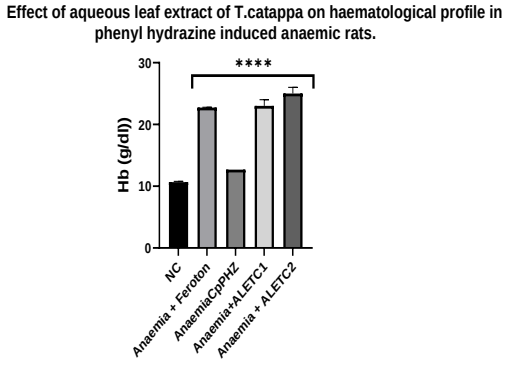

Fig 2: Effect of aqueous extract of Terminaliacatappa leaf on hemoglobin levels following phenylhydrazine-induced anemia in Wistar rats.

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with control. NC: Normal control, Standard: Anemic rats + Feroton (10mg/Kgbwt), AnaCpPHZ; Anemic control rats, induced with phenylhydrazine, Anemia + AETC1: Anemic rats + aqueous extract of Terminaliacatappa (100mg/Kgbwt), Anemic rats + AETC2: Anemic rats + aqueous extract of Terminaliacatappa (200mg/Kgbwt).

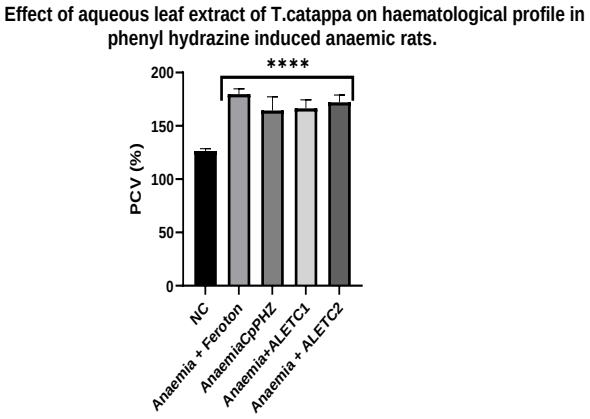

Fig 3: Effect of aqueous extract of *Terminaliacatappa* leaf on PCV following phenylhydrazine-induced anemia in Wistar rats.

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 vs. control NC: Normal control, Standard: Anemic Rats + Feroton (10mg/Kgbwt), AnaCpPHZ; Anemic Rats control, induced with phenylhydrazine, Anemia + AETC1: Anemic rats + aqueous extract of Terminaliacatappa (100mg/Kgbwt), Anemic rats + AETC2: Anemic rats + aqueous extract of Terminaliacatappa (200mg/Kgbwt).

In addition, aqueous extract of T. catappa leaves significantly (P<0.05) increased WBCs, lymphocytes and neutrophils but did not cause a significant difference in basophils compared with normal, standard and anemic control (fig. 4-7).

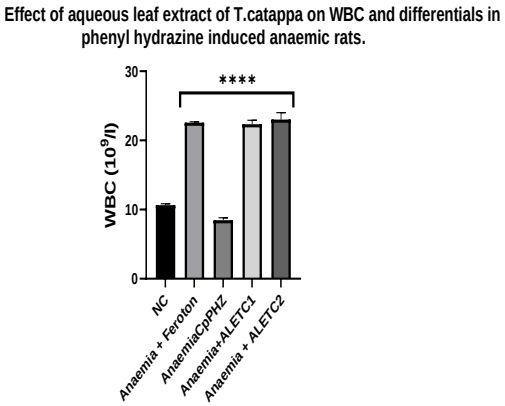

Fig 4: Effect of Terminaliacatappaleaf aqueous extract on white blood cell count following phenylhydrazine-induced anemia in Wistar rats.

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with control. NC: Normal control, Standard:Anemic Rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anemic Rats control, induced with phenylhydrazine, Anemia + AETC1: Anemic Rats + aqueous extract of Terminaliacatappa (100mg/Kgbwt) , Anemic Rats + AETC2: Anemic Rats + aqueous extract of Terminaliacatappa (200mg/Kgbwt).

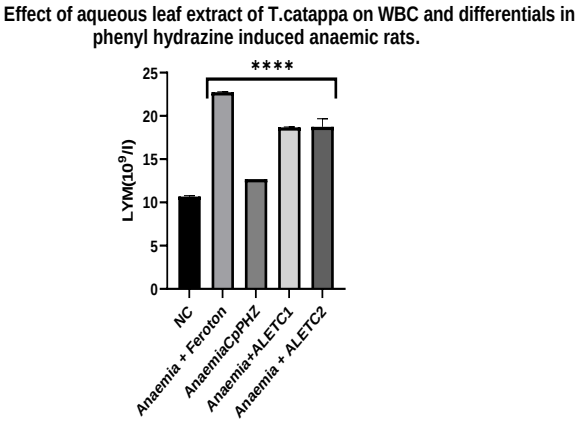

Fig 5: Effect of aqueous extract of Terminaliacatappa leaf on lymphocytes following phenyl hydrazine-induced anemia in Wistar rats.

Results were expressed as mean ± SD (n=5) *** significant at P<0. 05 vs. control NC: Normal control, Standard: Anemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ ; Anemic rats control, induced with phenylhydrazine, Anemia + AETC1: Anemic rats + aqueous extract of

Terminaliacatappa (100mg/Kgbwt) , Anemic rats + AETC2: Anemic rats + aqueous extract of Terminaliacatappa (200mg/Kgbwt).

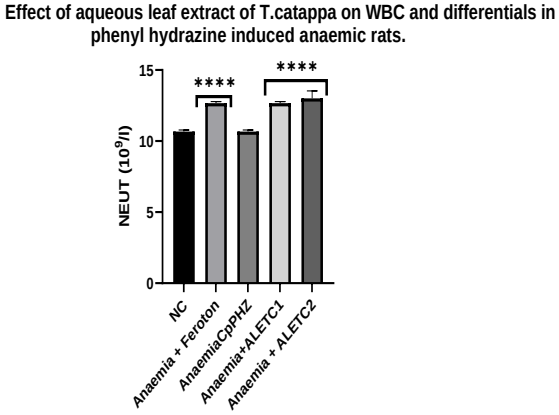

Fig 6: Effect of aqueous extract of Terminaliacatappa leaf on neutrophils following phenyl hydrazine-induced anemia in Wistar rats.

Les résultats ont été exprimés en moyenne ± SD (n=5) *** significatif à P<0.05 par rapport au contrôle NC : Contrôle normal, Standard: Rats anémiques+ Feroton (10mg/Kgbwt), AnaCpPHZ ; Rats anémiques contrôle, induit avec la phénylhydrazine, Anémie + AETC1 : Rats anémiques + extrait aqueux de Terminaliacatappa (100mg/Kgbwt) , Rats anémiques + AETC2 : Rats anémiques + extrait aqueux de Terminaliacatappa (200mg/Kgbwt).

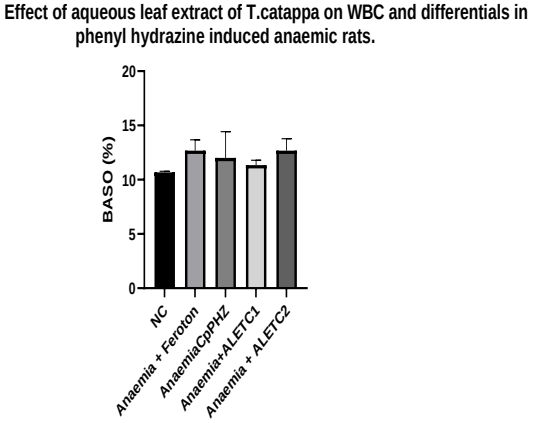

Fig. 7: Effect of aqueous extract of Terminaliacatappa leaf on white blood cell count following phenyl hydrazine-induced anemia in Wistar rats.

Results were expressed as mean ± SD (n=5) *** significant at P<0. 05 vs. control NC: Normal control, Standard: Anemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ ; Anemic rats control,

induced with phenylhydrazine, Anemia + AETC1: Anemic rats + aqueous extract of Terminaliacatappa (100mg/Kgbwt) , Anemic rats + AETC2: Anemic rats + aqueous extract of Terminaliacatappa (200mg/Kgbwt).

Effects of T.catappa aqueous extract on red blood cells and differentials following phenylhydrazine-induced anemia.

However, the extracts produced no significant difference on MCV, MCH and MCHC compared to normal, standard and anemic control (fig. 8-10).

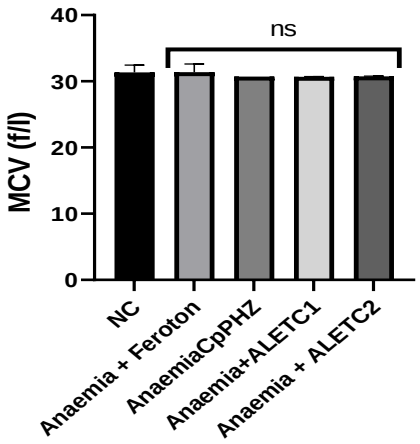

Fig 8: Effect of aqueous extract of Terminaliacatappa leaf on MCV following phenylhydrazine-induced anemia in Wistar rats.

Results were expressed as mean ± SD (n=5) *** significant at P<0. 05 vs. control NC: Normal control, Standard: Anemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ ; Anemic rats control, induced with phenylhydrazine, Anemia + AETC1: Anemic rats + aqueous extract of Terminaliacatappa (100mg/Kgbwt) , Anemic rats + AETC2: Anemic rats + aqueous extract of Terminaliacatappa (200mg/Kgbwt).

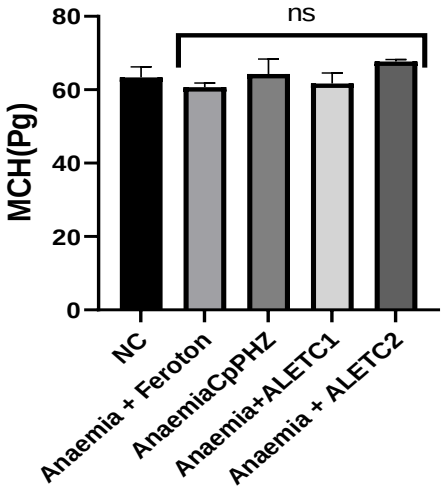

Fig 9: Effect of aqueous extract of Terminaliacatappa leaf on MCH after phenyl hydrazine-induced anemia in Wistar rats.

Results were expressed as mean ± SD (n=5) *** significant at P<0. 05 vs. control NC: Normal control, Standard: Anemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ ; Anemic rats control, induced with phenylhydrazine, Anemia + AETC1: Anemic rats + aqueous extract of Terminaliacatappa (100mg/Kgbwt) , Anemic rats + AETC2: Anemic rats + aqueous extract of Terminaliacatappa (200mg/Kgbwt).

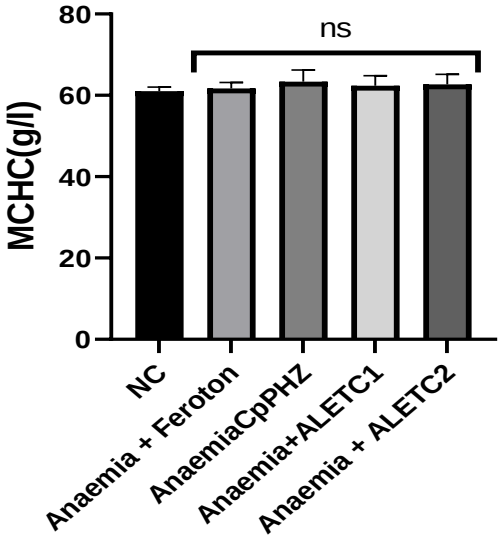

Fig 10: Effect of aqueous extract of Terminaliacatappa leaf on MCHC following phenylhydrazine-induced anemia in Wistar rats.

Results were expressed as mean ± SD (n=5) *** significant at P<0. 05 vs. control NC: Normal control, Standard: Anemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ ; Anemic rats control, induced with phenylhydrazine, Anemia + AETC1: Anemic rats + aqueous extract of Terminaliacatappa (100mg/Kgbwt) , Anemic rats + AETC2: Anemic rats + aqueous extract of Terminaliacatappa (200mg/Kgbwt).

4.Discussion

Pheylhydrazine (PHZ)-induced anemia is a model for studying hematological effects. Phenylhydrazinehas been reported to cause oxidative damage to red blood cells by increasing the formation of reactive oxygen species [8]. As expected, the significant reduction in red blood cell count, hemoglobin concentration and hematocrit scores following PHZ administration to our laboratory animals confirms the previously described and reported toxic effects of PHZ on red blood cells [19].

The results of this study showed that there was a significant increase in certain haematological parameters such as WBC, RBC, Hb and PCV treated with T. catappa. The main functions of white blood cells and their differentials are to fight infections, to defend the body by phagocytosis against invasion by foreign organisms, and to transport and distribute antibodies by immune response. Thus, animals with low white blood cell counts were exposed to a high risk of infection, while those with high white blood cell counts were able to produce antibodies in the process of phagocytosis and showed a high degree of disease resistance and better adaptability to the local environment but also to conditions of disease prevalence [17].

Cell volume (PCV), also called hematocrit (Ht or Hct) or erythrocyte volume fraction (EVF), is (narration time) the percentage (%) of red blood cells in the blood; anemia is (narration time) associated with low red blood cell production. The volume of packed cells is (narration time) also involved in the transport of oxygen and absorbed nutrients. The increase in PCV observed in this study suggests that plant extract, at different concentrations, could positively interfere with the osmoregulatory and hematopoietic systems of the blood, which may improve the management of anemia.

Increases in RBCs and hemoglobin were also observed, indicating erythrocyte synthesis. Therefore, the observed increase in RBC count and Hb could mean that the extract enhances

hematopoiesis and/or erythropoiesis. This is in line with the studies proposed by Dasofunjo K,Okwari O O, Ujong U P, Ati B U, Igwe C O [17] who noted the improvement in RBC count and hemoglobin when treated with medicinal plants. No significant difference was noted on MCV, MCH and MCHC of the treatment groups, when (narrative time) compared to normal, standard and anemic control. It appears that other changes in red blood cell parameters, such as red blood cell count, hemoglobin concentration or hematocrit level, may still occur even if MCV remained unchanged.

5. CONCLUSION

(To be completed) First of all, we need to say whether the objective set has been achieved, and whether the methodology adopted has enabled us to demonstrate the activity or otherwise of T catappa extract on the various haematological parameters. Then, the authors should summarize the results obtained from this work and, finally, suggest a course of action.

This work suggests that the extract, at different concentrations, can synergistically interfere with the osmoregulatory and hematopoietic systems of the blood and could be a panacea in the management of anemia.

ETHICAL APPROVAL

The use of animals for the study was approved by the Animal Ethics Committee of the Faculty of Basic Medical Sciences, Cross River State University, Nigeria. All animal experiments were conducted in accordance with the International Standard Laboratory Animal Use and Care of Laboratory Animals (1996) adopted and promulgated by the National Institute of Health (NIH publication No. 85(23), revised (1996), based on the Helsinki Convention and the guidelines and rules of the Faculty of Basic Medical Sciences, Cross River State University, Nigeria, regarding animal experimentation.

RÉFÉRENCES

Il faut que les auteurs mettent sous crochet les références afin d'être conforme par rapport au corps du texte. Exemple :

[1]Schreir S L. Approach to the Adult Patient with AnaemiaMentzer WC, Ed. Waltham, MA : Up To Date Inc. Science Association of Nigeria.Science, 2018;7(6) : 622-624.

[2]Balarajan Y, Ramakrishnan U, Özaltin E. Anaemia in low-income and middle-income countries. 2011 ; Lancet. 378 : 2123-2135.

[3] Black R E, Victora C G , Walker S P. Maternal and child undernutrition and overweight in low-income and middle-income countries. 2013 ; Lancet 382 : 427-451.

Introduction: Anaemia is an extremely common disease affecting up to one-third of the global population. In many cases, it is mild and asymptomatic and requires no management. The prevalence increases with age and is more common in women of reproductive age, pregnant women, and the elderly. The prevalence is more than 20% of individuals who are older than the age of 85. The incidence of anaemia is 50%-60% in the nursing home population. In the elderly, approximately one-third of patients have a nutritional deficiency as the cause of anaemia, such as iron, folate, and vitamin B_{12} deficiency.

Aim:This study investigated the effect of aqueous extract of *Terminaliacattapa on*haematological indices ofphenylhydrazine induced anaemicWistar rats.

Methodology: Twenty five (25) male Wistar rats were randomly divided into five (5) groups (A-E). Haemolyticanaemia was induced by intraperitoneal injection of phenylhydrazine (PHZ) at 10mg/kg body weight for 7 days.Anaemia was considered to be induced by comparing the PCV of the PHZ-induced animals with that of the normal control (non-induced) animals 24hours after the 7^{th} day of induction. Treatment was carried out orally using aqueous extracts of *Terminaliacattapa*lea**f** at 100mg/kg body weight and 200mg/kg body weight, once daily for 14 days.

Results: The results revealed a significant ($p< 0.05$) increase in the RBC,WBC,Hb and PCV but no significant ($p<0.05$) difference in MCH,MCHC, platelets, MCV, when compared with the normal standard and control.

Conclusion: The results of this study reveals that aqueous extract of *Terminaliacatappa* at varying concentrations may synergistically interfere with the osmoregulatory and haematopoietic system of the blood and might be a panacea in the management of anaemia.

Keywords:Anaemia, haematopoietic, intraperitoneal injection, osmoregulatory, phenylhydrazine

1. INTRODUCTION

Anaemia also known as reduced absolute number of circulating RBCs[1] or their oxygen-carrying capacity is insufficient to meet physiologic needs.Though, commonly diagnosed by a low heamoglobin concentration or low haematocrit[1].Anaemia can also be diagnosed using RBC count, mean corpuscular volume, blood reticulocyte count, blood film analysis, or Hb electrophoresis [2]. Anaemia is associated with increased morbidity and mortality in women and children[3] poor birth outcomes, [4,5]decreased work productivity in adults,[6] and impaired cognitive and behavioural development in children[7].Preschool children (PSC) and women of reproductive age (WRA) are particularly affected. Phenyl hydrazine (PHZ), a non-immunogenic drug causes alterations in the red cell membrane, leading to oxidative denaturation of haemoglobinwhich results in the reduction in the life span of the erythrocytes [8] which are eradicated by the spleen and liver, resulting in compensated haemolyticanaemia.

The effect of medicinal plants in the management of diseases has taken central stage of investigations and research by various scientists has shown that these plants have been found to have little or no side effects and relatively lower cost [9]. Tropical almond (*Terminaliacatappa*L.) is a large tree and is widely distributed throughout the tropics especially in coastal areas. *Terminaliacatappa*belongs to the family *Combretaceae* (*Combretum* family).*Terminaliacatappa*has been found to be rich in an array of phytochemicals and various studies have shown the anti-inflammatory, hepatoprotective, anti-diabetic, wound healing, anti-

cancer, hypocholestelemic, anti-oxidant and radical scavenging effects of the plant[10,11,12,13,14,15].A studyin 2014[16] isolated a novel foetalhaemoglobin- inducing compound (*Terminaliacatappa*distilled water active fraction) from the leaf of *Terminaliacatappa*, which work synergistically, and recommended a dual modulatory effect on inherent erythropoiesis.

Haematological parameters are useful markers to ascertain the adverse effect of plant extracts or drugs on blood constituents are good indicators of the physiological and biochemical status of animals[17] and researchers have shown that ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters[18].

2.MATERIALS AND METHODS

2.1 Collection and preparation of plant materials

Fresh leaves of *T. catappa* were collected from the UNICROSS environment, Okuku, Cross River State, Nigeria. Identification and authenticationwas carried out at the Department of Botany, University of Calabar, with voucher number of 206 for future reference at the department's herbarium. The leaves were then air dried at room temperature for a period of 21days until constant weight was obtained.

2.2 Extraction*T. catappa*

The dried leaves were pulverized to powdered form by a machine blender and sieved. Thereafter, 400g of the pulverized plant material (*T. catappa*) was dissolved in 1200ml of 70% petroleum ether for 72 hours. This was followed with vacuum filtration and extracts were concentrated using an evaporator water bath at 40°C to obtain a solvent free extract, and stored in a refrigerator at 4°C.

2.3 Animal management

Twenty five (25) male Wistar rats were obtained from the animal holding unit of the Department of Medical Biochemistry, Cross River University of Technology. The animals was allowed to acclimatize for a period of 7 days, in a well-ventilated room at room temperature and relative humidity of 29°C and 70% respectively with 12 hours natural light-dark cycle. They were

allowed food and water *ad libitum*. Good hygiene was maintained by daily cleaning and removal of faeces and spills from their cages.

2.4 Induction of haemolyticanaemia

Haemolyticanaemia was induced by intraperitoneal (I.P.) injection of phenyl hydrazine (PHZ) at 10 mg/kg for 7 days.Anaemia was considered to be induced by comparing the PCV of the PHZ-induced animals with that of the normal control (non-induced) animals after 24hours of the last induction.

2.5 Experimental design

The experimental rats were randomly divided into five (5) groups, with five animals per group and treated for a period of fourteen (14) days.

Group A: Normal control (non-anaemic control)

Group B: Anaemic rats (induced with phenyhdrazine) without treatment (anaemic control)

Group C: Anaemic rats treated with feroton (Standard control)

Group D: Anaemic rats treated with 100mg/kg body weight aqueous leaf extract *T. catappa*extract ($ALETC_1$)

Group E: Anaemic rats treated with 200mg/kg body weight aqueous leaf extract *T. catappa*extract ($ALETC_2$)

Treatment was made orally using oropharyngeal cannula once daily for 14days.

2.6 Sample collection

After sacrifice, blood sampleswere collected through cardiac puncture into labeledEDTA bottles and plane sterile tubes and kept at room temperature until processing, which occurred within 30 minutes of collection.

2.7 Determination of haematological parameters

The total Red blood cell count (RBC), White Blood cell count (WBC), and the differentials, platelets, red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular

haemoglobin concentration (MCHC) were analyzed using Sysmex, Kobe, Japan haematology analyzer.

2.8 Statistical analysis

The Statistical Package for Scientific Solutions (SPSS) Software version 20.0 was used for the analysis. The data obtained was analyzed using One Way Analysis of Variance (ANOVA) followed by post hoc test at $P<0.05$

3. RESULTS

The results below indicate the effects of *T. catappa* leaf on haematological profile in phenyl hydrazine induced anaemia rat following the administration of the extracts. The extracts was found to produce the significant increase ($P<0.05$) in RBC, Hb and PCV when compared with the normal , standard and anaemic control (fig.1-3).

Moreso, the aqueous leaf extract of *T. catappa* significantly increased ($P< 0.05$) the WBC, lymphocytes and neutrophils but elicits no significant difference on the basophil when compared with the normal, standard and anaemic control(fig 4- 7)

However, the extracts produced no significant difference on MCV, MCH and MCHC when compared with the normal, standard and anaemic control (fig 8-10).

Effect of aqueous leaf extract of T.catappa on haematological profile in phenyl hydrazine induced anaemic rats.

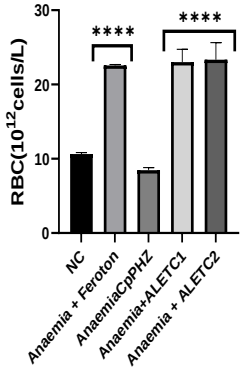

Fig1: Effect of aqueous extract on *Terminaliacatappa* leaf on RBC following phenyl hydrazine induced anaemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats + aqueous extract on *Terminaliacatappa* (200mg/Kgbwt).

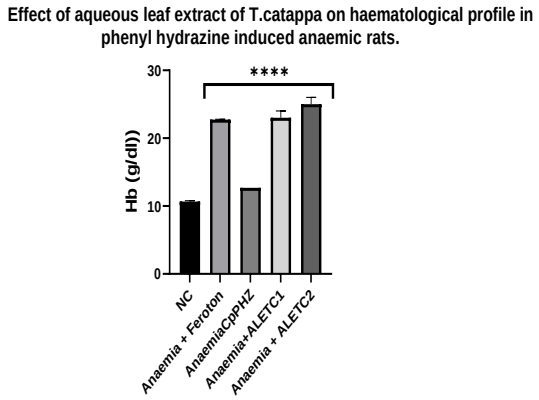

Fig 2: Effect of aqueous extract on *Terminaliacatappa* leaf on Hb following phenyl hydrazine induced anaemia in Wistar rat.

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control. NC: Normal Control, Standard: Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats

+ aqueous extract on *Terminaliacatappa* (200mg/Kgbwt)

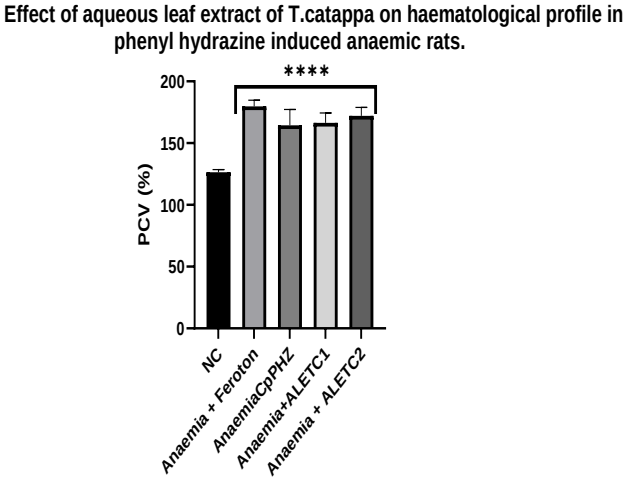

Fig 3: Effect of aqueous extract on *Terminaliacatappa* leaf on PCV following phenyl hydrazine induced anaemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats + aqueous extract on *Terminaliacatappa* (200mg/Kgbwt)

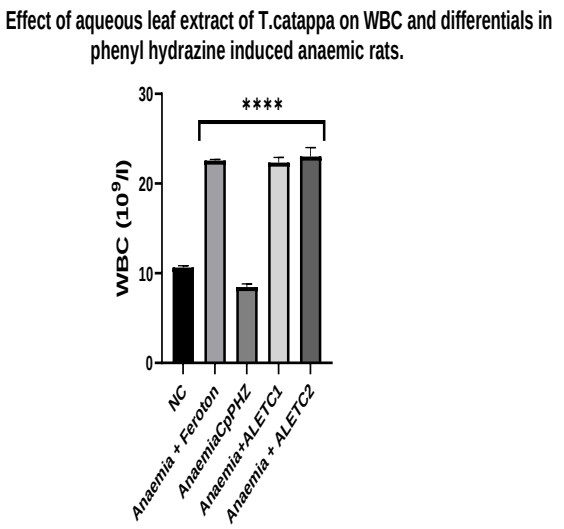

Fig 4: Effect of aqueous extract on *Terminaliacatappa*leaf on WBC following phenyl hydrazine induced anaemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats + aqueous extract on *Terminaliacatappa* (200mg/Kgbwt

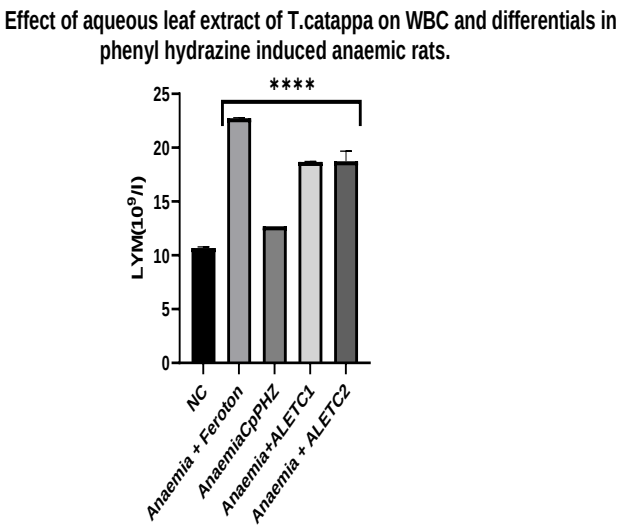

Fig 5: Effect of aqueous extract on *Terminaliacatappa* leaf on Lymphocytes following phenyl hydrazine induced anaemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats + aqueous extract on *Terminaliacatappa* (200mg/Kgbwt)

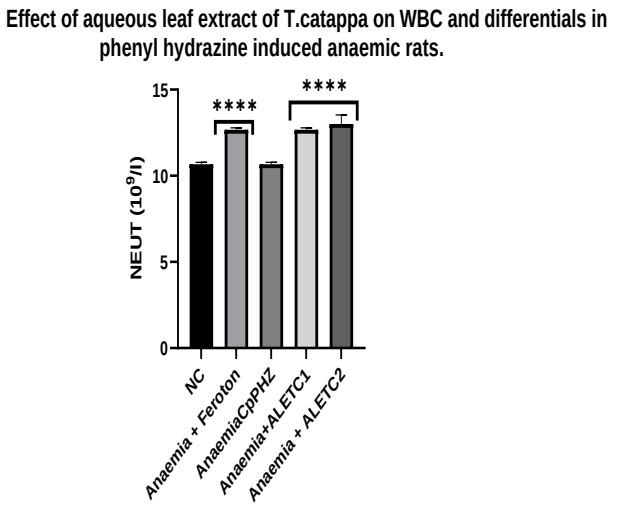

Fig 6: Effect of aqueous extract on *Terminaliacatappa*leaf on neutrophils following phenyl hydrazine induced anaemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats + aqueous extract on *Terminaliacatappa* (200mg/Kgbwt)

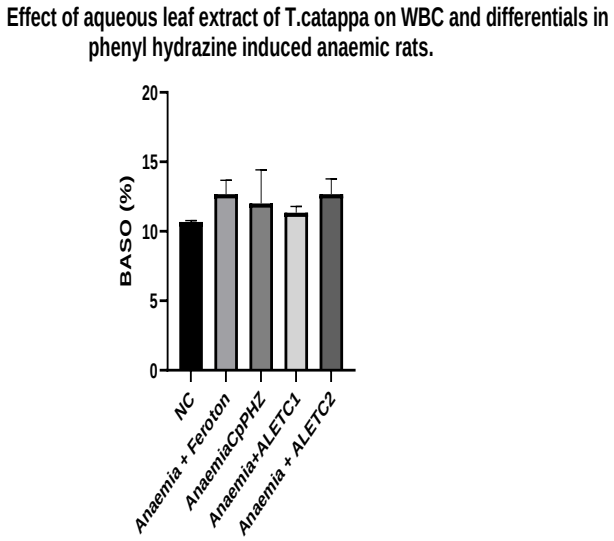

Fig 7: Effect of aqueous extract on *Terminaliacatappa* leaf on WBC following phenyl hydrazine induced anaemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats + aqueous extract on *Terminaliacatappa* (200mg/Kgbwt)

Effects of aqueous extract of *T.catappa* on RBC and differentials following phenyl hydrazine induced anaemia

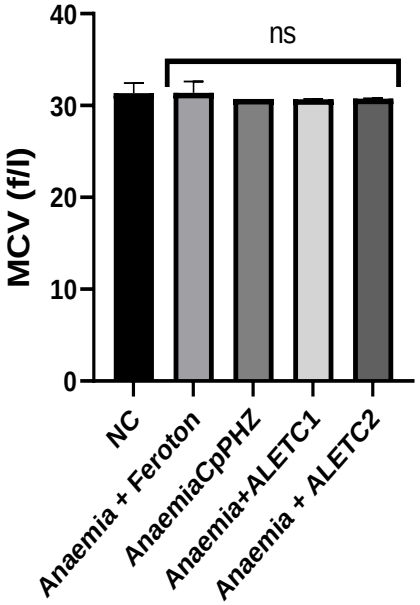

Fig 8: Effect of aqueous extract on *Terminaliacatappa*leaf on MCV following phenyl hydrazine induced anaemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats + aqueous extract on *Terminaliacatappa* (200mg/Kgbwt)

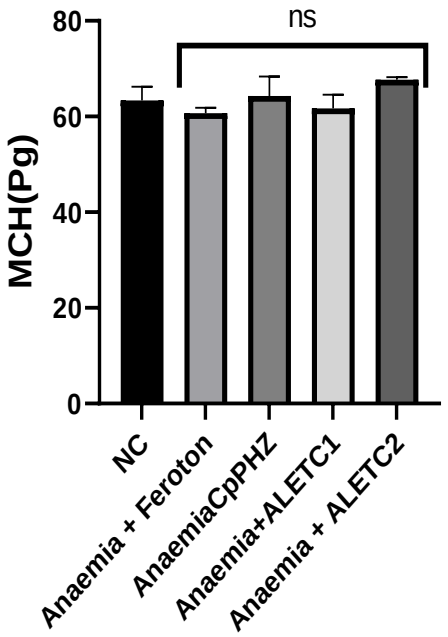

Fig 9: Effect of aqueous extract on *Terminaliacatappa* leaf on MCH following phenyl hydrazine induced anaemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats + aqueous extract on *Terminaliacatappa* (200mg/Kgbwt)

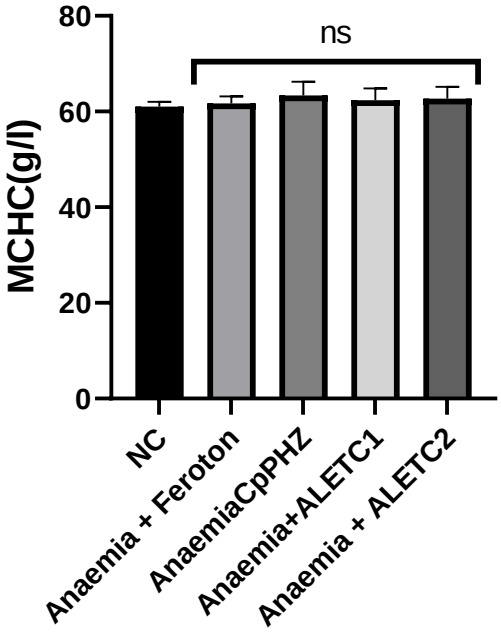

Fig 10: Effect of aqueous extract on *Terminaliacatappa* leaf on MCHC following phenyl hydrazine induced anaemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard:Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + AETC1: Anaemic rats + aqueous extract on *Terminaliacatappa* (100mg/Kgbwt) , Anaemic rats + AETC2: Anaemic rats + aqueous extract on *Terminaliacatappa* (200mg/Kgbwt)

4.0Discussion

Pheylhydrazine (PHZ) induced anaemia is a model, helping out for the investigation of haematinic impacts. It has been reported that phenyl hydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species [8].Expectedly, the significant reduction in the values of the red blood cell count, haemoglobin concentration and

haematocritscores, following the administration of PHZ to our experimental animals, fairly confirms the previously described and reported toxic effects of PHZ on the red blood cell[19].

The result of this study shows that, there was a significant increase in the some haematological parameters such as WBC, RBC, Hb and PCV treated with *T. catappa*. The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to transport and distribute antibodies by immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high WBCs counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions[17].

Packed cell volume (PCV) which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF) is the percentage (%) of red blood cells in blood. It measures the percentage volume of red blood cells in the blood; anaemic condition is associated with low production of red blood cells. Packed cell volume is also involved in the transportation of oxygen and absorbed nutrients. Increased PCV concentration shows a better transportation and thus results in an increased primary and secondary polycythemia [20].The observed marked increase in PCV in this study suggests that the plant extract at varying concentrations may positively interfere with osmoregulatory and haematopoietic system of the blood that can enhance management of anaemia. Increases in red blood cells (RBCs) and Hb were also observed, indicatingerythrocyte synthesis. Therefore, the increase observed in RBC count and Hb may connote that the extract enhances haematopoeisis and or erythropoiesis. This is in line with studies by[17]who noted the improvement in RBC and Hb on treatment with medicinal plants. No significant difference was noticed on MCV, MCH and MCHC of treatment groups, when

compared with the normal, standard and anaemic control. This is in line with findings of other studies [21].It appears that other changes in red blood cell parameters, such as the red blood cell count, haemoglobin concentration, or haematocrit level, could still occur even if MCV remains unchanged.

5.0 CONCLUSION

In conclusion, this present work suggests that the extract at varying concentrations may synergistically interfere with osmoregulatory and haematopoietic system of the blood and might be a panacea in the management of anaemia.

ETHICAL APPROVAL

Approval for the use of the animals for the study was obtained from the Animal Ethics Committee of the Faculty of Basic Medical Sciences, University of Cross River State, Nigeria. All animal experiments were conducted in accordance with internationally accepted Laboratory Animal Use and Care of Laboratory Animals (1996) as adopted and promulgated by the National Institute of Health (NIH publication No. 85(23), revised (1996), based on Helsinki convention and guidelines and rules of Faculty of Basic Medical Sciences, University of Cross River State, Nigeria for animal experimentation.

REFERENCES

1. Schreir S L. Approach to the Adult Patient with AnaemiaMentzer WC, Ed. Waltham, MA: Up To Date Inc. Science Association of Nigeria.*Science.*,2018;7(6): 622-624.
2. Balarajan Y,Ramakrishnan U,Özaltin E. Anaemia in low-income and middle-income countries. 2011;*Lancet.* 378: 2123–2135.

3. Black R E, Victora C G , Walker S P. Maternal and child undernutrition and overweight in low-income and middle-income countries. 2013;*Lancet*382: 427–451.
4. Haider B A ,Olofin I, Wang M. Anaemia, prenatal iron use, and risk of adverse Pregnancy outcomes: systematic review and meta-analysis. 2013;*BMJ* 346: f3443.
5. Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency Anemia and weight at birth, length of gestation and perinatal mortality? *Journal Nutrient*., 2001, 131: 590–603.
6. Haas J D, Brownlie T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *Journal Nutrient*2001;131: 676–688.
7. Walker S P, Wachs TD,Meeks G J. Child development: risk factors for adverse outcomes in developing countries. *Lancet*., 2007;369: 145–157.
8. Rashmi SP, Amrita M. Anti-anemic activity of D*hatryadighrita* in phenyl hydrazine-treated Wistar rats*Pharmacophore*. 2019;10(5): 29-36.
9. Yakubu O E , Nwodo O FC, Udeh SMC, Abdulrahman M, Ogri M O. Modulation of some biochemical complications arising from alloxan-induced diabetic conditions in rats treated with *Senna occidentalis* leaf extract. *J App Biol Biotech*., 2016;4 (04): 051-056. DOI: 10.7324/JABB.2016.40405
10. Mandloi S, Srinivasa R, Mishra R, and R. Varma,. Antifungal activity of alchoholic leaf extracts of *Terminaliacatappa*and *Terminaliaarrjuna* on some pathogenic and allergenic fungi. 2013; Adv Life Sci Technol., 8(1): 25-27.
11. Fan, Y. M., L. Z. Xu, J. Gao, Y. Wang, X. H. Tang, X. N. Zhao, and Z. X. Zhang. Phytochemical and anti-inflammatory studies on *Terminaliacatappa*. *Fitoterapia*2004;*75*(3-4): 253-260.

12. Khan A A ,Kumar V, Singh B K , Singh R. Evaluation of wound healing property of *Terminaliacatappa* on excision wound models in Wistar rats. Drug Res (stuttg). 2014; *64:* 225-228.
13. Divya N ,Anand A. V. Phytochemical investigation and invitro antidiabetic activity of *Terminaliacatappa* leaves. *Int. J of Phyto Pharm*. 2014;4:132-134.
14. Kotti P P ,Anand A V, Phytochemical analysis and invitro antioxidant activity of *Terminaliacatappa. World J.Pharm. Sci.*,2014; 2: 1485-1498.
15. Morioka T, SuzulM ,Nabandith V, Inamira M, Aniya Y, Nakayama T. Modifying effects of *Terminaliacatappa*on azoxymethane induced colon carcinogenesis in male F344 rats.*Eur. J. cancer Prev.*, 2005;14: 101-105.
16. Aimola I A, Ihuwa H M, Nok A.J, Mamman A I. Induction of foetalhaemoglobin synthesis in erythroid progenitor stem cells mediated by water soluble components of *Terminaliacatappa. Cell BiochemFunct.*,2014;32; 361-367.
17. Dasofunjo K, Okwari O O, Ujong U P, Ati B U, IgweC O. Biochemical implication of administration of methanol extract of *Ocimumgratissimum* leaf on haematological Profile of Wistar rats. Global Journal of Pure and Applied Sciences., 2020;**26**:93-98.
18. Ezugwu HC, Jankada PA, Ipav SS, Dasofunjo K. Anti-anaemic and hepato–renal activities of ethanol leaf extract of *Alchorneacordifolia* in phenyl hydrazine induced-anaemicWistar rats. Global journal of pure and applied sciences 2019;26: 93-98.
19. Damodara K M. Ethanolic extract of Nardostachysjatamansi potentiates haematopoietic system in albino wistar rats. Nitte Universal *Journal Health Science*,2013;3(1): 25-29.

20. Isaac L J, Abah G ,Akpan B, Ekaette I U. Haematological properties of different breeds and sexes of rabbits. Proceedings of the 18th annual conference of animalscience association of Nigeria 2013;pp 24-27.
21. Kangbeto B R, Attakpa SE,Guinnin F Senou M,LagnikaL . Toxicological assessment of ethanolic extracts of *Annonasenegalensis* and *Trichiliaprieureana* in the treatment of type 2 diabetes in *Benin. Journal of Physiology and Pathophysiology*.2002;13(2): 27-35.