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

ABSTRACT

Introduction: ~~Anaemia~~Anemia 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~~anemia 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 ~~anaemic~~anemicWistar rats.

Methodology: Twenty five (25) male Wistar rats were randomly divided into five (5) groups (A-E). ~~Haemolytic~~Hemolytic~~anaemia~~anemia was induced by intraperitoneal injection of phenylhydrazine (PHZ) at 10mg/kg body weight for 7 days.~~Anaemia~~Anemia 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*leafat 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~~anemia.

Keywords:~~Anaemia~~Anemia, ~~haematopoietic~~hematopoietic, intraperitoneal injection, osmoregulatory, phenylhydrazine

1. INTRODUCTION

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~~Anaemia~~Anemia 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 ~~haemoglobin~~hemoglobin concentration or low ~~haematocrit~~hematocrit[1]~~.~~. ~~A~~anaemia can also be diagnosed using RBC count, mean corpuscular volume, blood reticulocyte count, blood film analysis, or Hbelectrophoresis [2]. ~~Anaemia~~Anemia is associated with increased morbidity and mortality in women and children [3]~~3~~poor birth outcomes, [4,5]decreased work productivity in adults,[6] and impaired cognitive and ~~behavioural~~behavioral development in children[7].Preschool children (PSC) and women of reproductive age (WRA) are particularly affected by anemia.

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 ~~foetal~~fetal~~haemoglobin~~hemoglobin- 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~~Hematological 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~~hematological 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 authentication was 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**of *T. catappa*leaves**

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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 **~~haemolytic~~hemolytic~~anaemia~~anemia**

~~Haemolytic~~Hemolytic~~anaemia~~anemia was induced by intraperitoneal (i.p~~I.P.~~) injection of phenyl hydrazine (PHZ) at 10 mg/kg for 7 days.~~Anaemia~~Anemia 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~~anemic control)

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

Group C:~~Anaemic~~Anemic rats treated with feroton (Standard control)- what is the dose of Feroton administered and by what route?

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

Group E:~~Anaemic~~Anemic 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 on day 14, blood samplesfrom all the animals were collected through cardiac puncture into labeledEDTA bottles and plainesterile 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~~hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular ~~haemoglobin~~hemoglobin (MCH) and mean corpuscular ~~haemoglobin~~hemoglobin concentration (MCHC) were analyzed using Sysmex, Kobe, Japan ~~haematology~~hematology 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~~anemia 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).

~~More so~~More so, 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~~anemic control(fig 4- 7).

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

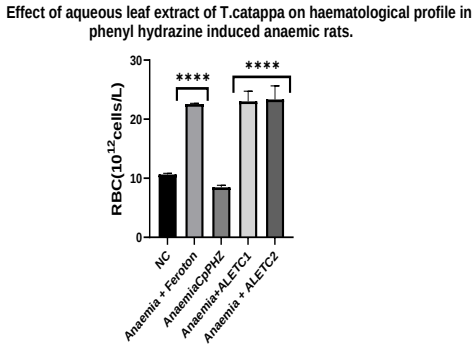

Fig1: Effect of aqueous extract on *Terminaliacatappa* leaf on RBC following phenyl hydrazine induced anaemia in Wistar rat

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

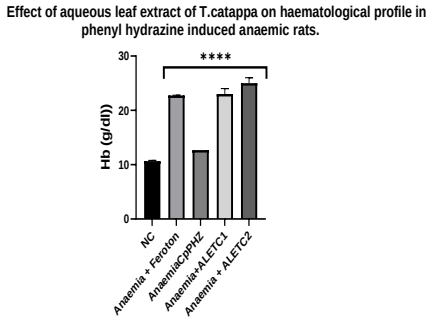

Fig 2: Effect of aqueous extract on *Terminaliacatappa* leaf on Hb following phenyl hydrazine induced ~~anaemia~~anemia in Wistar rat~~.~~s

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

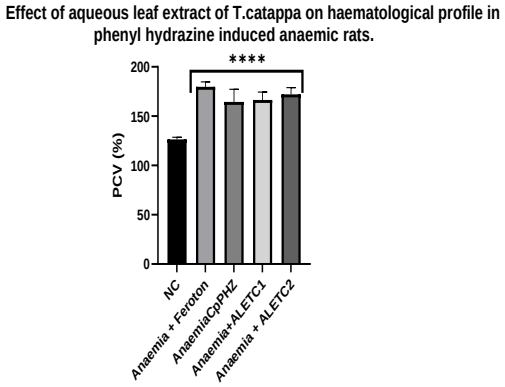

Fig 3: Effect of aqueous extract on *Terminaliacatappa* leaf on PCV following phenyl hydrazine induced ~~anaemia~~anemia in Wistar rat

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

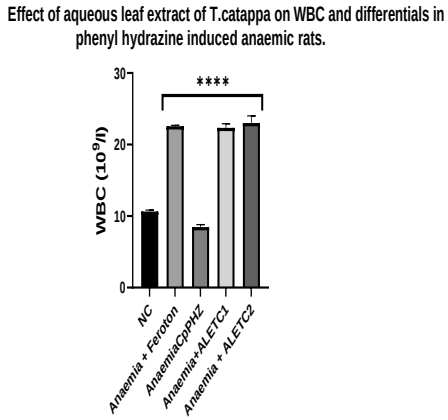

Fig 4: Effect of aqueous extract on *Terminaliacatappa*leaf on WBC following phenyl hydrazine induced ~~anaemia~~anemia in Wistar rats

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

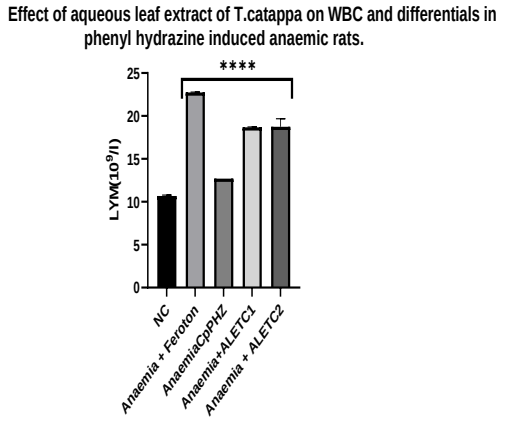

Fig 5: Effect of aqueous extract on *Terminaliacatappa* leaf on Lymphocytes following phenyl hydrazine induced ~~anaemia~~anemia in Wistar rats

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

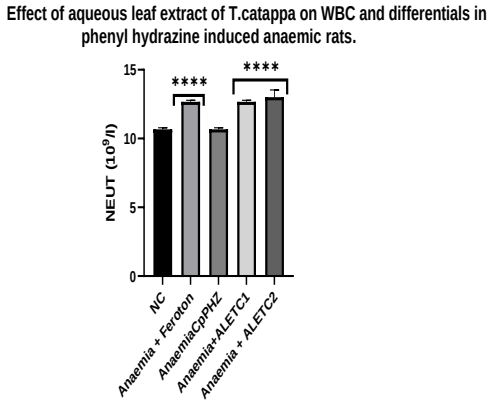

Fig 6: Effect of aqueous extract on *Terminaliacatappa*leaf on neutrophils following phenyl hydrazine induced anaemia in Wistar rat

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

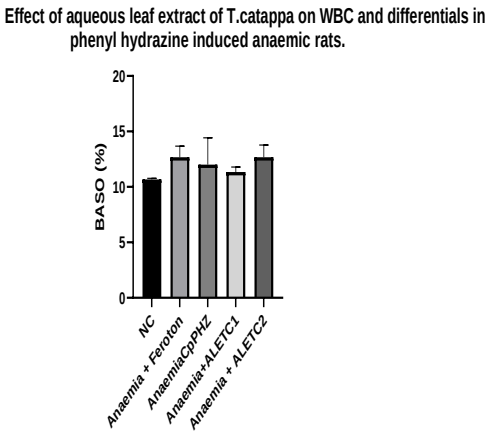

Fig 7: Effect of aqueous extract on *Terminaliacatappa* leaf on WBC following phenyl hydrazine induced ~~anaemia~~anemia in Wistar rats

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

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

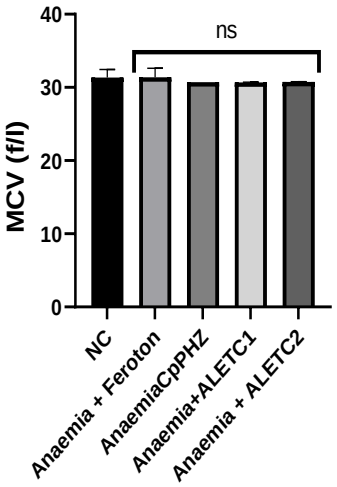

Fig 8: Effect of aqueous extract on *Terminaliacatappa*leaf on MCV following phenyl hydrazine induced ~~anaemia~~anemia in Wistar rats

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

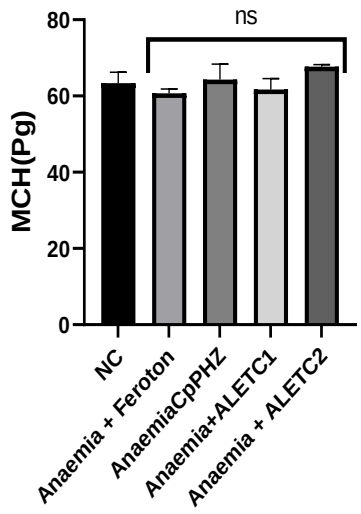

Fig 9: Effect of aqueous extract on *Terminaliacatappa* leaf on MCH following phenyl hydrazine induced ~~anaemia~~anemia in Wistar rats

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

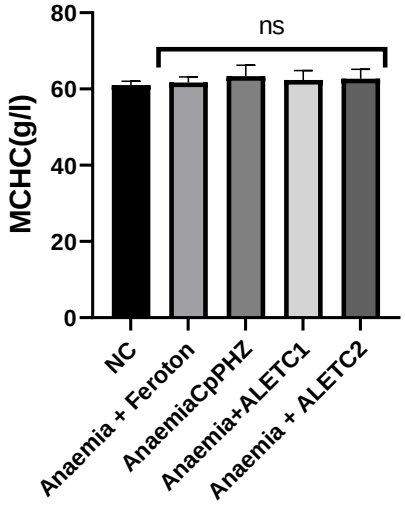

Fig 10: Effect of aqueous extract on *Terminaliacatappa* leaf on MCHC following phenyl hydrazine induced ~~anaemia~~anemia in Wistar rats

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~~anemia is a model, helping out for the investigation of ~~haematinic~~hematinic 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~~hemoglobinconcentration and ~~haematocrit~~hematocrit scores, 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~~hematocrit (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~~anemic 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~~anemia. 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~~hematopoiesis and or erythropoiesis. This is in line with studies ~~by~~[17]~~who noted~~ that reportsthe 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~~anemic 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~~hematocrit 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~~anemia.

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.

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