



Hematopoietic Effect of Aqueous Extract of *Terminalia catappa* Leaf in Phenyl Hydrazine Induced Hemolytic Anemia in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: One third of people worldwide are affected with anemia, a condition that is incredibly common. Many times, it is minor and asymptomatic and doesn't need to be managed. Prevalence rises with age and is more prevalent in elderly, pregnant, and females of reproductive age. In people above the age of 85, the prevalence is higher than 20%. In the population of nursing homes, anemia occurs 50% to 60% of the time. A dietary deficit, such as a lack of iron, folate, or vitamin B12, is the main cause of anemia in the elderly, accounting for about one-third of patients.

Aim: This study was designed to evaluate the effect of aqueous extract *Terminalia catappa* on hematological parameters in phenyl hydrazine induced anemic Wistar rats.

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Methodology: Five (5) groups (A-E) made up of twenty-five (25) male Wistar rats were assigned at random. Phenyl hydrazine (PHZ) was administered intraperitoneally for seven days at a dose of 10 mg/kg body weight to produce hemolytic anemia. Anemia was assumed to have been induced when the PCV of the PHZ-induced animals was compared to that of the healthy control (non-induced) animals 24 hours following the 7th day of induction. Treatment was administered orally over the course of 14 days using aqueous extracts of *Terminalia catappa* leaf at doses of 100 and 200 mg/kg, respectively.

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

Conclusion: The findings of this study suggest that *Terminalia catappa* aqueous extract, used in a variety of concentrations, may simultaneously interfere with the osmoregulatory and hemopoietic system of the blood and may be a panacea to anemia.

Keywords: Anemia; hematopoietic; intraperitoneal injection; osmoregulatory; phenyl hydrazine.

1. INTRODUCTION

Anemia is also referred to as a diminished absolute quantity of circulating RBCs [1] or an inadequate amount of oxygen-carrying capacity for physiologic demands. However, low hematocrit or hemoglobin concentration are frequently used to diagnose the condition [1]. "RBC count, mean corpuscular volume, blood reticulocyte count, blood film analysis, and hemoglobin electrophoresis are other methods for diagnosing anemia" [2]. Anemia is linked to higher rates of mortality and morbidity in women and children, 3 poorer outcomes during childbirth, [3,4,5] lower productivity at work in adults, [6] and reduced cognitive and behavioral growth in kids, according to research [7]. Women of reproductive age (WRA) and preschoolers (PSC) are severely impacted. On-immunogenic drug phenyl hydrazine (PHZ) alters the red cell membrane, resulting in oxidative denaturation of hemoglobin, which shortens the life expectancy of the erythrocytes [8], which are then eliminated by the liver and spleen, thereby causing compensated hemolytic anemia.

The use of plants with medicinal properties in the treatment of ailments has become a major focus of research, and studies by numerous scientists have revealed that these plants are considerably less expensive and have few to no adverse effects [9]. The tropical almond tree, or *Terminalia catappa* L., grows to a sizeable size and is found throughout the tropics, especially along the shore. The genus *Terminalia catappa* is a member of the *Combretaceae* (*Combretum* family). *Terminalia catappa* 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, hypocholesterolemic, antioxidant and radical scavenging effects of the plant [10,11,12, 13,14,15].

A study in 2014 [16] isolated "a novel fetal hemoglobin-inducing compound (*Terminalia catappa* distilled water active fraction) from the leaf of *Terminalia catappa*, which work synergistically, and recommended a dual modulatory effect on inherent erythropoiesis". The normal range of hematological parameters can be altered [17] by the consumption of medicinal compounds or drugs, according to research, which makes hematological parameters useful markers for identifying the negative effects of plant extracts or drugs on blood constituents [18]. This present work was designed to evaluate the effect of aqueous extract of *Terminalia catappa* on hematological parameters in phenyl hydrazine induced anemic Wistar rats

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

T. catappa fresh leaves were gathered from the UNICROSS surroundings in Okuku, Cross River State, Nigeria. At the University of Calabar's Department of Botany, identification and authentication were completed, and the voucher number 206 was assigned for the department's herbarium's future use. The leaves were then allowed to air dry at the ambient temperature for a further 21 days to achieve a constant weight.

2.2 Extraction of *T. catappa*

The leaves that had dried were sieved after being ground into a powder using a mechanical

blender. After that, 400g of the ground-up plant material (*T. catappa*) was allowed to dissolve for 72 hours in 1200 ml of 70% petroleum ether. Following vacuum filtration, extracts were concentrated in an evaporator water bath at 40°C to produce a solvent-free d extract, which was then kept chilled at 4°C in a refrigerator.

2.3 Animal Management

The animal holding facility of the Department of Medical Biochemistry at University of Cross River State provided twenty-five (25) male Wistar rats. The animals were given seven days to acclimate in a well-ventilated chamber with a relative humidity and temperature of 30% and 29°C, respectively, and a 12-hour natural light-dark cycle. They had unlimited access to water and food. By regularly cleaning their cages and getting rid of waste and spills, good hygiene was kept up.

2.4 Induction of Haemolyticanaemia

Phenyl hydrazine (PHZ) was injected intraperitoneally (I.P.) at 10 mg/kg for 7 days to cause hemolytic anemia. By comparing the PCV of the PHZ-induced animals with that of the healthy control (non-induced) animals after 24 hours from the last induction, anemia was assumed to have been induced.

2.5 Experimental Design

Five animals were placed in each of the five groups of experimental rats, and each group received care for fourteen (14) days.

Group A: Non-anemic control (Normal control)

Group B: Rats with phenyl hydrazine-induced anemia that weren't given any medication (the anemic control)

Group C: Feroton was administered to anemic rats (standard control).

Group D: Rats with anemia were given 100 mg/kg of *T. catappa* extract in aqueous form (ALETC 1).

Group E: Aqueous leaf extract of *T. catappa* 200 mg/kg body weight was administered to anemic rats (ALETC 2).

For 14 days, a once daily oral cannula treatment was administered.

2.6 Sample Collection

Following sacrifice, samples of blood were drawn from the heart using sterile, labeled EDTA vials and tubes, and they were left at ambient temperature pending processing, which took place 30 minutes after collection.

2.7 Determination of Hematological Parameters

The Sysmex, Kobe, Japan hematology analyzer was used to analyze the total red blood cell count (RBC), White blood cell count (WBC), differentials, platelets, hemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

2.8 Statistical Analysis

The study was performed using the Statistical Package for Scientific Solutions (SPSS) Software version 20.0. One Way Analysis of Variance (ANOVA) was used to examine the data, and then a post hoc test was performed using a value of $P < 0.05$.

3. RESULTS

The results below indicate the effect of *T. catappa* leaf on hematological profile in phenyl hydrazine induced anemia rat following the administration of the extracts. When compared to the normal, standard, and anemic control, the extracts were found to significantly ($P < 0.05$) increase RBC, Hb, and PCV (Figs. 1-3).

Furthermore, as compared to the normal, standard, and anemic control, the water-soluble extract of *T. catappa* considerably raised ($P < 0.05$) the WBC, lymphocytes, and neutrophils but elicited no significant impact on the basophil (Figs. 4- 7).

When compared to the normal, standard, and anemic control, however, the extracts did not significantly affect MCV, MCH, or MCHC (Figs. 8–10).

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

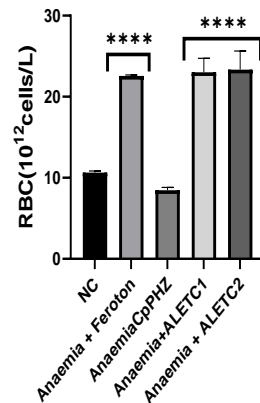


Fig. 1. Effect of aqueous extract on *Terminalia catappa* leaf on RBC following phenyl hydrazine induced anemia in Wistar rat

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

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

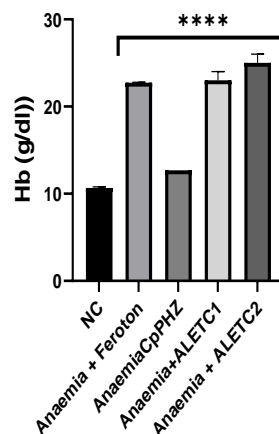


Fig. 2. Effect of aqueous extract on *Terminalia catappa* leaf on Hb following phenyl hydrazine induced anaemia in Wistar rat

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

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

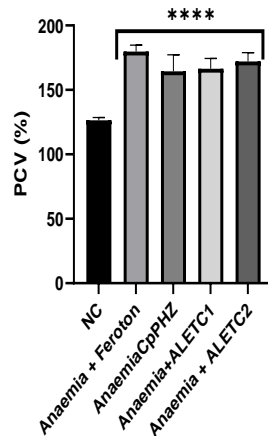


Fig. 3. Effect of aqueous extract on *Terminalia catappa* leaf on PCV following phenyl hydrazine induced anemia in Wistar rat

Results were expressed as mean \pm SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Standard: Anemic rats+ Feroton (10 mg/Kgbwt), AnaCpPHZ; Anemic rats control, induced with phenyl hydrazine, Anemia + AETC1: Anemic rats + aqueous extract on *Terminalia catappa* (100 mg/Kgbwt), Anemic rats + AETC2: Anemic rats + aqueous extract on *Terminalia catappa* (200 mg/Kgbwt).

Effect of aqueous leaf extract of *T.catappa* on WBC and differentials in phenyl hydrazine induced anaemic rats.

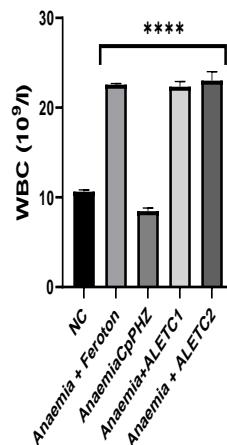


Fig. 4. Effect of aqueous extract on *Terminalia catappaleaf* on WBC following phenyl hydrazine induced anemia in Wistar rat.

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

Effect of aqueous leaf extract of *T.catappa* on WBC and differentials in phenyl hydrazine induced anaemic rats.

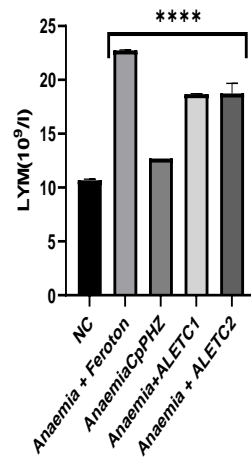


Fig. 5. Effect of aqueous extract on *Terminalia catappa* leaf on Lymphocytes following phenyl hydrazine induced anaemia in Wistar rat

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

Effect of aqueous leaf extract of *T.catappa* on WBC and differentials in phenyl hydrazine induced anaemic rats.

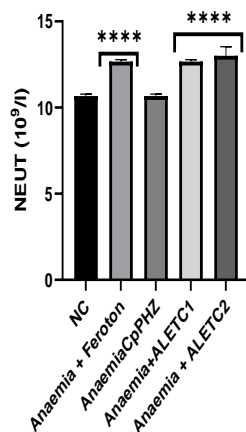


Fig. 6. Effect of aqueous extract on *Terminalia catappa* leaf on neutrophils following phenyl hydrazine induced anemia in Wistar rat

Results were expressed as mean \pm 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 phenyl hydrazine, Anemia + AETC1: Anemic rats + aqueous extract on *Terminalia catappa* (100mg/Kgbwt), Anemic rats + AETC2: Anemic rats + aqueous extract on *Terminalia catappa* (200mg/Kgbwt)

Effect of aqueous leaf extract of *T.catappa* on WBC and differentials in phenyl hydrazine induced anaemic rats.

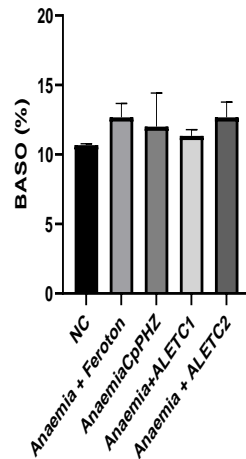


Fig. 7. Effect of aqueous extract on *Terminalia catappa* leaf on WBC following phenyl hydrazine induced anemia in Wistar rat

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

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

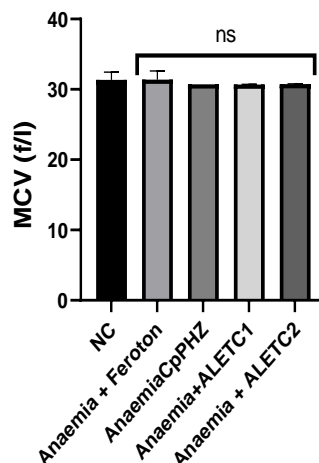


Fig. 8. Effect of aqueous extract on *Terminalia catappa* leaf on MCV following phenyl hydrazine induced anemia in Wistar rat

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

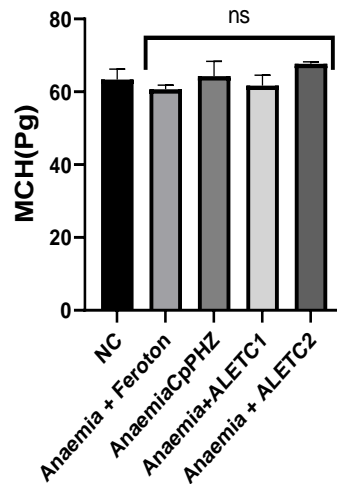


Fig. 9. Effect of aqueous extract on *Terminalia catappa* leaf on MCH following phenyl hydrazine induced anemia in Wistar rat

Results were expressed as mean \pm SD (n=5) *** significant at $P < 0.05$ compared with the control. NC: Normal Control, Standard: Anemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anemic rats' control, induced with phenyl hydrazine, Anemia + AETC1: Anemic rats + aqueous extract on *Terminalia catappa* (100mg/Kgbwt), Anemic rats + AETC2: Anemic rats + aqueous extract on *Terminalia catappa* (200mg/Kgbwt).

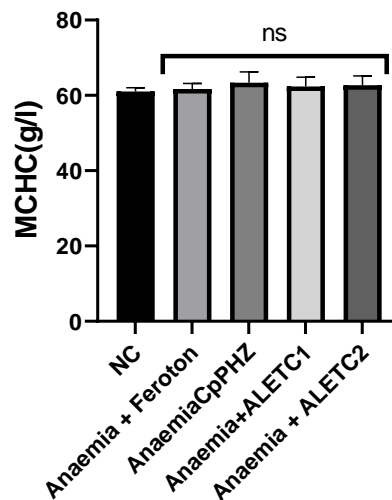


Fig. 10. Effect of aqueous extract on *Terminalia catappa* leaf on MCHC following phenyl hydrazine induced anemia in Wistar rat

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

4. DISCUSSION

Anemia caused by phenylhydrazine (PHZ) is a model that aids in the exploration of hematinic effects. According to studies, phenyl hydrazine increases the production of reactive oxygen species, which leads to red blood cell oxidative damage [8]. Following the treatment of PHZ to our experimental animals, there was a considerable decrease in the results of the red blood cell count, hemoglobin concentration, and hematocrit scores, which similar to report of PHZ on the red blood cell [19]. "The findings of this study indicate that treatment with *T. catappa* significantly increased various hematological parameters including WBC, RBC, Hb, and PCV. The primary roles of white blood cells and their differentials are to combat infections, protect the body from invasion by foreign organisms through phagocytosis, and transport and disseminate antibodies through immune response. Therefore, animals with low white blood cell counts are at a higher risk of contracting a disease, whereas those with high WBC counts can produce antibodies during the phagocytosis process, have a high level of disease resistance, and have improved adaptability to local environmental and disease-prevalent conditions" [18].

"The packed cell volume (PCV), also referred to as the hematocrit (Ht or Hct) or the erythrocyte volume fraction (EVF), is the proportion (%) of red blood cells in the blood. Anemia is characterized by a low production of red blood cells, and this test examines the volume of red blood cells as a percentage of total blood volume. Transport of oxygen and ingested nutrients also involves packed cell volume. A better transportation is demonstrated by higher PCV concentrations, which leads to an increase in secondary as well as primary polycythemia" [20]. The study's observed significant rise in PCV raises the possibility that plant extract, at various dosages, may affect positively with the blood's thereby enhancing the osmoregulatory and hemopoietic systems which might be a panacea in the management of anemia. Increases in hemoglobin (Hb) and red blood cells (RBCs) were also seen, suggesting erythrocyte production. As a result, the increase in RBC count and Hb may indicate that the extract improves erythropoiesis and/or hematopoiesis. This is consistent with research by [18] who found that using medicinal plants improved RBC and Hb levels. When compared to the normal, standard, and anemic control groups, no appreciable

difference was seen in MCV, MCH, and MCHC. This is consistent with the results of additional research [21]. It seems possible that even if MCV doesn't change, other changes in red blood cell differentials, including the red blood cell count, hemoglobin concentration, or hematocrit level may still take place.

5. CONCLUSION

In conclusion, the alterations from the present work implies that the extract may interfere synergistically with the osmoregulatory and hemopoietic systems of the blood at different doses and may be a panacea for anemia.

ETHICAL APPROVAL

The Animal Ethics Committee of the Faculty of Basic Medical Sciences, University of Cross River State, Nigeria, gave its approval for the study's use of animals. All animal testing was done in compliance with internationally recognized laboratory standards. The National Institutes of Health adopted and published Animal Use and Care of Laboratory Animals in 1996 (NIH publication No. 85(23), revised in 1996), based on the Helsinki Convention and the policies and procedures of the Faculty of Basic Medical Sciences at the University of Cross River State in Nigeria for animal experimentation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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