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Regenerative Hematinic Properties of *Mondia whitei* Root Aqueous Extract in Phenylhydrazine Hydrochloride-Induced Anemia in Female Wistar Rats

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Abstract

Anaemia, a medical condition worldwide, is of significant concern as it lowers hemoglobin or red blood cell levels, causing potential risk or death to patients. This study investigates the hematinic properties of *Mondia whitei* on phenylhydrazine hydrochloride-induced anaemic rats. Freshly prepared root of *M. whitei* was chopped into pieces, washed, shade-dried, and processed into aqueous extract. Phenylhydrazine hydrochloride (40 mg/kg) was administered across the groups for 3 days to induce anaemia in female Wistar rats. *M. whitei* extract (25, 50,100 mg/kg) and reference drug (folate 5 mg/kg) were orally administered for 7 days across the treatment groups, and hematological, peripheral blood smear, and histopathology parameters were analyzed. The results obtained from this study showed that the root extract exhibited a significant difference in the hematinic properties (red blood cells and their components, peripheral blood smear, and regeneration and rapid maturation of blood cells with normal blood morphology) across graded doses of the extract when compared with the untreated control $p>0.05$. The plant's traditional use in managing anaemia was scientifically validated, hence further studies are warranted to elucidate the implicated compound-to-compound isolation-guided bioassay.

Keywords: Hematinic; *Mondia Whitei;* Phenylhydrazine Hydrochloride; Anemia; Rats..

INTRODUCTION

Prehistoric people were driven by instinct, taste and experience, to use plants, animal parts and minerals that weren't typically part of their diet to treat illnesses (Idu *et al*., 2007). Plant phytochemicals have demonstrated a pivotal role in the development of new pharmaceuticals (Nostro *et al*., 2000), because of the Phyto-constituents present in plants it sparked scientific interest in biological activities (Moghadamtousi *et al*., 2009; 2015; Al-daihan *et al*., 2013). They are used for several prophylactic, curative and suppressive treatment purposes because of their therapeutic importance (Idu *et al*., 2022).

Using thin layer chromatography, Inkoto *et al*. (2018) reported that while alkaloids were absent, phenolic compounds, coumarins, anthraquinone, anthocyanins, tannins and irridoids were present in the root of *M. whitei*. The ethanolic fresh root bark extracts of *M. whitei* that was prepared showed a significant presence of saponins, phenols, tannins and alkaloids (Ndukui *et al*., 2013). Moreover, Amaechi and Egesi (2017) reported that the fruit of M. whitei showed the presence of tannins, phenol, phytate, sterol, carotenoid, oxalate, saponins, flavonoids, alkaloids and even measured the amount of hydrogen cyanide (mg/kg-1) on a fresh weight basis. It has been reported that the leaves of *M. whitei* do not contain cyanogenetic glycosides, anthraquinone, alkaloids and cardiac glycosides and contains saponins, flavonoids, tannins, and resins. According to Wacho *et al*. (2013) using the aqueous and hexane root extracts reported the presence of reducing sugars, triterpenes and steroids.

In traditional medicine, various parts of the *Mondia whitei* plant are used for the treatment and management of several diseases and maladies such as impotence and sexual dysfunction (Watcho *et al*., 2013; Segawa and Kasenen, 2009), constipation, abdominal discomfort, appetite stimulant (Kokwaro, 1976), urinary infections, gonorrhea pains (Matu and Van-staden, 2003), and inducing labor. It is also used in the treatment of kidney disease, backache, and gastritis (Ngbolua *et al*., 2016). The seeds are used in the preparation of arrow poisons (Neuwinger, 2000), antimalarials and anti-anemia (Hermans *et al*., 2004; Odugbemi *et al*., 2007).

MATERIALS AND METHODS

Collection of Plant Material

Mondia whitei roots were obtained from Osun state in the wild in the month of July and were identified and authenticated by Prof. Odaro Timothy in Life Sciences with voucher number UBH-M113.

Preparation of Plant Material

The roots of the *M. whitei* plant were air-dried for some days. The dried roots were then pulverized into powder using the British milling machine. The weight of the powdered plant was further weighed using maceration techniques in the aqueous extraction process. The filtrate was concentrated via water bath and the extract was stored in a sterile container and kept for further use.

Experimental Animals

Fifteen (15) healthy female rats which were weighed between 175 – 248 g were used for this experiment. They were housed in Life Sciences animal house, in a well-ventilated plastic cage under normal laboratory conditions (12 hours' light/dark cycle: 23 ± 2ºC) and fed with standard diet. All selected animals were acclimatized for 14 days. The use and proper handling of animals was approved by Life Sciences ethical committee, Edo state Nigeria with an ethical number LSC24052.

Experimental Protocol

Female albino rats were obtained and randomly divided into five (5) groups. The groups received 25, 50, 100 mg/kg of the extract orally and the remaining two groups were used as the positive control (5 mg/kg of folic acid) and negative control (untreated). Group 1-3 received 25, 50 and 100 mg/kg of the *Mondia whitei* extract orally, group 4 and 5 received 5 mg/kg Folic acid and 40 mg/kg of Phenylhydrazine hydrochloride was induced into the albino female Wistar rats for three (3) days, before the administration of the treatment groups for seven (7) days. The animals were sacrificed under mild chloroforms anesthetic. Blood samples and bone marrow were analyzed.

Haematological Indices

Haematological assays were done on the entire blood collected in tubes of ethylene diamine tetra-acetic acid (EDTA). Red blood cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RCDW), White blood cell (WBC), Monocytes (MO), Lymphocytes (LY), Platelets (PLT), Platelets crit (PCT), Platelet density width (PDW), Mean platelet volume (MPV) and granulocytes (GR) were resolute via Automated Sysmex KX-21 hematology analyzer (Sys-mex Corporation, Kobe, Japan) (Idu *et al*., 2022).

Peripheral Blood Smear

The blood is collected into a tube containing an anticoagulant, such as EDTA, to prevent clotting. A drop of blood is placed on a clean glass slide. A second slide is used as a spreader to spread the blood thinly across the first slide, creating a thin "feathered edge". The wedge technique is commonly used, where the spreader slide is held at an angle and pushed across the blood drop to create a uniform smear. Once the smear is air-dried, it's stained with a special stain, such as Wright's stain or Giemsa stain, to highlight the different components of the blood cells. These stains allow for visualization of red blood cells (RBCs), white blood cells (WBCs), and platelets. The stained smear is examined under a microscope, typically using the oil immersion lens, to assess the morphology and number of blood cells. A trained laboratory professional (clinical laboratory scientist or pathologist) evaluates the smear for any abnormalities in cell morphology, such as shape, size, and staining characteristics. They also assess the number of different types of blood cells and platelets. The results of the peripheral blood smear are used to help diagnose various conditions, including anemias, leukemias, infections, and other blood disorders. The findings are reported along with the results of other blood tests, such as a complete blood count (CBC).

Determination of Body Weight

The weights of the rats were determined using My Weigh 70001DX Multi-Purpose Digital Scale on day zero (0) and at the end of a ten (10) days period of the experiment. The Net change in body weight (difference between final body weight and initial body weight) was calculated using the formula: Net change in Body Weight = Final Body Weight - Initial Body weight

Histopathological Study

Bone marrow was obtained from the femur bones, which were isolated and fixed in neutral buffered formalin. The fixed femur was completely dehydrated using absolute ethanol, followed by 96% ethanol, 70% ethanol and then rinsed with distilled water. Then, slides were prepared from them after they were decalcified using a decalcifying machine, the slide pictures were taken using Photomicrographs at × 40 magnification using a digital camera and were interpreted.

Statistical Analysis

The results are expressed as mean ± SEM (Standard error of the mean) using GraphPad Prism 6 version. Data for the groups were compared using one-way analysis of variance (ANOVA), and the significant difference was displayed as p *-value* < 0.05.

RESULTS AND DISCUSSION

Hematology

As displayed in Table 1, a significant increase in white blood cells, mid, and granulocyte specifically at 50 and 100 mg/kg leaf biherbal extract compared with the untreated control. This displayed that the extract at 50 and 100 mg/kg was implicated in the release or rapid production of matured blood cells, which could be attributed to the presence of the phytochemicals.

Anaemia is a medical condition characterized by lowered hemoglobin level and decreased Red Blood Cell Count (RBCC) leading to inadequate oxygen transport to body tissues. This condition is a blood disorder that frequently impacts people across all age brackets. There are more than 400 known types of anaemia, ranging from hemolytic to sickle cell anemia and many others (Ogbe *et al*., 2010), among the various types of anemia, hemolytic anemia being the most prevalent (Fasidi *et al*., 2008). Over 50% of the global population experiences some type of anaemia in their life time (Duff, 2011), global statistics indicates that 1.9 billion people worldwide are affected (Gabriel and Idu 2021), 50% of pregnant women in developing countries suffer from anemia (Gabriel and Idu 2019), 40% of children under 5years in developing countries are also anaemic (Agea *et al*., 2008). The incidence of anaemia is more prevalent in the third world countries where there are limited health care access and resources when compare to developed countries (Ogbe *et al*., 2010). Individuals with a higher risk of anemia includes; adolescent women of child-bearing age, older adults and infants (Diallo *et al*., 2008). A Nigerian rural study in a rural revealed alarming rates of anaemia among 19.7% the children which were anemic leading to impaired cognitive development, stunted physical growth, increased susceptibility to infection, which may decrease academic performance (Akinkugbe, 1991). Anaemia during pregnancy in women results in several complications affecting fetal development such as elevated maternal mortality rates, Impaired fetal growth (low birth weight), premature birth, increased likelihood of anaemia in children (Qinghua *et al*., 2019). The prevalence of anemia has been attributed to various combinations of aggravating factors such as nutritional deficiencies (iron and vitamin deficiency such as B_{12} and folate), high prevalence of blood parasites (malaria caused by plasmodium, trypanosomiasis caused by Trypanosome and helminthiasis caused by helminthes) (Ogbe *et al*., 2010), underlying chronic medical conditions (HIV/AIDS, cancer, Kidney disease), Genetic disorders (sickle cell anaemia, thalassemia and leukaemia such as acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL), additional factors include prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) and exposure to toxic chemicals such as phenylhydrazine (Sharda *et al*., 2011). Anaemia is one of the major causes of death worldwide, resulting from a lack of essential mineral nutrient, if untreated is capable of triggering blood related diseases like anemia which can lead to fatigue and weakness, shortness of breath, pale skin, impaired cognitive function, increased infection risk, cardiovascular problems, pregnancy complications and stunted growth and development. The high prevalence and possibility of anaemia, escalates its potential risks (Duff, 2011), there is an urgent need to prevent it or seek for more effective measures and cost-efficient treatment strategies. Some effective prevention and treatment strategies for anaemia overtime include dietary modification through implementation of micronutrients supplementation (iron, and incorporation of vitamin B_{12} and folate), blood transfusions, health education and awareness amongst others. Thus, conventional medicine has faced several limitations due to its increased cost of treatment, numerous adverse effect and growing drug resistance (Gabriel and Idu, 2023).

Table 1. Effect of *Mondia whitei* aqueous root extract on white blood cells and its differentials in phenylhydrazine induced Wistar rats.

Parameters	Phenylhydrazine hydrochloride (40 mg/kg)	Folic Acid (5 mg/kg)	*M. Whitei* (25 mg/kg)	*M. Whitei* (50 mg/kg)	*M. Whitei* (100 mg/kg)
WBC $10^3/\mu L$	3.17 ± 0.33^a	4.23 ± 0.32^b	3.80 ± 0.12^a	4.77 ± 0.92^b	6.90 ± 1.33^b
LYM %	96.17 ± 0.38^b	92.50 ± 1.42^a	94.80 ± 0.32^a	93.60 ± 1.90^a	89.00 ± 2.90^a
MID %	2.23 ± 0.19^a	5.03 ± 1.14^b	3.17 ± 0.18^a	3.67 ± 0.57^b	6.47 ± 0.93^b
GRAN %	1.60 ± 0.20^a	2.47 ± 0.29^a	2.03 ± 0.15^a	2.73 ± 0.35^a	4.53 ± 1.98^b
LYM $10^3/\mu L$	3.07 ± 0.33^a	3.93 ± 0.32^a	3.60 ± 0.12^a	4.47 ± 0.92^b	6.10 ± 0.98^b
MID $10^3/\mu L$	0.10 ± 0.00^a	0.20 ± 0.06^a	0.10 ± 0.00^a	0.17 ± 0.03^a	0.47 ± 0.18^b
GRAN $10^3/\mu L$	0.00 ± 0.00^a	0.10 ± 0.00^a	0.10 ± 0.00^a	0.13 ± 0.03^b	0.33 ± 0.19^b

The values were expressed in Mean ± SEM and the significant difference was spotted as *p -value* < a 0.05.

As shown in Figure 1 had a significant increase in red blood cell count and haemoglobin level on days 14 specifically at 100 mg/kg biherbal extract when compared with the untreated control. This displayed that the extract at100 mg/kg instigated the rapid production and maturation of RBC and HGB, which could be traced to the present of phytochemical.

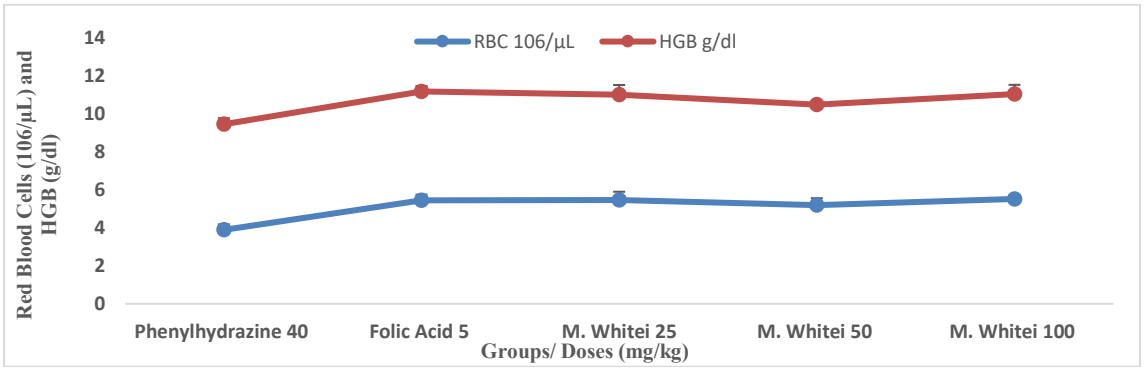

Figure 1. Fourteen days acute effect of *Mondia whitei* aqueous root extract on red blood cells and hemoglobin in phenylhydrazine induced Wistar rats.

Figure 2 elicited a slightly significant increase in HCT and MCV levels on days 14 across graded doses (25, 50 and 100 mg/kg) of the biherbal extract when compared with the untreated control. This displayed that the extract at across the treatment groups could propelled the production and release of HCT and MCV, which could be traced to the presence of flavonoids and phenol implicated.

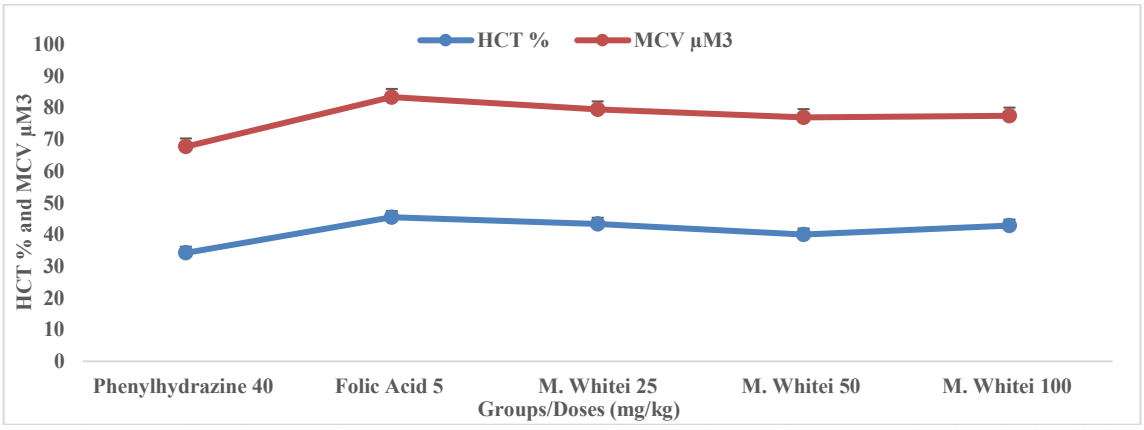

Figure 2. Fourteen days acute effect of *Mondia whitei* aqueous root extract on packed cell volume and mean corpuscle volume in phenylhydrazine induced Wistar rats.

The results obtained as shown in Figure 3 elicited a slight significant increase in MCHC level on days 14 across graded doses (25, 50 and 100 mg/kg) of the biherbal extract when compared with the untreated control. While MCH had no significant difference when compared with the untreated control. This displayed that the extract at across the treatment groups could possibly propelled the production and release of MCHC released.

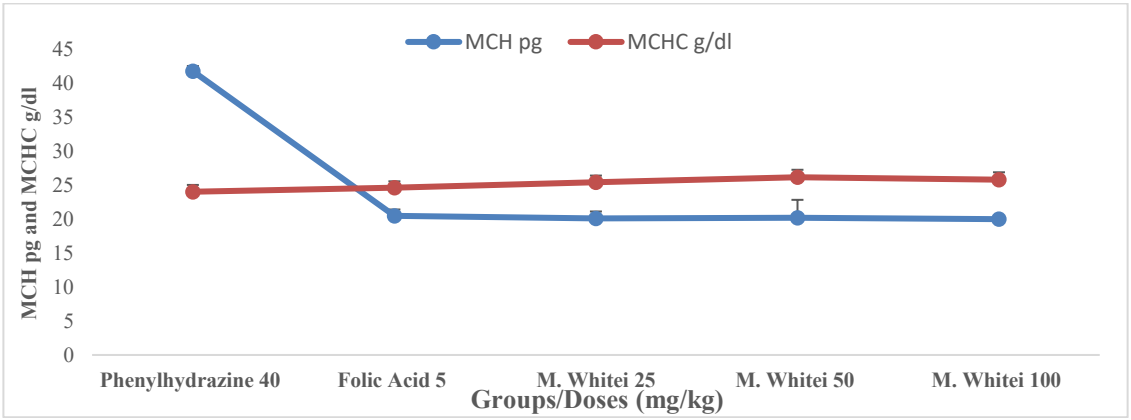

Figure 3. Fourteen days acute effect of *Mondia whitei* aqueous root extract on mean concentration hemoglobin and mean corpuscle hemoglobin concentration in phenylhydrazine induced Wistar rats

The results obtained from Figure 4 elicited a slight significant increase in RDWS and RDWC level on days 14 across graded doses (25, 50 and 100 mg/kg) of the biherbal extract when compared with the untreated control. This displayed that the extract across the treatment groups could possibly propelled the production and release of RDWS and RDWC level.

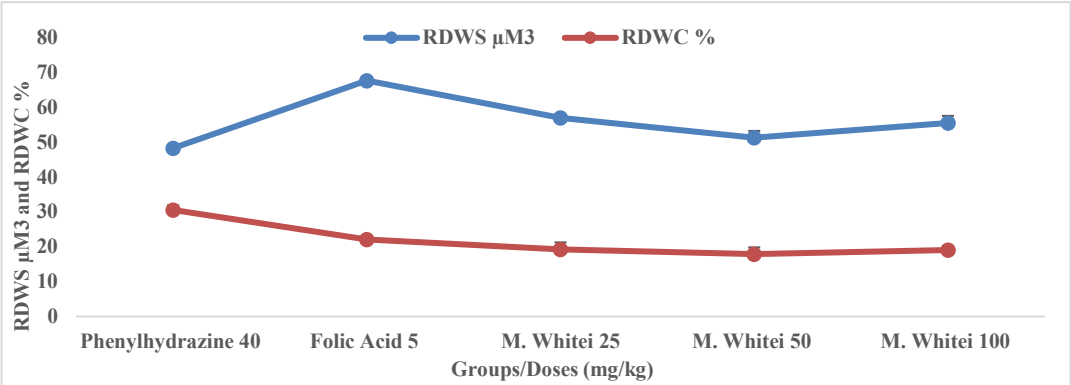

Figure 4. Fourteen days acute effect of *Mondia whitei* aqueous root extract on RDWS and RDWC in phenylhydrazine induced Wistar rats.

The results obtained from Figure 4 elicited no significant difference in platelet and its factors on days 14 across graded doses (25, 50 and 100 mg/kg) of the biherbal extract except at 25 mg/kg that showed slight significant increase when compared with the untreated control.

The Preliminary phytochemical screening of *Mondia whitei* root extract showed the presence of tannins, phenol, phytate, sterol, carotenoid, oxalate, saponins, flavonoids, alkaloids and even measured the amount of hydrogen cyanide. Antioxidant phytochemicals such as polyphenols, flavonoids, tannins and alkaloids have been documented to enhance blood cell regeneration in animals (Claro *et al*., 2006; Sahreen *et al*., 2013), and may indirectly stimulate the action of the hypothalamus and pituitary glands leading to an increased erythropoietin production in the kidneys which further activates myelo-erythroid cells in the bone marrow (Sen *et al*., 2005).

Anti-anemic study showed that *Mondia whitei* plant extract enhanced blood production in the treatment groups across the female animals used in the experiment. The present result from this study showed that the red blood cell and its components (RBC, Hgb and HCT) had a significant increase across the treatment groups (5.53 ± 0.07; 11.07 ± 0.66 and 42.80 ± 1.84) when compared with untreated group (phenylhydrazine hydrochloride) (3.90 ± 0.95, 9.47 ± 2.13 and 34.23 ± 11.52), these are results from the graded concentration of 100 mg/kg. A significant increase across the graded doses (25, 50 and 100 mg/kg) of *Mondia whitei* extract compared to the untreated group ($p < 0.05$). The recovery effect of blood cells probably could be a result other mechanism of action from erythropoiesis in the liver, stimulating the bone marrow to regenerate blood cells. Haemogloblin (oxygen carrying capacity of the blood), MCV, MCH and MCHC exhibited a significant increase in the treated groups; this finding concurs with the work of Idu *et al*. (2022) on the hematinic property of Mojeaga herbal remedy using an animal model.

Table 2. Effect of *Mondia whitei* aqueous root extract on platelet and its factors in phenylhydrazine induced Wistar rats.

Parameters	Phenylhydrazine hydrochloride (40 mg/kg)	Folic Acid (5 mg/kg)	*M. Whitei* (25 mg/kg)	*M. Whitei* (50 mg/kg)	*M. Whitei* (100 mg/kg)
PLT $10^3/\mu L$	1702.00 ± 108.00^a	1357.00± 783.00^b	2047.00± 300.30^b	2743.00 ± 290.00^b	3172.00± 92.59^b
MPV μM^3	1141.00 ± 114.00^a	2.73 ± 0.73^a	5.77 ± 0.91^a	2.73 ± 0.70^a	00.00 ± 0.00^a
PDW %	3.50 ± 0.35^a	3.07 ± 0.67^a	6.13 ± 3.10^b	3.17 ± 0.17^a	0.00 ± 0.00^a
PCT %	0.44 ± 0.04^a	0.37 ± 0.04^a	1.00 ± 0.50^b	0.62 ± 0.06^b	0.00 ± 0.00^a
P-LCR %	6.37 ± 0.36^a	3.20 ± 0.20^a	8.53 ± 0.67^b	3.27 ± 0.67^a	0.00 ± 0.00^a

The values were expressed in Mean ± SEM and the significant difference was spotted as *p -value* < a 0.05.

Peripheral blood smear

The results displayed from the peripheral blood smear as shown in Plate 4 elicited no deteriorative effect on days 14 across graded doses (25, 50 and 100 mg/kg) of the biherbal extract when compared with the positive and untreated control.

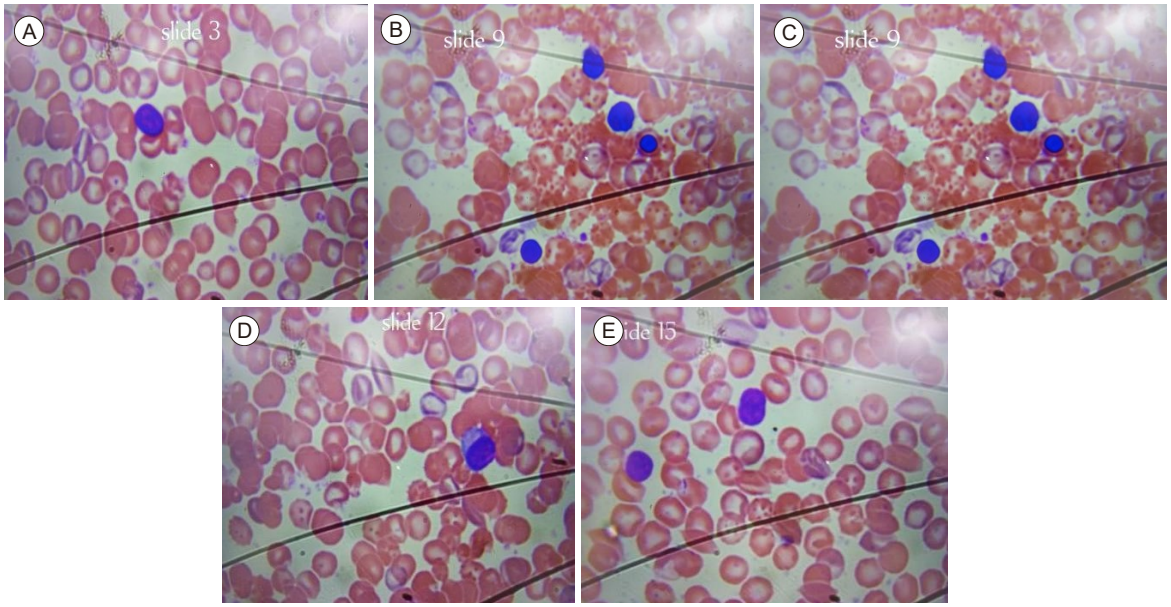

Plate 1. Effects of *Mondia whitei* aqueous extract in peripheral blood smear in Female Wistar rats.

A. 25 mg/kg *Mondia whitei* peripheral blood smear: **WBC** leukocytes appeared adequate in number with relative lymphocytosis; small lymphocytes (++); large lymphocytes (+); neutrophils (+). absent atypical cell seen. **RBC** erythrocytes showed normochromic cells (++); normocytic cells (++); stomatocytes (++); microcytic cells (+); polychromatic cells (++) and few target cells (+). **PLT** platelet appeared adequate in number and normal in size.

B. 50 mg/kg *Mondia whitei* peripheral blood smear: **WBC** leucocytes showed adequate number with moderate lymphocytosis (++); eosinophils (+) and neutrophils (+); absent atypical cell seen. **RBC** erythrocytes showed normocytic cells (++); normochromic cells (++); polychromatic cells (++); target cells (+) and few crenated cells. **PLT** platelet appeared adequate in number and normal in size.

C. 100 mg/kg *Mondia whitei* peripheral blood smear: **WBC** leucocytes appeared adequate in number with relative lymphocytosis; large lymphocytes (+); small lymphocytes (++); neutrophils (+) with absent plasma cell seen. **RBC** erythrocytes showed normocytic cells (++); normochromic cells (++); target cells (+); hypochromic cells (+); polychromatic cells (++); nucleated RBC (+); stomatocytes (+). **PLT** platelet appeared adequate in number and normal in distribution.

D. 5 mg/kg folic acid peripheral blood smear: **WBC** leucocytes appeared adequate in number with absent lymphocytosis; large lymphocytes (++); small lymphocytes (+++); with no atypical cell seen. **RBC** erythrocytes showed normocytic cells (++); ecliptocytes (+); microcytic cells (+); normochromic cells (++); polychromatic cells (+); stomatocytes (++); target cells (++); hypochromic cells (+) and tear drop cells (+). **PLT** platelet appeared adequate in number and normal in size.

E. Untreated control peripheral blood smear: **WBC** leucocytes appeared adequate in number with absolute lymphocytosis; large lymphocytes (++); small lymphocytes (+++); with no plasma cells seen. **RBC** erythrocytes showed macrocytic cells (+++); normocytic cells (++); hypochromic cell (+++); stomatocytes (++); polychromatic cells (++) with target cells (++). **PLT** platelet showed moderate thrombocytosis.

The peripheral blood smear result had an increase in red blood cell count across graded doses (25, 50 and 100 mg/kg) of the root extract of *Mondia whitei*, the RBC had normal cell shape, size and haemoglobin levels indicating a healthy red blood cell, when compared with the untreated group which had large RBC and low hemoglobin levels indicating unhealthy red blood cells. The treated group displayed a normal red blood cells; normocytic, normochromic, and homochromatic cell morphology and structures across graded doses of the treatment groups of *Mondia whitei* root extract when compared with the untreated control that displayed an abnormal red blood cells; macrocytic, hypochromic, polychromatic cells and Sickle blood shape; this corresponds with the work of Gabriel and Idu (2023) on the Mineral composition, in-vivo hematinic and antioxidant potential of *Jatropha gossypiifolia* n-hexane root extract in hemolytic anaemic rats.

Bone marrow

The results obtained from plate 2 showed the regenerative effect of the extract in blood regeneration via instigating hemopoitein of the kidney and myelo erythroid cell in the bone marrow to trigger the release of blood cells on days 14 across graded doses (25, 50 and 100 mg/kg) of the biherbal extract when compared with the untreated control.

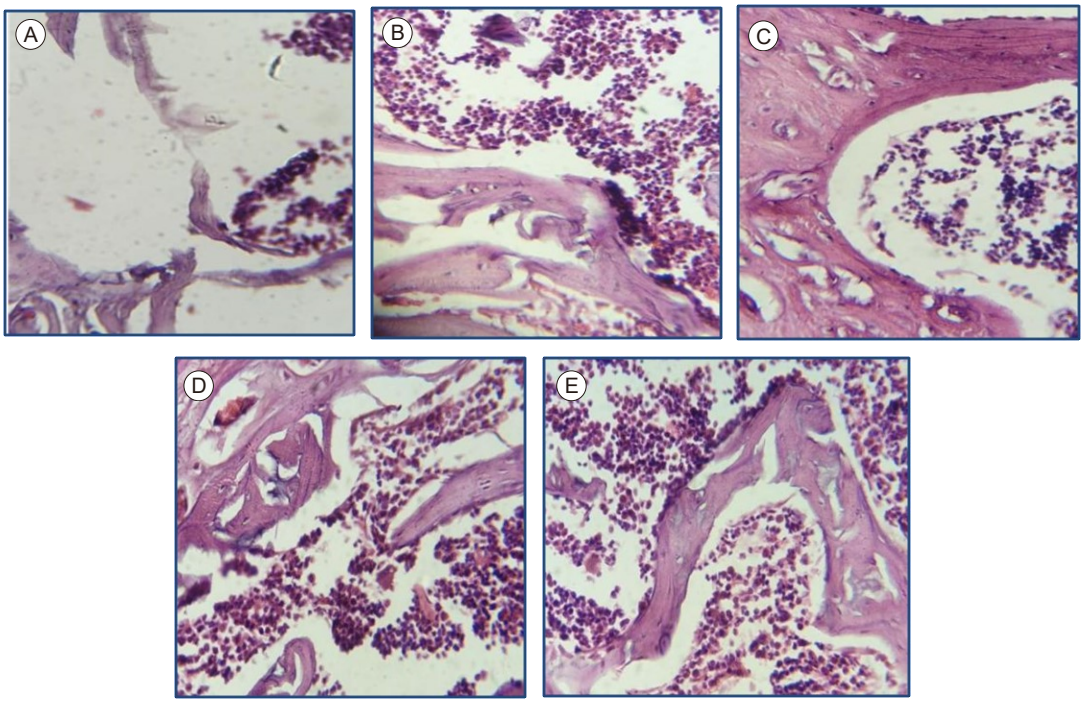

Plate 2. Effect of *Mondia whitei* extract on Myelo-Erythroid Cell in Female Wistar rats.

A. Control (untreated): bone marrow cavity had seen depleted scanty marrow cells, (long arrow) with unremarkable histologic cartilage and bone trabeculae appearance (short arrow).
B. 5 mg/kg Folic acid: bone marrow cavity with pronounced marrow cells, (long arrow). Seen histologic cartilage and bone trabeculae appearance (short arrow).
C. 25 mg/kg *Mondia whitei* extract: bone marrow cavity declines a pronounced marrow cell, (long arrow). Visible histologic cartilage and bone trabeculae appearance (short arrow).
D. 50 mg/kg *Mondia whitei* extract: bone marrow cavity had visible marrow cells, (long arrow). pronounced histologic appearance of the cartilage and bone trabeculae (short arrow).
E. 100 mg/kg *Mondia whitei* extract: bone marrow cavity showed augmented marrow cells, (long arrow). Visible histologic cartilage and bone trabeculae (short arrow).

The bone marrow in the treatment groups of the *Mondia Whitei* root extract revealed an increase in mylo-erythroid cell proliferation in the bone marrow contrasting with the anemic control that had reduced mylo-erythroid cells indicating enhanced erythropoiesis. The bone marrow examination elicited well defined myelo-erythroid cell structures with an increased cellularity and normal hematopoiesis across the treated groups (25, 50 and 100 mg/kg) of the female animals when compared to the untreated group (phenylhydrazine hydrochloride induced anaemic rats) which displayed severe anaemic state. These findings consent with the earlier report Gabriel and Idu (2023) on the Mineral composition, *in-vivo* hematinic and antioxidant potential of *Jatropha gossypiifolia* n-hexane root extract in hemolytic anemic rats and Idu *et al*. (2022) on the hematinic property of Mojeaga herbal remedy using animal model on the significant increase in red blood cells production and myelo-erythroid cells.

CONCLUSION

In conclusion, the present study showed that plant extract in various doses increased blood levels possibly due to the regeneration or quick maturation of the blood cells from the bone marrow. Hence, this study requires further research on compound isolation and the determination of mechanism of action.

Conflict of interest: The authors declare that there is none conflict of interest.

Ethical approval: Ethical approval for this study was obtained from Institutional Review Board of Life Sciences, Edo state Nigeria.

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