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Attenuation of *Plasmodium Berghei berghei* -induced Bone Marrow Suppression by Bark Extract and fractions of *Chrysophyllum albidum* on bone marrow response to haemolytic conditions in male albino mice

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ABSTRACT

Chrysophyllum albidum is a tropical plant in Southern Nigeria. Methanol extract of *Chrysophyllum albidum* bark (*MeCaB*) has been reported to have antiplasmodial, haematinic and membrane stabilizing properties. The mechanism by which *MeCaB* exerts these properties is yet to be elucidated. This study therefore seeks to investigate the probable mechanism of its haematinic potential on the bone marrow in two separate anaemic studies. Mice were inoculated with *plasmodium berghei bergehi* while others were made anaemic by bleeding out 0.25mls of blood through the retro-orbital plexus. Smears of the femoral bone marrow from each group were prepared on days 3 and 7 of extract/drug treatment for blood precursor cell evaluation (myeloid: erythroid ratio; M:E). Groups parasitized and treated continuously for 3 days with *MeCaB (PMeCaB)* had significantly reduced M: E ratio (*1.88±0.03*) compared with parasitized untreated *group (PUn) (2.33±0.00)*. Withdrawal of treatment with *MeCaB*, it's fractions *(CFr 1,2,3*) and chloroquine from parasitized animals between days 4 to 7 showed significant increase in M:E ratio of *PUn* (*5.13±0.59*) and *PCq (4.11±0.37) compared* with P*CFr 1 and 3* (*1.21±0.08 and 1.69±0.00* respectively). The *MeCaB* only treated and group bled and treated with *MeCaB* (*BMeCaB*) continuously for 7 days had significantly reduced M:E ratio (*1.67±0.00 and 1.35±0.00* respectively) compared with control, bled and treated with haematinic (*2.13±0.19, 2.25±0.21 respectively*). This study thus establishes that *Chrysophyllum albidum* exerts haematinic properties on bone marrow cells by stimulating the production of more erythroid series which reverted anaemia induced by *plasmodium berghei berghei* and bleeding out.

Keywords: *Chrysophyllum albidum,* bone marrow, anaemia, Mice.

INTRODUCTION

Anaemia is a haemolytic condition which develops when accelerated removal of erythrocytes is not compensated by production from the bone marrow (Lamikanra *et al,* 2007). The fact that some malaria patients develop severe anaemia, whereas others retain normal or near normal haemoglobin (Hb) could be explained by the amount of erythrocyte destruction occurring during the period until return of normal bone marrow function (Ekvall, 2003). Suppression of bone marrow activity has been reported in all malaria patients (Kurtzhals *et al*

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1997) and in asymptomatic *P. falciparum* infection (Kurtzhals *et al,* 1999). Data on the duration of bone marrow activity suppression after malaria attack are conflicting. Some studies reported hypo-proliferative erythropoiesis and dyserythropoiesis for weeks following treatment (Camacho *et al,* 1998, Phillips *et al,* 1986), while other studies have shown that suppression of bone marrow activity is reversed rapidly after treatment (Abdalla *et al,* 1980, Kurtzhals *et al,* 1997).

Bone marrow is a primary lymphoid tissue and a major haematopoietic organ responsible for the production of about 500 billion blood cells daily in form of Red blood cells, White blood cells, Platelets (Barbara, 2006, Abboud and Lichtman, 2001, Hoffman *et al,* 2000, Cline and Golde, 1979,). It accounts for approximately 3% of the body weight in adult rats (Schermer, 1967), 2% in dogs (Jain, 1986b) and 5% in humans (Picker and Siegelman, 1999).

Chrysophyllum albidium G.Don_Holl.(*Sapotaceae*) also known as African star apple is known for its various ethno-medicinal uses (Amusa *et al,* 2003, Pearson, 1976 and Dalziel,1937). The bark is used as a remedy for yellow fever and malaria while the leaves are used as emollients (Adisa, 2000, Adewusi *et al,* 1997). Eleagnine, an alkaloid isolated from *C. albidum* seed cotyledon has been reported to have antinociceptive, anti–inflammatory and antioxidant activities (Idowu *et al,* 2006). Previous studies on methanol extract of *C.albidum* stem bark have revealed its antiplasmodial (Adewoye *et al,* 2010), antimicrobial (Adewoye *et al,* 2011), haematinic (Adewoye *et al,* 2012) and membrane stabilizing potentials (Adewoye *et al,* 2013).

The mechanism by which *C. albidum* stem bark maintains a normal blood cell count in haemolytic conditions are yet to be elucidated. This study therefore seeks to evaluate the activity of the plant bark extract and its active chromatographic fractions on the bone marrow response in *plasmodium berghei berghei* infection and induced- anaemia in albino male mice.

MATERIALS AND METHODS

Reagents: All reagents are of analytical grade and were obtained from BDH chemicals LTD, Poole England.

Drug: Chloroquine was obtained from Sigma (UK) and was used as a positive control drug to evaluate the *in vivo* efficacy of methanolic extract of C. *albidum.*

Plant Materials: Collection and Identification: The fresh bark of *C. albidum* was collected between the months of November 2011 and April 2012 from its natural habitat at Igbo Owe cash crop farm at Moniya, Akinyele Local Government Area of Oyo State, South-western Nigeria. The plant was identified and given a voucher number FHI 107514 at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The plant bark were dusted and dried at room temperature for 3 weeks and then grounded to powder using a dry electric mill (Moulineux, UK).

Preparation of extract partition and fraction: *Chrysophyllum albidum* bark (1.5kg) was soaked in 2.5 litres of n – hexane for 72 hours and the filtrate was collected as hexane partition. The residue was spread out evenly and allowed to dry for 24 hours. To this residue, 2.5 litres of dichloromethane was added and was well macerated for 72 hours after which the filtrate (dichloromethane partition) was decanted and the residue was again spread out to air dry for 24 hours. Methanol (2.5litres) was added to the dried residue stirred and left for 72 hours, after which the whole mixture was filtered and the filtrate was labelled methanol partition. The mixture was filtered with What-man's filter paper (No. 1). All the 3 different filtrates were evaporated to dryness *in-vacuo* and stored at 4°C until use.

The methanol partition (*MeCaB*) was then allowed to pass through a silica gel column chromatography using different solvent mixture of n-hexane, dichloromethane and methanol (in varying polarity gradient) to obtain 3 fractions *(CFr1,2 and 3).*

Parasites: Chloroquine-sensitive *P. berghei berghei* (NK 65) was obtained from the National Institute of Malaria Research (NIMAR) Yaba, Lagos State. The parasite was maintained in mice by serial passage of infected blood to uninfected mice in the animal house. Parasitized red blood cells used for inoculation in the experiment were obtained by cardiac puncture from an infected donor mouse. The blood was diluted to desired parasite density in 0.9% NaCl solution (Kendall McGraw, Laboratories, Inc, USA). Each mouse was inoculated with 1.0×10^6 parasitized red blood cells contained in 0.2mls of the NaCl solution.

In this study, the day of inoculation was defined as day zero D_0 while subsequent days were named D_1, D_2, D_3 etc.

Animal Grouping

Animals: One hundred and sixty five (165) Swiss male albino mice weighing between 19 - 22 g were used in this experiment. They were obtained from the Animal House of the College of Medicine, University of Ibadan,

Nigeria and were maintained under standard conditions (12 h light and 12 h dark) with free access to mice chow and clean water.

Extract administration

Drugs and extracts were administered orally using orogastric tube. Two separate studies were conducted: studies A and B

- Study A – investigated the effect of methanol extract of *C.albidum* bark (*MeCaB*) and its 3 fractions *(CFr 1, 2 and 3)* on bone marrow of *Plasmodium bergehi berghei* infected mice.
- Study B – investigated the effect of methanol extract of *C.albidum* extract *(MeCaB)* on bone marrow of anaemia induced mice.

Study A – We investigated the effect of *C. albidum* bark extract and its 3 fractions *(CFr 1, 2 and 3)* on the bone marrow of *Plasmodium bergehi berghei* infected mice. This study contained six groups of 15 mice each.

- Group 1 animals were infected and untreated, (*PUn*).
- Group 2 mice were infected and treated with 30mg/kg *b.w.* Chloroquine for 3 consecutive days, (*PCq*).
- Group 3 consist of mice infected and treated with 1000mg/kg *b.w* of *C. albidum for 3 consecutive days*, (*MeCaB*).
- Group 4 mice were infected and treated with 250mg/kg *b.w* of fraction 1 for 3 consecutive days, *(PCFr1)*.
- Group 5 animals were infected and treated with 250mg/kg/day *b.w* of fraction 2 for 3 consecutive days, (*PCFr2*).
- Group 6 consists of mice infected and treated with 250mg/kg *b.w* of fraction 3 for 3 consecutive days, (*PCFr3*).

Study B – In this study, we investigated the effect of methanol extract of *C. albidum (MeCaB)* on bone marrow in anaemia-induced mice. The study contained five groups of 15 animals.

Drugs and extract administrations were given orally. Group 1 consists of *Nor*mal mice that were not bled and did not receive *C albidum* extract, (control). Group 2 consists of mice not bled but received methanolic extract of *C.albidum* (1000mg/kg b.w), (*MeCaB*). Group 3 animals were bled (0.25ml of blood) and administered ferrous sulphate (100mg/kg), (*BHaem*). Group 4 animals were bled (0.25ml of blood) and administered *MeCaB* (1000mg/kg b.w), (*BMeCaB*). Group 5 animals were bled (0.25ml of blood) and untreated, (*Bla*).

Morphological studies of Bone Marrow:- The air-dried bone marrow smears on glass slides were fixed with methanol for 2-5 minutes at room temperature. After air-drying for 20- 30 minutes, the fixed smear was stained with Wright's-Giemsa stain and the morphology of hematopoietic cells was investigated under a light microscope. The bone marrow smear was differentially counted (at least 500 cells per slide) into erythroid, granulocytic, agranulocytic, and megakaryocytic series. The myeloid: erythroid ratio (M:E ratio = total myeloid cells/total erythroid cells) was calculated from the proportion of total myeloid lineage cells and total erythroid lineage cells.

Statistical analysis

Experimental data were analyzed using one way analysis of variance (ANOVA) and multiple range tests to determine significant differences between means. Difference between means were regarded as significant at $p<0.05$.

RESULTS

Effect of *Chrysophyllum albidum* on myeloid-erythroid ratio in parasitized animals.

The effect of the methanolic extract of *Chrysophyllum albidum* (*MeCaB*) on myeloid-erythroid ratio in parasitized animals by days 3 and 9 is shown in figure 1. The myeloid-erythroid ratio of parasitized animals treated with fractions 1, 2 and 3 *(PCFr1, PCFr 2 and PCFr 3)* significantly increased ($p<0.05$) by day 3 compared with parasitized untreated (PUn) and parasitized chloroquine treated (*PCq*) groups. By day 9 of the experiment, the myeloid –erythroid ratio of *PCFr1* and *PCFr* 3 treated groups significantly decreased ($p<0.05$) compared with *PUn* and *PCq* groups.

Histology of bone marrow smear in parasitized animals treated with *Chrysophyllum albidum* and its' fractions.

Plate 1 shows the histology of the bone marrow of *PUn*, *PCFr1* and *PCq* treated groups. The bone marrow showed more erythroid series than myeloid in *PCFr* 1 compared with *PUn* and *PCq* which showed presence of malaria parasites.

Myeloid-Erythroid ratio of animals bled and treated with *Chrysophyllum albidum*.

The effect of methanolic extract of *Chrysophyllum albidum bark (MeCaB)* on myeloid-erythroid ratio in bled animals by day 7 is shown in Figure 2. The myeloid-erythroid ratio of *MeCaB* and bled mice treated with haematinic (*BMeCaB)* significantly decreased compared with bled untreated (*Bla)* and bled mice treated with haematinic (*BHaem).*

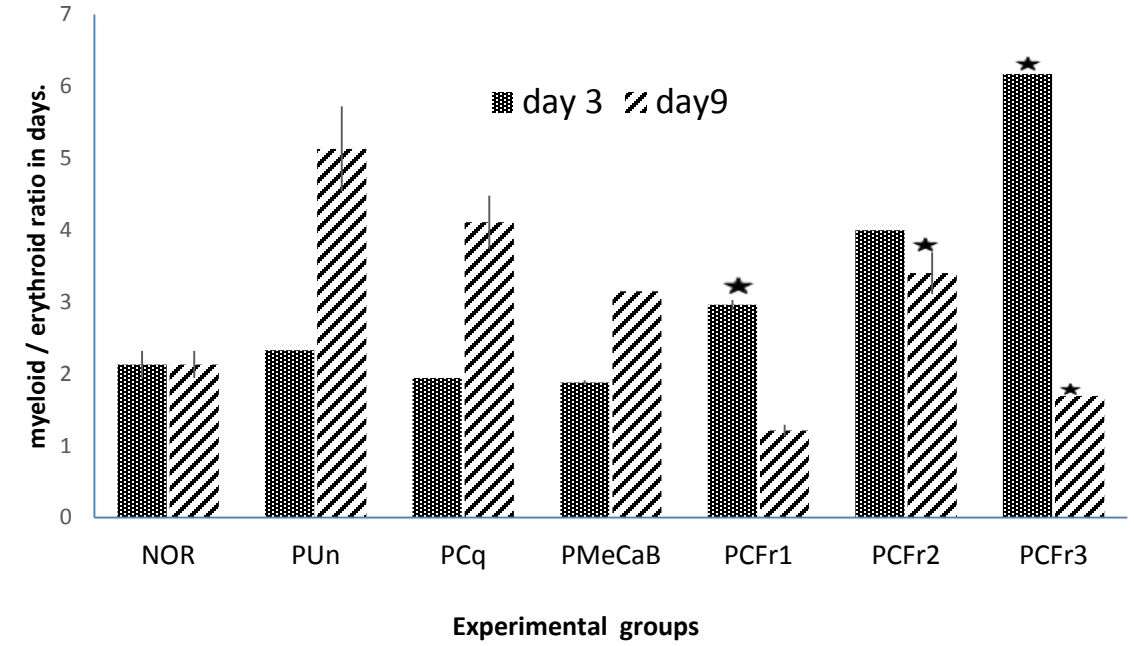

Figure 1:
Myeloid-Erythroid ratio for parasitised groups treated with *C.albidum* extract *and its' fractions (PCFr 1, PCFr 2 and PCFr 3)*

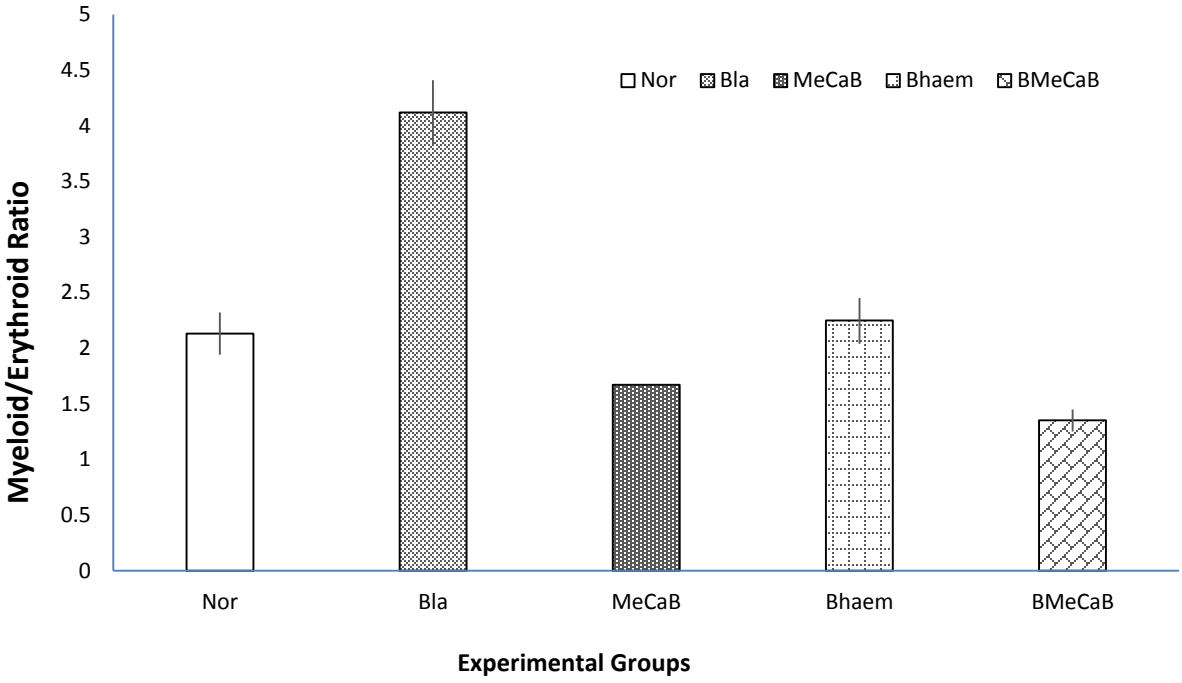

Figure 2:
Myeloid-Erythroid ratio for animals bled but treated with *C.albidum* extract (*MeCaB*).

Histology of bone marrow smear from animals bled and treated with *Chrysophyllum albidum.*

Plate 2 is the histology of bone marrow smear from normal mice, bled mice treated with haematinic (*BHaem*) and bled mice treated with methanolic extract of *Chrysophyllum albidum* bark (*BMeCaB*). The *Bhaem* and *BMeCaB* groups revealed presence of more erythroid series compared with the control group.

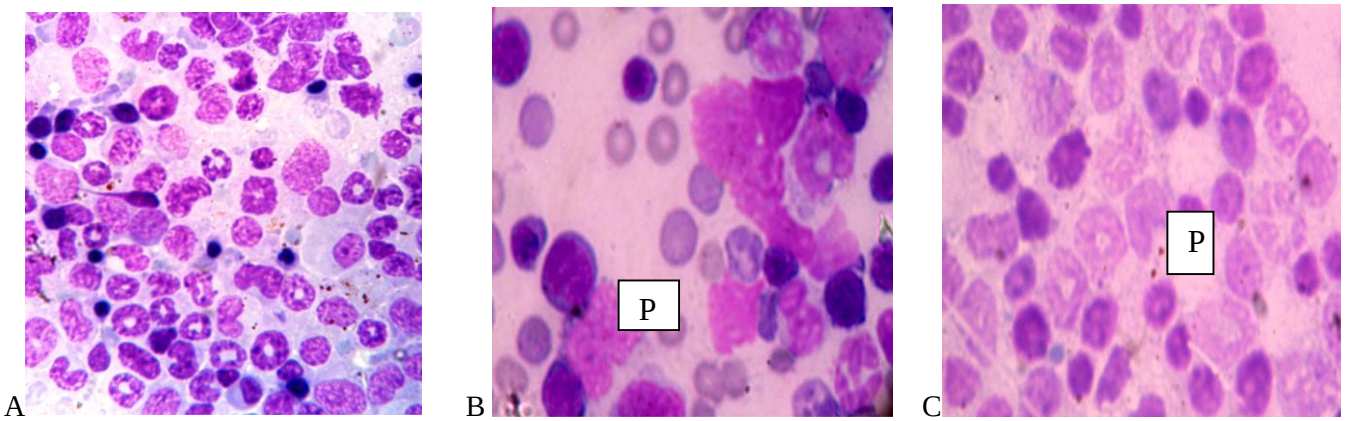

Plate 1

Plate 1 showing the bone marrow of parasitized mice untreated and treated with PCFr1 and chloroquine**. A.** Parasitised group treated with fraction 1 of C. *albidum (PCFr 1)* bark extract. (Mag x 400 H&E). The erythroid cells(E) series are more than the myeloid cells (M). **B**. Parasitised untreated group, (PUn). (Mag x 1000 H&E). Note the presence of malaria parasite (P). **C.** Parasitized rats treated with chloroquine (Mag x 1000 H&E). Note the presence of malaria parasite (P).

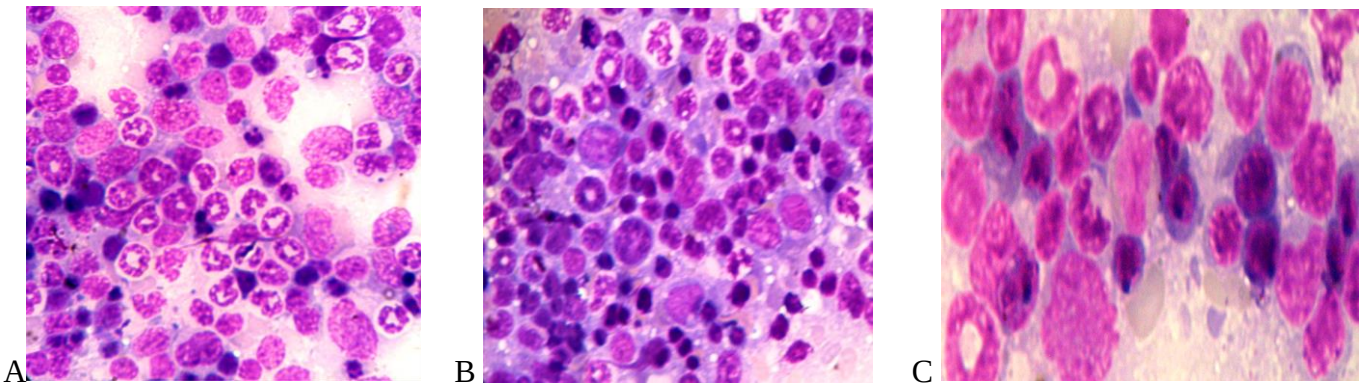

Plate 2

Plate 2 showing bone marrow of normal mice and mice bled but treated with haematinics and *C.albidum* extract**. A** Normal picture of the cells in the bone marrow of a mice (Mag x 400 H&E) **B**. Group bled and treated with haematinic (*Bhaem*). (Mag x 400 H&E)**.**Note the presence of more erythroid series (E and R) compared with myeloid series (M). **C.** Group bled treated with MeCaB (BMeCaB). (Mag x 1000), H&E.Note the presence of more erythroid series (E) than myeloid series (M).

DISCUSSION

This present study investigated the effects of methanolic extract of *C. albidum* stem bark on the bone marrow. Maggio-Price *et al,* 1985 reported the presence of granular leukocytes with or without little erythroblasts 1 to 3 days post plasmodium infection in mice. By day 5, immature erythroblasts were seen and by day 10, there was intense erythropoiesis and granulopoiesis in ratio 1:1. The anemic conditions observed in these studies might be the result of depletion of murine marrow BFU-E occuring in response to haemolysis or blood loss anaemia (Adamson *et al,* 1978, Hara and Ogawa 1977 and 1976).

Figures 1 and 2 show the significant difference in myeloid-erythroid ratio of *MeCaB, BMeCaB, PCFr1 and 3* compared with other groups (*PUn, PCq and BHaem*) by day 7 of the experiment. The increased granulopoiesis observed in bone marrow of *PCFr 2,3* treated and *PUn* between days 1-3 of treatment is similar to the reports of Maggio Price *et al*, 1985. Earlier studies (Adewoye *et al,* 2010, Jubbs *et al,* 1996) reported leucocytosis in infected mice as an indication of enhanced granulopoiesis and lymphocytosis as cellular and humoral responses respectively to the protozoan infection.

On treatment with *MeCaB* for 3 consecutive days and subsequent withdrawal of treatment between days 4 to 7 led to an increase in erythropoiesis in *PCFr1 a*nd *3*

treated groups compared with *PUn* and *PCq* (Figure 1, Plate 1). The ratio 1:1 reported by Maggio Price *et al*, 1985 was however not the same in this study. The ratio shifted from 1:1 to 1:2. after treatment. It is most likely that *PCFr 1* and *3* exhibited prolonged effects on the bone marrow thereby producing more erythroid series than *MeCaB.* This shift in ratio, which is a result of more erythroid series being produced in the bone marrow could be responsible for the resistance to developing anaemia in parasitized and extract treated animals (Adewoye *et al*, 2010) compared to chloroquine treated animals.

Adewoye *et al*, 2012 reported a significant haematinic (increased Red blood cell count, haemoglobin concentration and packed cell volume) and anti – inflammatory potential (a significant decrease in Neutrophil/Lymphocyte ratio) in the groups treated with this plant extract. Similarly, some plants have been reported to possess erythropoietic properties: Udut *et al*, 2005, reported the erythropoiesis stimulating effect of *Scutellaria baicalensis* extract through the activation of erythropoiesis precursor cells in the bone marrow of mice. George A Koffuor *et al*, 2012, reported the haematinic properties of ethanol root bark extract of *Carissa edulis* in phenylhydrazine-induced anaemic Sprague Dawley rats. Aimola *et al*, 2013, also reported the erythropoietic ability of *Terminalia catappa* extract in which it stimulated normal erythroid differentiation in phenylhydrazine-induced anaemic mice.

In this study, increased production of erythroid series observed in the extract treated groups have shown a possible mechanism by which *C. albidum* exerts its haematinic property. It may well be that one or more of the active ingredients (flavonoids, tannins, alkaloids and saponins) earlier reported by Adewoye *et al*, 2011 stimulated erythropoietic process (i.e. hyperplasia) during anaemia. Increase in the erythroid series observed in the extract treated groups corroborate the findings of Adewoye *et al*, 2010 where haematological values remained within normal range even with parasite infection when treated with the extract.

In conclusion, Erythrocyte hyperplasia (production of more erythroid cells) observed in this study, exhibited in the bone marrow of mice treated with methanolic extract of *Chrysophyllum albidum* bark (*MeCaB), mice bled and treated with methanolic extract of Chrysophyllum albidum bark (BMeCaB), mice parasitized and treated with Chrysophyllum albidum fractions 1 and 3 (PCFr 1 and 3)* could most likely be the mechanism by which the plant reverted or prevented anaemia here and also in previous studies. These observations confirms the potential of the methanolic extract of *Chrysophyllum albidum* stem bark as a possible source for drug development

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