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Afr. J. Biomed. Res. Vol.15(May 2012);85 - 91

Research Article

Effect of Methanolic Extract of *Chrysophyllum albidum* Bark on Hematological Indices in Mice with Experimental Hemorrhagic Anemia

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ABSTRACT: *Chrysophyllum albidum* is a common plant in the tropical Central, East and West Africa region. Its fruits are widely consumed and plant parts are vastly utilized. The broad utilization and consumption of this plant parts make it pertinent to examine its effect on blood parameters. This study was therefore designed to investigate the effect of methanolic extract of *Chrysophyllum albidum* on haematological indices in mice. Seventy five Swiss albino male mice (19g – 22g) were used for this study. 45 of which were anaesthetized with diethyl ether and 0.25mls (40%) of their blood drained through the retro-orbital plexus. Haemoglobin concentration (*Hb*) was determined 24hours after. Animals with *Hb* of <11.12 g/dl were considered anaemic and randomly divided into five groups of fifteen animals. Group 1 - control animals (*Nor*), Group 2 - animals treated with 1000mg/kg b.w of *Chrysophyllum albidum* alone (*MeCaB*), Group 3- animals bled and treated with ferrous sulphate (*BHaem*), Group 4 - animals bled and treated with *MeCaB* (*BMeCaB*) and Group 5 animals were bled and untreated (*Bla*). Complete blood count was done using standard methods on days 3 and 7. The body weight of *BMeCaB* group showed significant ($p < 0.05$) increase compared with control and *Bla*. The *BMeCaB* group showed significant increase ($p < 0.05$) in RBC and Packed Cell Volume within 48 hours of treatment and up to day 7 compared with *Bla* and *BHaem*. There was significant decrease ($p < 0.05$) in the Neutrophil lymphocyte ratio of *BMeCaB* and *MeCaB* compared with *Bla*. This study established the haematinic potentials of *Chrysophyllum albidum*

Keywords: *Chrysophyllum albidum*, blood cells, anemia, Mice.

INTRODUCTION

Anaemia, a haemolytic condition characterised by insufficiency in quality and quantity of the red blood cell is common in the tropical countries. Children are more vulnerable with a higher prevalence of 30% to 40% in Africa. It occurs also in nursing and pregnant women making it a dreaded disease among the population (Gentilini 1993).

Anaemia may be the result of malnutrition, that is, it could be a result of deficiency of iron, zinc, selenium, copper, folic acid and vitamins; it could also occur as a result of impaired immune responses which could lead to infection and bactericidal activity of macrophages, monocytes and neutrophils, obstetrical complications resulting in abnormal blood loss or inherited disorders such as haemoglobinopathies or glucose 6 – phosphate dehydrogenase deficiency (Cheesbrough 2002, Ekiz *et al* 2005). Anaemia constitutes a serious health problem in many tropical countries because of the prevalence of malaria and other parasitic infections (Dacie and Lewis 1994; Diallo *et al* 2008). Anaemia is associated with hypoxia, ischemia, increased morbidity and mortality while ischemia associated with anaemia may alter leukocyte count. White blood cell (WBC) count is reported to be an independent risk factor for coronary heart disease (CHD), stroke, vascular diseases, and total morbidity and mortality (Gokula *et al* 2009,

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Received: February 2012; Accepted: April 2012

Jaremo 2007). Recently, neutrophil : lymphocyte ratio (NLR) has emerged as a useful inflammatory index in critically ill patients and ischemic heart diseases (IHDs) (Chia *et al* 2009).

Medicinal plants contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. A good number of medicinal plants are traditionally employed to alleviate anaemia. Some of these plants include *Telfeira occidentalis*, *Combretum dolichopetalum*, *Psorospermum ferbrifugum*, *Jatropha curcas*, *Flacourtia flavescens* and *Brillantasia nitens* (Dina *et al* 2006, Alada 2000).

The *Chrysophyllum albidum* G.Don_Holl.(Sapotaceae) is primarily a forest tree species common throughout the tropical Central, East and West Africa regions. Its sweet edible fruits and various ethno-medical uses are widely known (Amusa *et al*). *Chrysophyllum albidum* fruits known as African star apple and 'Agbalumo' in Yoruba land are widely eaten in southern Nigeria (Bada *et al* 1997). The plant has in recent times become a crop of commercial value in Nigeria. The fruit has been found to have the highest content of ascorbic acid per 100g of edible fruit or about 100 times that of orange and 10 times that of guava or cashew. It has also been reported to be an excellent source of vitamins, iron and food flavours (Adisa *et al* 2000).

The stem bark is used as remedy for yellow fever and malaria, while the leaves are used as emollients and for the treatment of skin eruptions, diarrhoea and stomach-ache (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). Methanol extract of *C.albidum* stem bark has been found to have antiplasmodial (Adewoye *et al* 2010) and antimicrobial activities (Adewoye *et al* 2011). In spite of the rich component of the fruits and vast local use of *Chrysophyllum albidum* plant parts, there is dearth of information on its effect on blood parameters. This study was designed to investigate the effect of methanolic extract of *C. albidum* bark on haematological indices in Swiss albino mice.

MATERIALS AND METHODS

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

Plant materials, collection and identification: The fresh bark of *C. albidum* was collected from its natural habitat at Igbo Owe cash crop farm at Moniya,

Akinyele Local Government Area of Oyo State, South-western Nigeria between the months of November and April 2007. The plant was identified and given a voucher number FHI 107514 at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The plant materials were dusted and dried at room temperature for 3 weeks and then ground to powder using a dry electric mill (Moulineux, UK).

Preparation of Plant extract.: The powdered bark (1.5kg) of *C. albidum* was soaked in 2.5 litres of 70% n – hexane for 72 hours and the filtrate was collected as hexane fraction. The residue was spread out evenly and allowed to dry for a day. To this residue, 2.5 litres of 70% dichloromethane was added and was well macerated for 72 hours after which the filtrate dichloromethane fraction was decanted and the residue properly air dried for a day. A 2.5 litres of 70% methanol was added to the residue for 72 hours and stirred for about 20 minutes daily. The whole mixture was filtered with Whatman's filter paper (No. 1) and labelled methanol fraction. All the 3 different filtrates were evaporated to dryness *in-vacuo* and were stored at 4°C until use.

Previous studies (Adewoye *et al* 2010; Adewoye *et al* 2011) revealed that the methanolic extract of *C.albidum* at 1000mg/kg b.w. exhibited the most potent activity as an antimalarial drug.

Phytochemical screening

Standard screening tests for the extract were carried out for various constituents like alkaloids, saponins, tannins, flavonoids, glycosides and volatile oils using standard procedures (Harbone 1998; Trease and Evans 2002).

Animals

Seventy-five (75) Swiss albino male mice weighing between 19 - 22 g were used in this study. The mice were obtained from the Animal House of the College of Medicine, University of Ibadan, Nigeria. They were maintained under standard conditions (12 h light and 12 h dark) and had free access to mice chow and clean water. The animals were divided into five groups of fifteen animals each. All treatments were administered orally. Group 1 (control) consists of normal mice that were not bled and did not receive *C albidum* extract. Group 2 (*MeCaB*) consists of mice not bled but received methanolic extract of *C.albidum* (1000mg/kg b.w). Group 3 (*BHaem*) animals were bled (0.25mls) and administered ferrous sulphate (500mg/kg). Group 4 (*BMeCaB*) animals were bled (0.25mls) and administered *MeCaB* (1000mg/kg). Group 5 (*Bla*) animals were bled (0.25mls) and untreated.

Analysis of Haematological Parameters

Each mouse was sedated by ether suffocation and blood was collected through ocular puncture on days 3 and 7 into heparinised tubes for haematological studies. Parameter studied are packed cell volume (PCV), red blood cell (RBC) counts, haemoglobin (Hb) concentration, total white blood cell (WBC) counts (total and differential counts), reticulocytes and platelets counts according to the methods described by Dacie and Lewis 1994. Red blood cell indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated (Jain 1986).

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

The effect of the methanolic extract of *Chrysophyllum albidum* on body weight of bled animals by days 3 and 7 is shown in Table 1. The body weights of all bled mice were significantly decreased 24 hours post bleeding. Weight of mice in *MeCaB* and *BMeCaB* groups increased significantly ($p < 0.05$) by days 3 and 7 compared with *Bla* group.

All bled animals showed significant decrease in body weight, RBC, Hb, PCV and reticulocyte count 24 hours post bleeding compared with control (Table 2). The WBC, platelet and lymphocyte counts of bled animals showed significant increase ($p < 0.05$) within 24 hours post bleeding compared with control (Table 2).

The results of the administration of methanolic bark extract of *Chrysophyllum albidum* on red blood cell count, haemoglobin concentration and packed cell volume on bled animals by days 3 and 7 is shown in Table 3. By day 3, the RBC count, Hb and PCV of the extract-treated bled animals were significantly higher than those of the untreated, bled group ($p < 0.05$).

Table 1:

Effect of methanolic extract of *Chrysophyllum albidum* on body weight of bled animals by days 3 and 7.

Groups	Body Weight (g)	
	Day 3	Day 7
Nor	20.98 ± 1.35	21.38 ± 1.33
MeCaB	23.14 ± 0.34*	23.72 ± 0.44*
BHaem	20.21 ± 0.25	20.55 ± 1.22
BMeCaB	21.20 ± 0.83	21.64 ± 0.79
Bla	19.43 ± 0.35*	20.46 ± 0.65

Values are mean ± S.E.M.; * $p < 0.05$, n= 15.

Nor – Normal Control animals,

MeCaB – animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum*,

BHaem – animals bled and administered with ferrous sulphate,

BMeCaB – animals bled and administered with 1000mg/kg of methanol extract of *C.albidum*,

Bla – animals bled untreated.

By day 3 of treatment with ferrous sulphate, the *BHaem* group showed significant decrease ($p < 0.05$) in RBC count, Hb and PCV compared with *Bla* group. By day 7 of treatment with the extract, *MeCaB* and *BMeCaB* groups showed no significant difference in RBC count, Hb and PCV compared with *Bla*. *BHaem* treated group also did not show any significant difference in RBC counts, Hb and PCV by day 7 when compared with *Bla*.

Table 2: -

Profile of Haematological parameters of bled animals (24 hours after bleeding).

Groups	RBC ($10^6 \mu\text{L}$)	Hb (gdL^1)	PCV (%).	Retic (%).	Platelet Count (mm^3).	White Blood Cells (mm^3)			MCV (fl).	MCHC (g/dl).	MCH (pg).
						TLC	L	N			
Normal animals	7.27 ± 0.06	13.29 ± 0.22	43.60 ± 0.40	1.20 ± 0.20	114600 ± 9097.25	8770 ± 2301.12	71.40 ± 3.01	20.80 ± 1.74	59.91 ± 0.47	30.89 ± 0.32	19.13 ± 0.20
Bled animals	5.84 ± 0.17*	11.12 ± 1.05*	36.00 ± 0.45*	1.00 ± 0.07	141000 ± 9066*	20360 ± 11152*	76.00 ± 0.63*	19.20 ± 0.74	61.64 ± 2.65*	32.42 ± 0.11	19.04 ± 0.29

Values are mean ± S.E.M. * $p < 0.05$, n=5

TLC = Total Leucocyte Count; L = Lymphocyte; N = Neutrophil

Table 3: -

Effect of *MeCaB* administration on Red Blood Cell Count, Haemoglobin Concentration and Packed Cell Volume of bled animals by day 3 and day 7.

Groups	RBC($\times 10^6 \mu\text{L}^{-1}$)		Hb(g dL^{-1})		PCV %	
	Day 3	Day 7	Day3	Day 7	Day 3	Day 7
Nor	7.27 \pm 0.06	6.91 \pm 0.13	13.92 \pm 0.22	13.50 \pm 0.33	43.60 \pm 0.40	42.4 \pm 1.12
MeCaB	7.16 \pm 0.19	7.04 \pm 0.26	13.64 \pm 0.29	14.02 \pm 0.87	43.20 \pm 0.96	43.00 \pm 2.17
BHaem	5.69 \pm 0.49*	6.67 \pm 0.14	11.98 \pm 1.18*	12.56 \pm 0.44	37.60 \pm 3.44*	39.20 \pm 0.49
BMeCaB	6.68 \pm 0.18	6.99 \pm 0.85	12.68 \pm 0.34	13.72 \pm 0.27	40.60 \pm 1.08	42.20 \pm 1.07
Bla	6.26 \pm 0.26*	6.26 \pm 0.26	11.67 \pm 0.05*	11.94 \pm 0.49*	38.2 \pm 1.43*	35.00 \pm 3.08*

Values are mean \pm S.E.M.; * $p < 0.05$, $n=15$

Table 4:

Effect of *MeCaB* administration on Reticulocyte and Platelet count of bled animals on day 3 and 7

Groups	Reticulocyte (%)		Platelets (mm^3)	
	Day 3	Day 7	Day3	Day 7
Nor	1.20 \pm 0.20	1.40 \pm 0.25	114600.00 \pm 9097.25	105400.00 \pm 7089.43
MeCaB	1.60 \pm 0.04	1.60 \pm 0.25	123400.00 \pm 16868.91	96800.00 \pm 10136.07
BHaem	2.00 \pm 0.32	2.20 \pm 0.37	133400.00 \pm 10988.18	92400.00 \pm 5473.57
BMeCaB	2.20 \pm 0.37*	2.20 \pm 0.49	121800.00 \pm 8003.75	95600.00 \pm 4945.71
Bla	1.60 \pm 0.25	2.20 \pm 0.37	82400.00 \pm 4445.22*	82600.00 \pm 9553.01

Values are mean \pm S.E.M.; * $p < 0.05$, $n=15$

Nor – Normal Control animals, *MeCaB* – animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum*, *BHaem* – animals bled and administered with ferrous sulphate, *BMeCaB* – animals bled and administered with 1000mg/kg of methanol extract of *C.albidum*, *Bla* – animals bled untreated.

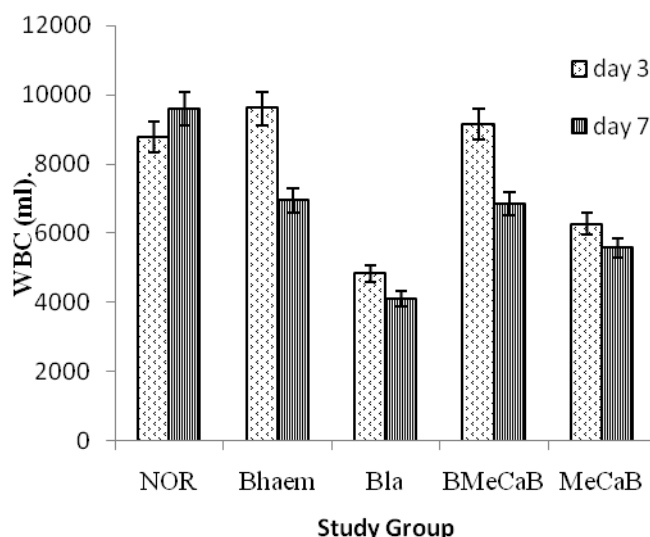


Figure 1:

Total White Cell Count of animals bled and treated with oral administration of 1000mg/kg b.w of methanol extract of *Chrysophyllum albidum*, (*MeCaB*).

Values are mean \pm S.E.M.; * $p < 0.05$, $n=15$

Total White Blood Cell Count in *BMeCaB* and *MeCaB* groups increased significantly by days 3 and 7 (Figure 1) while a significant decrease was observed in NLR of the same groups by day 3 (Figure2) compared with *Bla*.

The results of *methanol extract of Chrysophyllum albidum* on Reticulocyte and Platelet count of bled animals by days 3 and 7 are shown in Table 4. In all the animals bled, there was significant decrease in the reticulocyte and platelet count 24 hours post bleeding. In *BMeCaB* group, there was significant increase in reticulocyte count by day 3 compared with *Bla* group. There was an increase in the platelet count of *BMeCaB* and *MeCaB* by day 7 of treatment compared with *Bla* group. By day 3 and 7 post bleeding, *Bla* showed no significant difference in the reticulocyte count compared with control but there was a significant decrease in the platelet count compared with control.

Table 5:-

Effect of *MeCaB* administration on Mean corpuscular Volume (MCV) Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin on bled animals on day 3 and 7.

GROUP	MCV (fl)		MCHC (g/dL)		MCH (pg)	
	DAY 3	DAY 7	DAY 3	DAY 7	DAY 3	DAY 7
Nor	59.91 ± 0.47	61.34 ± 0.86	31.92 ± 0.32	31.85 ± 0.21	19.13 ± 0.20	19.54 ± 0.32
MeCaB	60.36 ± 0.92	61.68 ± 4.93	31.58 ± 0.15	32.53 ± 0.42	19.07 ± 0.35	20.13 ± 1.81
BHaem	60.76 ± 0.30	60.27 ± 0.94	31.23 ± 0.26	32.53 ± 0.31	18.98 ± 0.17	19.60 ± 0.25
BMeCaB	60.71 ± 4.96	58.74 ± 0.48	31.76 ± 0.29	32.01 ± 0.77	21.23 ± 1.69	18.79 ± 0.34
Bla	61.07 ± 0.51	59.33 ± 10.97	30.55 ± 0.29	31.73 ± 0.40	17.68 ± 0.28*	18.96 ± 3.75

Values are mean ± S.E.M.; * p< 0.05, n=15

Nor – Normal Control animals, **MeCaB** – animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum*, **BHaem** – animals bled and administered with ferrous sulphate, **BMeCaB** – animals bled and administered with 1000mg/kg of methanol extract of *C.albidum*, **Bla** – animals bled untreated.

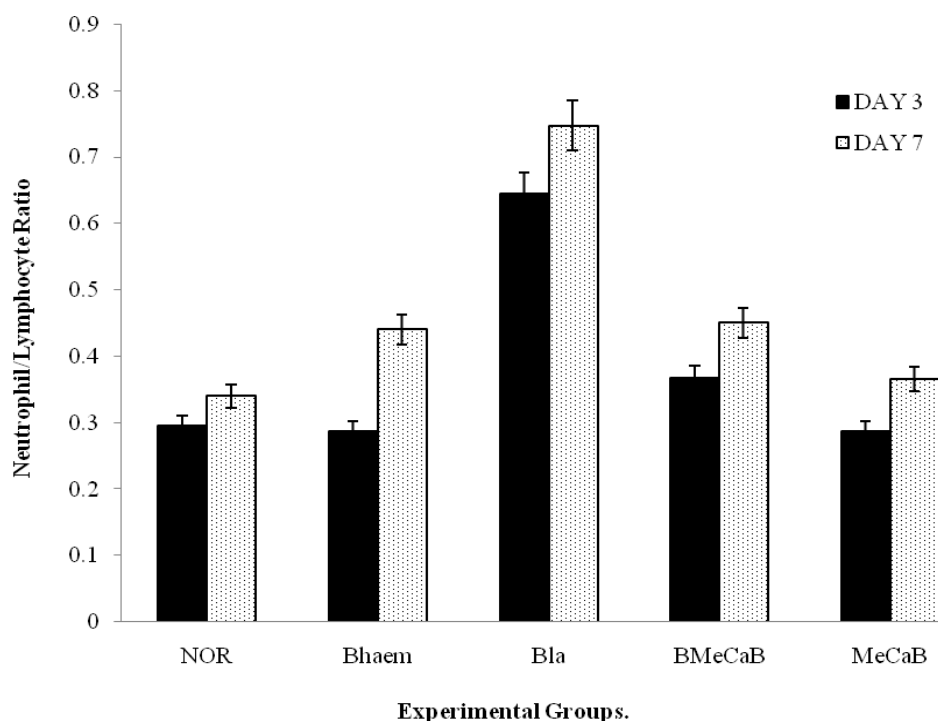


Figure 2

Neutrophil /Lymphocyte ratio (NLR) of animals bled and treated with oral administration of 1000mg/kg b.w of methanol extract of *Chrysophyllum albidum*, (*MeCaB*).

Values are mean ± S.E M.; * p< 0.05, n=15

Nor – Normal Control animals, **MeCaB** – animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum*, **BHaem** – animals bled and administered with ferrous sulphate, **BMeCaB** – animals bled and administered with 1000mg/kg of methanol extract of *C.albidum*, **Bla** – animals bled untreated.

Table 5 shows the effect of *MeCaB* administration on Mean corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) of bled

animals by days 3 and 7. There was no significant change in the MCV, MCHC and MCH of *BMeCaB* and *MeCaB* groups compared with *Bla* by days 3 and 7.

DISCUSSION

The increase in the body weight of mice treated with *C. albidum* could be as a result of tannins present in the extract. Recent study (Marcus *et al* 2003) revealed that tannins present in small quantities in medicinal plants stimulate increase in body mass. Another report (Xueging Liu *et al* 2005) suggests a decrease in body mass though not conclusive. Phytochemical analysis of *MeCaB* (Adewoye *et al* 2010; Adewoye *et al* 2011) revealed the presence of small quantities of tannins among other things and this could be responsible for the increased body weight observed in this study.

The significant increase observed in RBC, Hb and PCV within 3 days of treatment with *C.albidum* extract in the *MeCaB* and *BMeCaB* groups showed the possible haematinic potentials of this plant. Previous studies (Adewoye *et al* 2010; Adewoye *et al* 2011) showed that *MeCaB* contains tannins, flavonoids, saponins, alkaloids, anthraquinone and cardenolides. Some species of plants known to contain similar phytochemical constituents as *C.albidum* have been reported to possess anti-anaemic potential (Chia *et al* 2009, Dina *et al* 2006, Amusa *et al* 2003). It is likely that these phytochemical constituents might be responsible for the anti-anaemic properties observed in this study. The increase in the blood indices was progressive giving a notable effect on the seventh day of treatment. Under normal condition, the body generates new red blood cells (RBCs) to replace the lost red cells and this process takes a much longer time (Agbor and Odetola *et al* 2001). The quick attainment of normal RBC count could well be an indication of accelerated erythropoiesis occurring as a result of *C.albidum* treatment (Amusa *et al* 2003). This result suggests that *C.albidum* stem bark might have directly stimulated increased production of red blood cell precursors thereby reversing the anaemic condition (Chia *et al* 2009, Dina *et al* 2006).

Flavonoid has been suggested as a possible factor responsible for the increased erythrocyte count in Wistar rat (Esomonu *et al* 2005, Dhakar *et al* 2012). It might well be that the flavonoid content in *C.albidum* was responsible for the erythropoietic ability observed in this study.

The significant reduction in platelet count shows that *MeCaB* may possess the ability to potentiate thrombocytopenia. Anti-platelet agents have been utilized in the treatment of acute myocardial infarction, myocardial ischemia and or unstable angina while platelets have been reported to have major role in the stability of atherosclerotic plaques (George 2000). Since *MeCaB* showed anti platelet activity as indicated

by the low platelet count, *C. albidum* could be a plant source for the treatment of some heart complications.

Flavonoids have been reported to exhibit anti-inflammatory, anti-allergic effects, antibacterial, antithrombotic and tumour protective principles (Akaneme 2008). The antioxidant potentials have also been reported (Aruna 2001) as well as its ability to prevent platelet thrombosis (Harnafi and Amrani 2007, Roy *et al* 1999, Lilian *et al* 1999). The reduction in platelet count observed in this study could therefore be as a result of the flavonoid content of the plant.

Bla showed reduced leukocyte count by day 3 while a further significant reduction was observed in *BHaem* and *BMeCaB* groups by day 7 (Fig. 1). These observations suggest that *C.a.* might possess potentials that reduce leukocytes. Recently neutrophil / lymphocyte ratio (NLR) has been described as significant inflammatory index in Ischemic Heart Diseases (Chia *et al* 2009) and even in different types of cancers and stress related conditions. The NLR seems to be a superior index to WBC count, as it also explains the differential roles played by neutrophils and lymphocytes in different pathological conditions [9]. Decrease in T lymphocyte number, T lymphocyte blastogenesis and mitogenesis were also reported in iron deficiency anaemia (Ekiz *et al* 2005). The significant ($P<0.05$) reduction in NLR in *BMeCaB* and *MeCaB* compared with *Bla* (Fig.2) could be as a result of the anti-inflammatory potential present in *C.albidum*.

In conclusion, the extracts of *Chrysophyllum albidum* stem bark helped to reverse anaemia induced by bleeding out 40% (0.25mls) of the blood volume in the mice (Schalm *et al* 1975). It might also be possible that *Chrysophyllum albidum* has the ability to balance between the rate of destruction and production of blood cells as evident by the increased RBC count. The increased haematological parameters observed in anaemic mice treated with *Chrysophyllum albidum* could be a result of the flavonoid present in the plant while the tannin content might have been responsible for the increase in body weight of the animals. It may be concluded that *Chrysophyllum albidum* could be a good plant source for haematinics, antiplatelets and drug development.

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