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Full length Research Paper

Aqueous extract and methanol fractions of the leaves of *Brillantaisia nitens* Lindau. reverses phenylhydrazine – induced anaemia in rats

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Anaemia is a serious health problem especially in developing countries. In the tropics, rural dwellers had often resort to herbal treatments in some cases of anaemia. The essence of this work was to ascertain the rational for the use of the leaves of *Brillantaisia nitens* as a traditional hematinic. The aqueous extract (AE), methanol extract (ME), n-Hexane fraction (HF), chloroform fraction (CF) and methanol fraction (MF) of the leaves were tested for hematinic effects using phenylhydrazine (PHZ) - induced anaemia in rats. Blood parameters such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration (Hb) and haematocrit were assayed as indices of anaemia. Analysis of the vitamin and mineral contents of the ME and MF were carried out while phytochemical analysis of the extracts and fractions as well as the LD_{50} of the ME were determined. The results indicated that the oral administration of AE, ME, CF, HF and MF (400 - 3200 mg/kg/day) exhibited a significant ($P < 0.05$) hematinic activity by ameliorating PHZ - induced decrease in blood parameters viz, Hb, PCV, WBC and RBC. The hematinic potency was in the order of MF > ME > AE > CF > HF. Vitamin analysis showed that both ME and MF contain adequate amounts of water soluble vitamins (Vitamins B_6, B_{12}, C, and Folic acid), Vit. E and iron in varying concentrations. Phytochemical analysis of the extracts and fractions indicated high concentration of glycosides, alkaloids and resins while flavonoids, terpenoids, carbohydrates and saponins occurred in trace amounts. The oral LD_{50} value of the ME was greater than 5000 mg/kg, indicating the high safety profile of *B. nitens.* These results conclude the presence of hematinic principles in the leaves of *B. nitens*, thus justifying its use in the management of anemia especially in children.

Key words: *Brillantaisia nitens,* hematinic effects, phenylhydrazine, blood parameters, rats.

INTRODUCTION

Certain medical diseases, such as malaria, malnutrition, protozoa infections and pregnancy are among various conditions that may lead to anaemia in both adult and children. Anaemia is a reduction from the normal quantity of circulating hemoglobin in the blood less than 13 g/dl for male and less than 12 g/dl for female adults (Okochi et al., 2003). Epidemiological studies indicated that WHO estimates for the number of anaemic people globally for the year 2004 was 2 billion, representing 30% of the world's population (Adusi-Poku et al., 2008). Also over 50% of pregnant women and over 40% of infants worldwide are anaemic with a prevailing significant morbidity and mortality particularly in the developing world (Holden and Acomb, 2007). Hence anaemia is one of the leading health disorders posing a great threat to global healthcare. Medicinal plants are currently being used in various parts of the world especially in the tropics for the treatment of various forms of anaemia. In south eastern Nigeria, with high prevalence of both malaria

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induced and iron deficiency anaemia, aqueous decoctions of leaves of *Brillantaisia nitens* Lindau (Acanthaceae) is very popular among village women in combating malaria induced anaemia in children (Akah et al., 2009). *Brillantaisia nitens* Lindau (Acanthaceae) is an herbaceous shrub of about 1.5 m high found in central and West Africa (Mtopi et al., 2007). *B. nitens* have been reported to have antihypertensive (Adjanohoun et al., 1996; Mtopi et al., 2007), smooth muscle relaxant (Dimo, 2007) effects, as well as haematinic activity (Akah et al., 2009), while *B. palisolti*, another specie, has antinociceptive effects (Matheus et al., 2005).

MATERIALS AND METHODS

Plant material

Fresh leaves of *B. nitens* were collected in March, 2008 from Nsukka, Enugu State, Nigeria and were botanically identified by Mr. A. Ozioko of Bioresource Development and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria. The fresh leaves were air dried under shade for 7 days and pulverized into coarse powder. About 1 kg of the powdered leaves was extracted with 2.5 L of methanol by cold maceration for 48 h and filtered (Trease and Evans, 2002). The filtrate was dried in rotary evaporator to obtain the methanol extract (ME; 365.08 g; 36.51% w/w). The ME (200 g) was further fractionated, using chromatographic techniques, with the following solvents in the order of increasing polarity viz n-hexane, chloroform, and methanol. The yield afforded n-hexane (HF; 49.45 g; 24.7% w/w), chloroform (CF; 52.51 g; 26.2% w/w) and methanol (MF; 86.4 g; 43.2% w/w) fractions. Also 1 kg of the dried powdered leaves was extracted with distilled water by cold maceration for 48 hours (Trease and Evans, 2002). The filtrate was freeze dried to obtain the aqueous extract (AE, 104 g; 10.4%). The fractions (HF, CF and MF) and AE were subjected to phytochemical screening using standard methods (Harbone, 1988).

Animals

Adult albino rats of both sexes (110 - 200 g) bred in the Laboratory Animal Center of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, were used for the studies. The animals were maintained under standard laboratory conditions and had free access to standard pellets (Guinea Feeds, PLC, Nigeria) and water. On transfer to the work area, animals were allowed two weeks of acclimatization before the commencement of the experiments. All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub. No8. 5-23, Revised 1985).

Acute toxicity and lethality (LD_{50}) test

The oral acute toxicity and lethality test (LD_{50}) of the methanol extract (ME) was determined in mice as described by Lorke (1983).

Analysis of vitamin and mineral contents

Haematopoietic vitamins such as riboflavin (vitamin B_2), pyridoxine (vitamin B_6), cyanocobalamin (vitamin B_{12}), folic acid and iron contents of the ME and MF were analyzed using the methods described by Pearson (1976) and Onwuka (2005). The ascorbic acid or vitamin C content was determined by the method of Olokodana (2005).

Induction of anaemia

Anaemia was induced in rats by daily oral administration of phenylhydrazine (PHZ, 10 mg/kg) for 8 days while the control group received normal saline. Blood samples were collected from the rat by ocular puncture on the 8th day of treatment for haematological analysis. Rats that developed anaemia with hemoglobin concentration lower than 13 g/dl were recruited for the study (Yeshoda, 1942; Berger, 2007; Agbor et al., 2005).

Treatment of the animals

Stage 1

The anaemic rats were randomly divided into six groups (n = 6/group). Group I received the vehicle (10 ml/kg; Tween 20). Group II received Vit. B_{12} syrup (1 ml/kg; Campharm Pharmaceutical Ltd., Orlu, Nigeria) while groups III, IV, V and VI received 400, 800, 1600 and 3200 mg/kg of the aqueous extract (AE) respectively daily for 4 weeks.

Stage 2

Anaemic rats were randomly divided into five groups (n = 6/ group). Group I received the vehicle (10 ml/kg, Tween 20). Group II received Vit. B_{12} syrup (1 ml/kg; Campharm Pharmaceutical Ltd., Orlu, Nigeria). Rats in groups III, IV and V received 400 mg/kg of MF, CF and HF respectively daily for 4 weeks. All administrations were through the oral route.

Analysis of hematological parameters

Blood sample (0.25 - 0.45 ml) was collected from the animal by ocular puncture after overnight fast at the 1, 2, 3, and 4th week of treatment and the red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration (Hb) and haematocrit were assayed using an automatic counter (Sysmex K21, Tokyo, Japan), (Dacie and Lewis, 1991; Duello et al., 2008).

Statistical analysis

Data obtained were analyzed using One Way Analysis of Variance (ANOVA) (SPSS Version 14) software and expressed as Mean ± SEM. Differences between means were regarded significant at $P < 0.05$ (LSD post hoc test).

RESULTS

Phytochemical constituents of extract and fractions

Phytochemical analysis of the extract and fractions revealed the presence of carbohydrates, glycosides, flavonoids and saponins whereas reducing sugars, tannins and acidic components were absent (Table 1).

Acute toxicity and lethality (LD_{50}) test

Acute toxicity test showed that the extract was not lethal

Table 1. Phytochemical constituents of extract and fractions.

Constituent	AE	ME	HF	CF	MF
Alkaloids	+	++	-	++	+
Glycosides	+++	++	-	-	+++
Reducing sugar	-	-	-	-	-
Saponins	+	+	-	-	+
Tannins	-	-	-	-	-
Resins	-	+++	-	++	++
Flavonoids	-	+	+	-	-
Terpenoids	+	+	+	-	+
Steroids	-	+	+	-	+
Acidic components	-	-	-	-	-
Carbohydates	+	+	-	-	+

+++ = Conspicuously present; ++ = moderately present; + = present; - = absent.

to the animals at the doses used. Thus, the oral LD_{50} in mice was greater than 5,000 mg/kg.

Analysis of vitamin and mineral constituents

Analysis of the ME and MF revealed the presence of the vitamins; Vit.B_{12}, folic acid, Vit. E, Vit. B_6 and Vit. C, with mineral iron and Vit. B_{12} occurring in the highest concentration in both sample (Table 2).

Hematinic effects of aqueous extract (AE)

PHZ-induced anaemia in rats was reversed by AE after one week of administration with the blood indices reaching normal within the first two weeks of treatment. The result was dose- dependent up to two weeks of administration. There was a progressive increase in the concentration of the hemoglobin (Hb), red blood cells (RBCs) and packed cell volume (PCV) (Figures 1 - 3). The increament in the Hb, RBC and PCV was significant ($P > 0.05$) at the doses tested compared to the control group (Group 1). However the AE exhibited no appreciable further increase in the parameters above 1200 mg/kg dose which is the particular dose manifesting same degree of activity with Vit. B_{12} syrup (Figures 1 and 2).

Hematinic effects of the fractions

The effects of *B. nitens* fractions on the hematological parameters of PHZ-induced anaemic rats were shown in Figures 4, 5 and 6. The Hb, RBC and PCV returned to normal ranges after one week of treatment with methanol fraction while only Hb and PCV returned to normal after one week of treatment with chloroform fraction. The n-hexane fraction did not reverse the parameter after one week of treatment. The methanol fraction evoked a significant ($P < 0.05$) increase in the concentrations of the Hb, RBC and PCV while the n-hexane fraction indicated insignificant or poor activity. The chloroform fraction also showed a little significant increase higher than negative control in Hb, RBC, WBC and PCV in 2nd, 3rd and 4th weeks of treatment (Figures. 4, 5 and 6).

DISCUSSION

Phenylhydrazine (PHZ) has been documented to have the ability of inducing hemolytic anaemia in rats both when administered parenterally and orally by decreasing the concentrations of the blood parameters such as Hb, WBC, RBC and PCV (O'Riordan et al., 1995; Criswel et al., 2002). The methanol extract of *B. nitens* has been reported to exhibit significant hematinic effects in rats with PHZ-induced anaemia (Akah et al, 2009). However in this study, the aqueous extract (AE) and the various fractions were tested for hematinic effects. The AE and the methanol fraction (MF) significantly ($P < 0.05$) normalized the concentration of the blood parameters; Hb, PCV and RBC in PHZ treated rats. The MF is the most effective and active fraction among the fractions. The n-Hexane fraction (HF) and the chloroform fraction (CF) manifested no significant hematinic activity compared with the control. This is an indication that the active phytochemical constituents responsible for this activity should be very polar in nature as the greatest activity resides with the AE and MF. This is more so since the vitamin and mineral constituents were mainly water soluble vitamins which are mostly the B-complex vitamins and Vitamin C. This of course explains why the methanol fraction, which is more polar, could possess the highest hematinic effect when compared with other fractions. The result of the vitamin analysis showed that AE and MF contains a reasonable amount of Vit B_{12} and Folic acid and other mineral constituents necessary for erythropoiesis such as iron, Vitamin B_6 and Vitamin C (Table 2). Deficiency of Vit. B_{12} and folic acid in the erythropoiesis has been reported to cause macrocytic, megaloblastic and pernicious anaemia (Rang et al, 2007). It has been shown that folic acid is effective in relieving the symptoms in patients who have nutritional macrocytic anaemia, macrocytic anaemia of pellagra, megaloblastic anaemia of pregnancy, and megaloblastic anaemia of infancy (Chanarin et al., 2004; Spies, 1962).These hematinic agents may have contributed in the faster reversal of the PHZ hemolytic anaemia in the treated rats within the first two weeks of treatment which exhibited progressive recovery of the blood parameters (Figures 1 - 6). Iron, an essential constituent of the haem-moiety of hemoglobin, is also another hematinic agent found present in both the AE and MF in reasonable quantity. Deficiency of Iron in humans and animals often leads to iron deficiency anaemia. Iron deficiency has been reported to cause anaemia in children of 6 months to 2 years, (FAO/WHO, 1988), pregnant women and menstruating

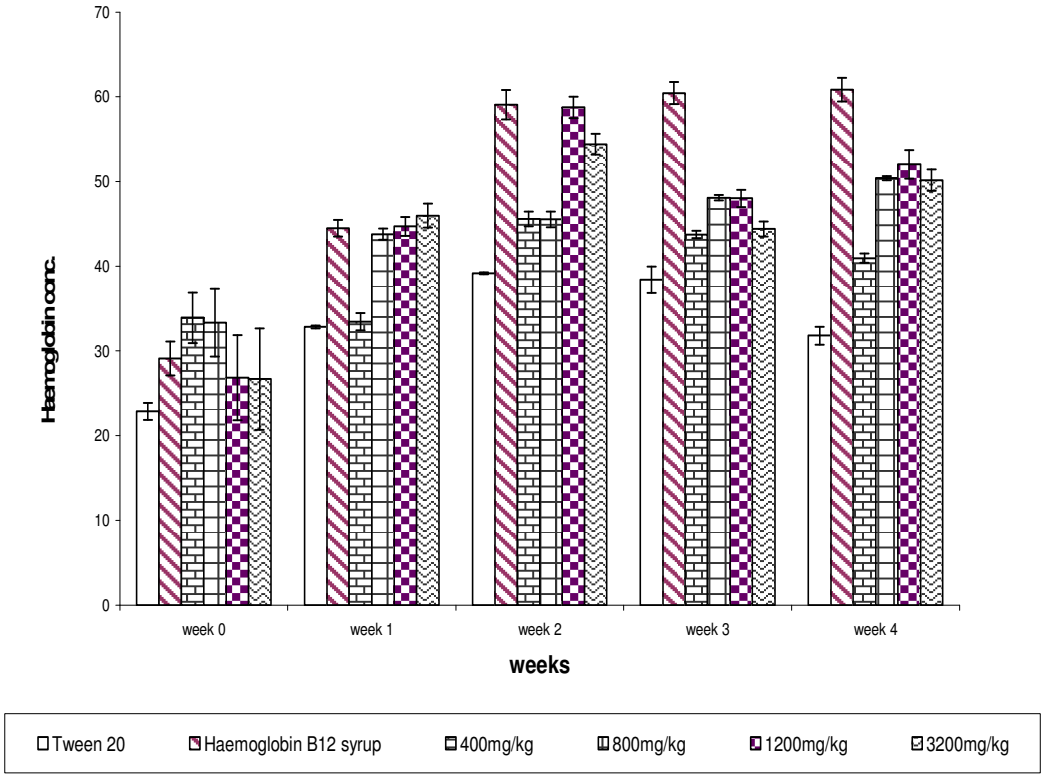

Figure 1. Effects of AE on hemoglobin concentrations of PHZ – induced anaemic rats. All values were signifcant ($P < 0.05$) vs. control from week 1 of treatment.

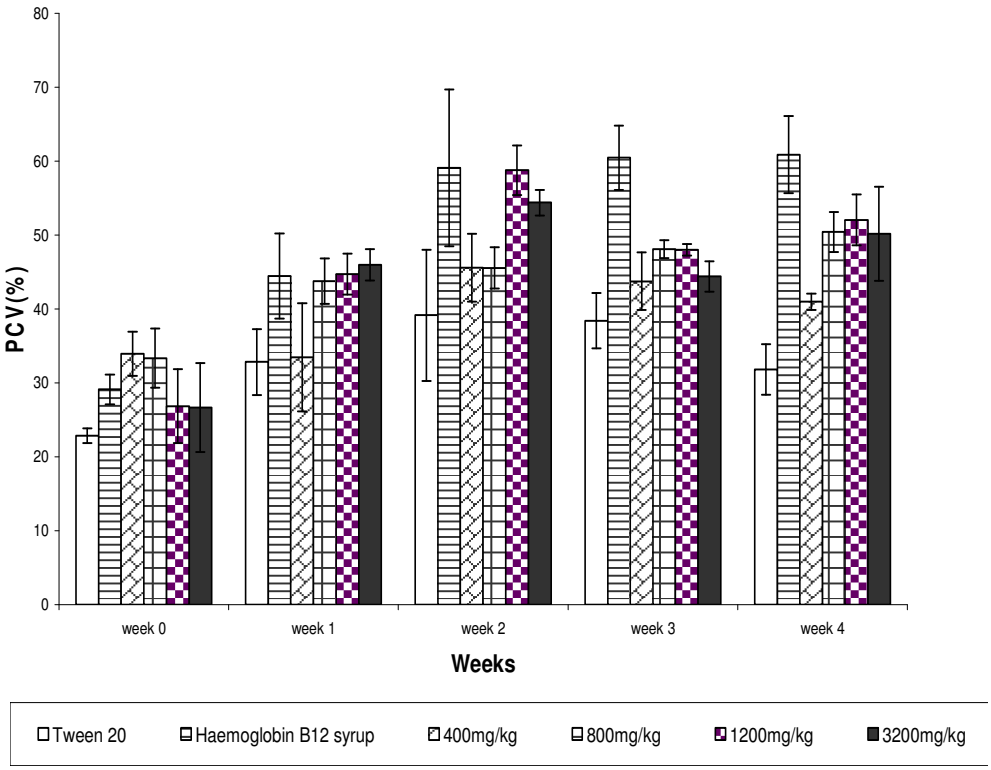

Figure 2. Effects of AE on PCV of PHZ - induced anaemic rats. Data were significant ($P < 0.05$) vs. control from week 1 of treatment.

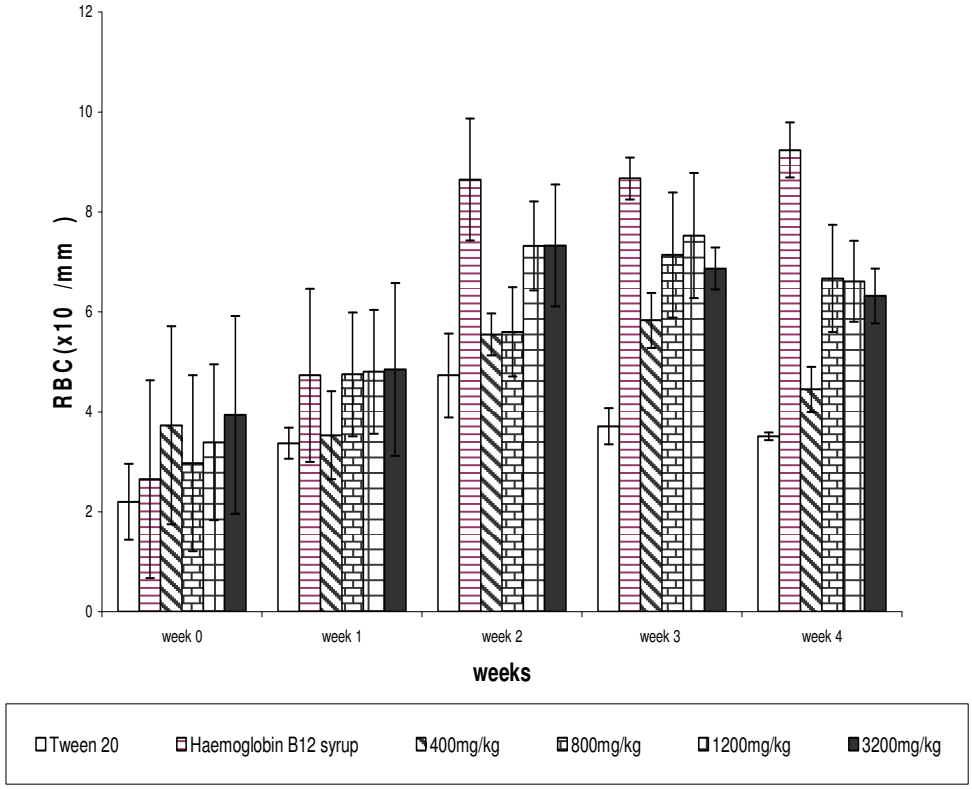

Figure 3. Effects of AE on RBC of PHZ – induced anaemic rats. Data were significant (P<0.05) vs. control from week 2 of treatment.

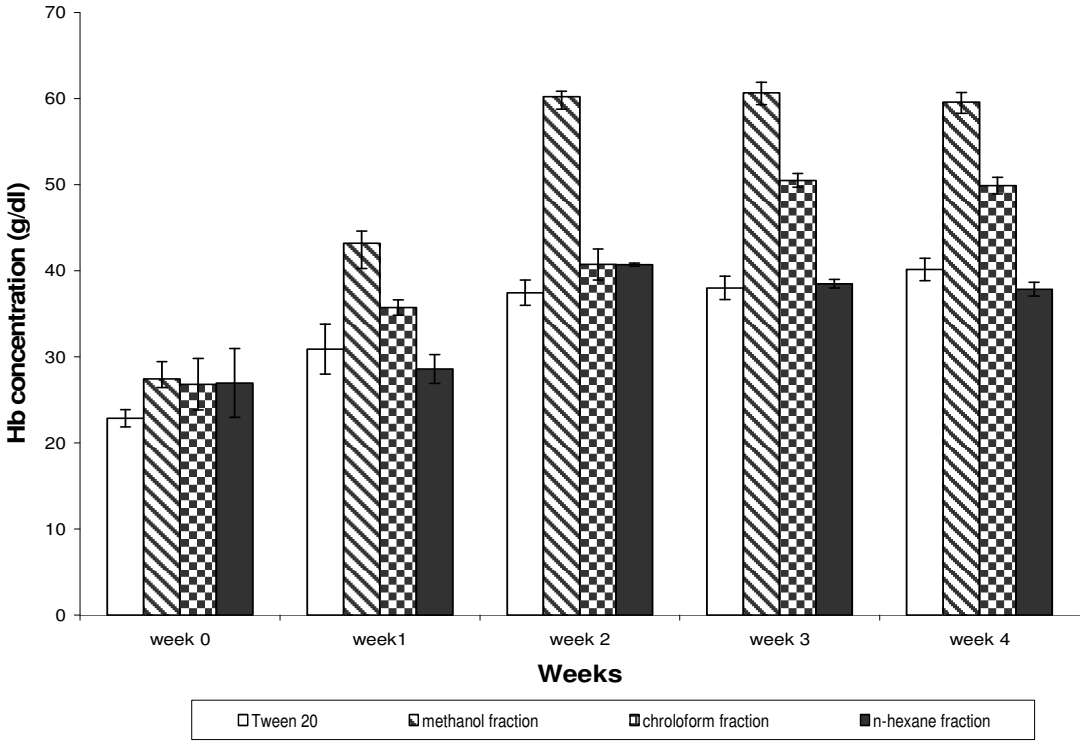

Figure 4. Effects of fractions on hemoglobin (Hb) concentrations of PHZ – induced anaemic rats. Methanol fraction (MF) produced significant effect (P < 0.05) vs. control from week1.

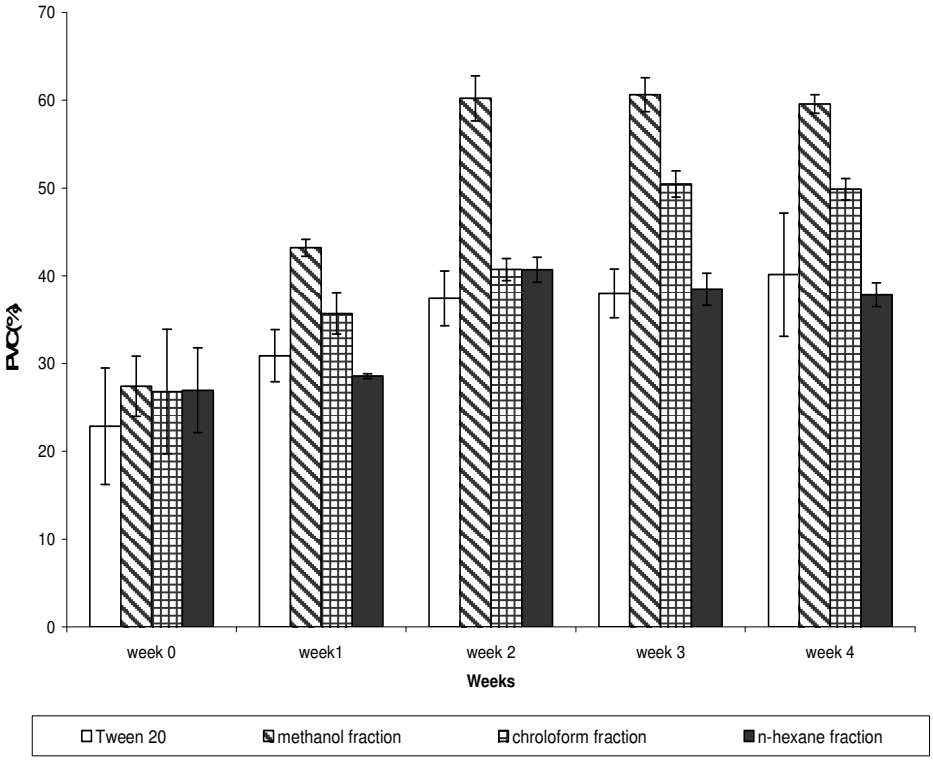

Figure 5. Effects of fractions on PCV of PHZ – induced anaemic rats. MF vs. control ($P < 0.05$) from week 1.

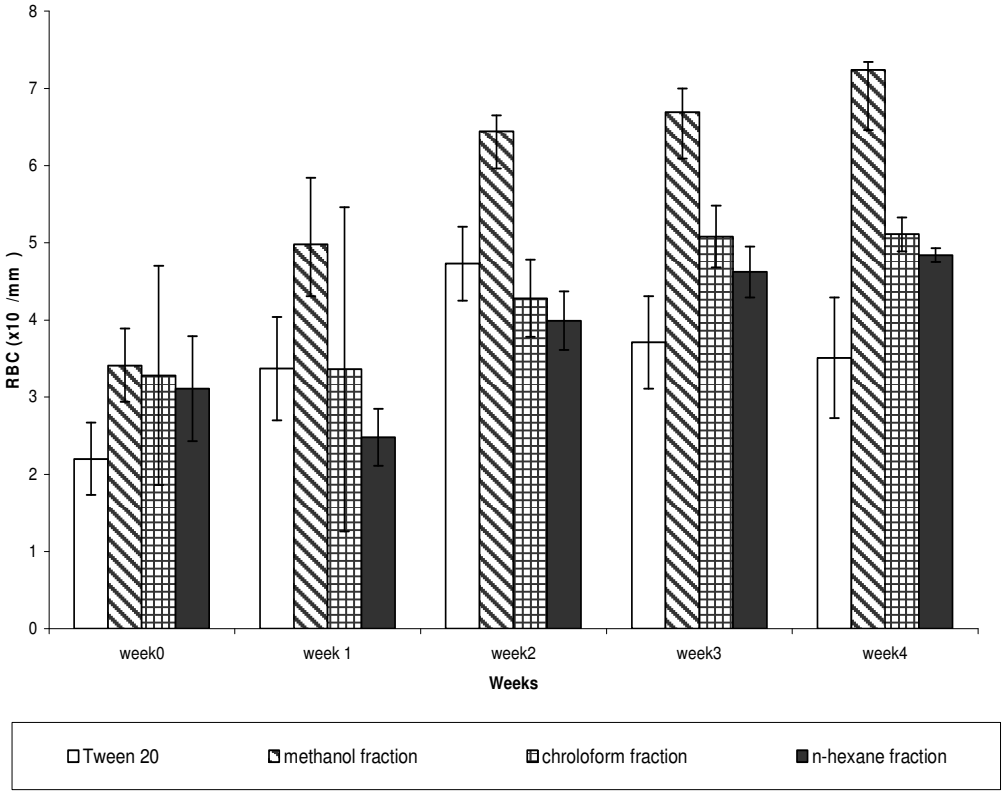

Figure 6. Effects of fractions on RBC of PHZ – induced anaemic rats. MF vs. control ($P < 0.05$) from week 1.

Table 2. Vitamin and mineral constituents of ME and MF.

Vitamin/Mineral	Concentration (g/100mg)	
	ME	MF
Vit. B_6	3.50	11.00
Vit. B_{12}	30.75	16.20
Vit. C	2.95	11.90
Folic acid	20.50	10.80
Iron.	14.00	9.32
Vit E	12.20	9.28

women (Frenchman and Johnston, 1949). Interestingly, many foods and plants are very rich in iron and can be absorbed easily. The increase in the hematological indices observed with the AE and the MF might not be unconnected with the vitamin, mineral and chemical composition of the leaves of *B. nitens.* The possible combined hematinic effects of Vit. $B_{12,}$ folic acid, iron as well as Vit. E constituents of the AE and MF should be taken to be the mechanism responsible for the haematinic activity of the AE and MF witnessed in the treated rats. The recovery was progressive and dose dependent with AE and MF after the first two weeks of continuous treatment while the blood indices were higher in the *B. nitens* treated rats than the control. However the result of the LD_{50} which was greater than 5 g/kg and as such very much safe for consumption may have been part of the reason the rural dwellers use the decoctions of *B. nitens* to treat and prevent anaemia.

In conclusion the extracts and fractions of *B. nitens* leaves reversed anaemia induced by phenylhydrazine in rats and this is in line with the traditional use of *B. nitens* in folkloric medicine.

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