

Full Length Research Paper

The haematinic activity of the methanol leaf extract of *Brillantasia nitens* Lindau (Acanthaceae) in rats

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Accepted 17 December, 2008

Ethnopharmacological information indicates that the leaves of *Brillantasia nitens* are used in the treatment of anaemia in the south eastern states of Nigeria. In this study, the methanol extract of the leaves of *B. nitens* was tested for haematinic activity in rats using phenylhydrazine (PHZ- 10 mg/kg. po)-induced anaemia. The red blood cell count (RBC), haemoglobin concentration (Hb), white blood cell count (WBC) and haematocrit (PCV) were analyzed as indices of anaemia. The phytochemical and mineral contents, as well as the acute toxicity (LD₅₀) of the extract were determined. Oral administration of *B. nitens* extract (400 - 3200 mg/kg/day) to rats previously treated with PHZ increased the Hb, RBC, WBC and PVC within one week. Phytochemical analysis showed the presence of alkaloids, glycosides, saponin, terpenoids, carbohydrates and resins. The extract also contained substantial amounts of vitamins B₆, C and E, as well as folic acid and iron. The LD₅₀ value of the extract was greater than 5000 mg/kg. These results lend credence to the traditional use of *B. nitens* leaves in the treatment of anaemia.

Key word: Haematinic activity, *Brillantasia nitens*, rats, anaemia, phenylhydrazine.

INTRODUCTION

Anaemia constitutes a serious health problem in many tropical countries because of the prevalence of malaria and other parasitic infections (Dacie and Lewis, 1994). In anaemia there is decreased level of circulating haemoglobin, less than 13 g/dl in male and 12 g/dl in females (Okochi et al., 2003). In the tropics, due to endemicity of malaria, between 10 to 20% of the population presents less than 10 g/dl of Hb (Diallo et al., 2008). Children are more vulnerable.

A good number of medicinal plants are traditionally employed to alleviate anaemia. Some of these plants include *Telfeiria occidentalis*, *Combretum dolichopetalum*, *Psorospermum ferbrifugum*, *Jatropha curcas*, *Flacourtia flavescens* and *Brillantasia nitens* (Alada, 2000; Dina et al., 2006). The leaves of *B. nitens* are commonly used as haematinic and are claimed to be very effective in the treatment of malaria-induced and other types of

anaemias. The *Brillantasia nitens* Lindau (Acanthaceae) is a herbaceous shrub of about 1.5 m high found in central and west Africa. It is widely used in African traditional medicine to treat skin infections and toothache. The root taken in soup is used in Southern Nigeria to reduce pain during pregnancy (Burkett, 1968). The decoction of *B. nitens* dried leaves has been administered orally to treat arterial hypertension in Central Province of Cameroon.

The methylene chloride/methanol leaf extract of *B. nitens* was reported to lower arterial blood pressure and heart rate of normotensive Wistar rats (Mtopi et al., 2007). The relaxant effect of *B. nitens* extracts on rat vascular smooth muscle has also been demonstrated (Dimo, 2007). Some other species have been shown to possess antinociceptive (Matheus et al., 2005) and antihypertensive effects (Adjanohoun et al., 1996). This present study was undertaken in order to evaluate the haematinic effect of the methanol extract of *B. nitens* leaves extract using phenylhydrazine-induced anaemia in rats.

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MATERIALS AND METHODS

Plant material

Fresh leaves of *B. nitens* were collected in October 2007 from Nsukka, Enugu State, Nigeria and were botanically identified by Mr. A. Ozioko of Bioresource Development and Conservation Programme (BDPC) Laboratory, Nsukka. The fresh leaves were air dried under shade for 7 days and milled into coarse powder using a manual blender. 1 kg of the powdered leaves was extracted with 2.5 L of methanol by cold maceration for 48 h (Trease and Evans, 2002). The filtrate was freeze-dried to obtain the methanol extract (ME, 125 g, 12.5% w/w).

Analysis of the phytochemical and mineral contents

The extract (ME) was subjected to phytochemical investigation using the methods described by Harbone (1988). Haematopoietic vitamins such as vitamin B₁₂, B₆, B₂ and folic acid were analysed as described by Pearson (1976) and Onwuka (2005). The vitamin C content was determined by the method of Olokodana (2005). Iron content was obtained by phenanthroline method of Pearson (1976).

Acute toxicity test

The acute toxicity and lethality test (LD₅₀) of the extract was determined in mice as described by Lorke (1983).

Induction of anaemia

Anaemia was induced in rats by daily oral administration of phenylhydrazine (PHZ) at 10 mg/kg for 8 days (Yeshoda et al., 1942; Berger, 1985). Rats that developed anaemia with haemoglobin concentration lower than 13 g/dl were recruited for the study (Agbor et al., 2005).

Treatment of the animals

The anaemic rats were randomly divided into six groups (5 rats per group) and treated daily for 4 weeks as follows (Dimo et al, 2007). The first group received Tween 20 (10 ml/kg) (negative control). The group 2 animals received Vit B₁₂ syrup (Campharm Pharmaceutical Ltd, Orlu, Nigeria) (1 ml/rat). Animals in groups 3, 4, 5 and 6 received the ME at 400, 800, 1600 and 3200 mg/kg respectively. All administrations were by oral intubation.

Analysis of haematological parameters

Blood was collected by ocular puncture after overnight fast. The blood was collected before induction of anaemia, after induction of anaemia with PHZ and during 1, 2, 3 and 4 weeks of treatments. The volume of blood collected (0.25 to 0.45 ml) did not affect blood parameters as earlier reported (Diallo et al 2008). The red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (Hb) and haematocrit were determined at weeks 0, 1, 2, 3 and 4 using automatic counter Sysmex (K21, Tokyo, Japan).

Statistical analysis

Experimental data were analyzed using one way analysis of variance (ANOVA) and LSD multiple range test to determine signi-

significant differences between means. Difference between means were regarded as significant at $p < 0.05$.

RESULTS

The phytochemical screening of *B. nitens* methanol leaf extract revealed abundance of resins, alkaloids, and glycosides, and trace amounts of saponins, terpenoids, and carbohydrate. The extract was also rich in vitamins, B₆, C and E as well as folic acid and iron. The acute toxicity testing revealed no death up to doses of 5000 mg/kg.

In the control rats phenylhydrazine induced significant ($p < 0.5$) decrease in Hb concentration (46.3%), RBC (76.1%), WBC (65.2%) and haematocrit (42.7%), indicating anaemia. The administration of the extract evoked a significant ($p < 0.5$) increase in the haematological parameters. The PHZ-induced anaemia was significantly ($p < 0.05$) reversed within 1 week of treatment with the extract, reaching maximum by the second week (Figures 1 - 3). In the control rats, the Hb for instance increased naturally and progressively from 8.25 ± 2.49 g/dl at day zero to 10.75 ± 0.83 g/dl at week 4. For 400 mg/kg extract-treated rats, the Hb increased from 8.67 ± 2.13 g/dl at day zero to 18.00 ± 1.58 g/dl (week 2), 17.25 ± 1.29 g/dl (week 3) and 16.25 ± 1.08 g/dl (week 4). Similar positive and significant ($p < 0.05$) changes were recorded in the other haematological parameters and at the other doses of the extract (Figures 1 - 3). The effects of Vit. B₁₂ syrup was comparable to those of the extract.

DISCUSSION

This study aimed to evaluate the effect of *B. nitens* leaves extracts on the haemolytic anaemia induced by phenylhydrazine in albino rats. It has been demonstrated previously that intraperitoneal administration of phenylhydrazine decreased haemoglobin concentration, red blood cells number and haematocrit (O'Riordan et al., 1995; Criswell et al., 2002). Also Agbor and colleagues (2001) demonstrated that oral administration of 10 mg/kg phenylhydrazine for 8 days reduced haematological indices by 50%. In this study, PHZ altered the function of RBC by haemolysis characterized by 76.06% decrease in RBC, 46.27% decrease in Hb concentration, 65.24% decrease in WBC and 42.68% decrease in PCV. However, these parameters were restored to normal range after treatment with *B. nitens* leaves extracts suggesting that *B. nitens* leaves have some haematinic effect.

The results of this study indicated that the whole methanol extract of *B. nitens* increased significantly the concentration of haemoglobin, red blood cell count, white blood cell count and the packed cell volume mainly one week after of treatment. The increase in the blood indices

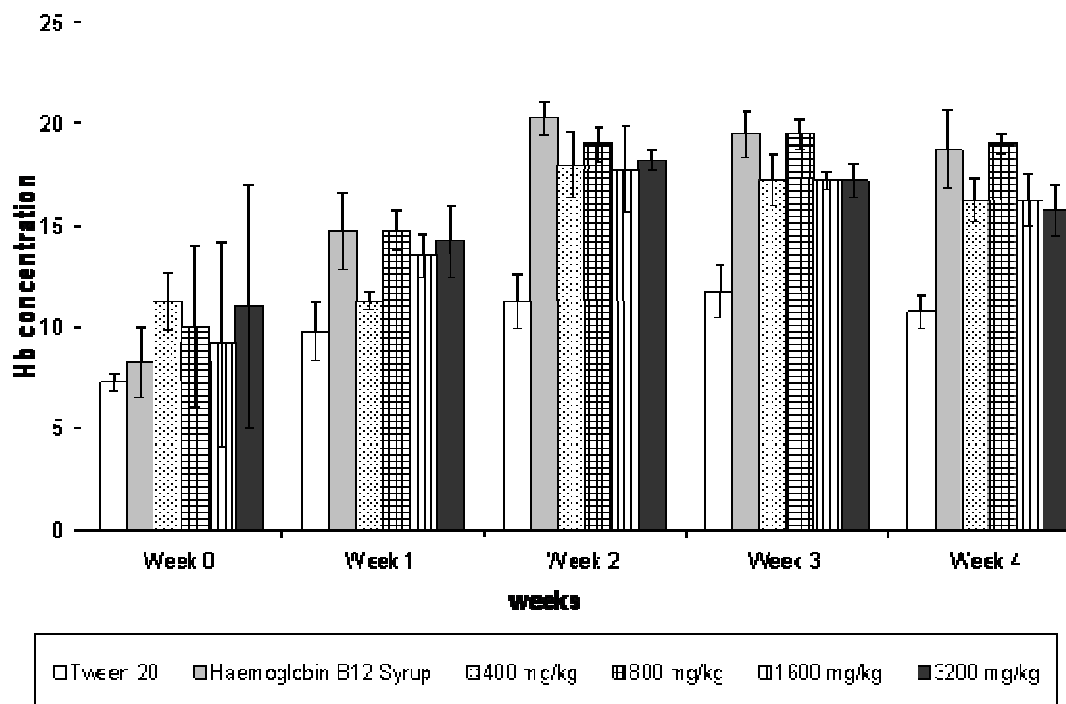


Figure 1. The effect of methanol leaf extract of *B. nitens* on haemoglobin concentration in rats with phenylhydrazine-induced anaemia.

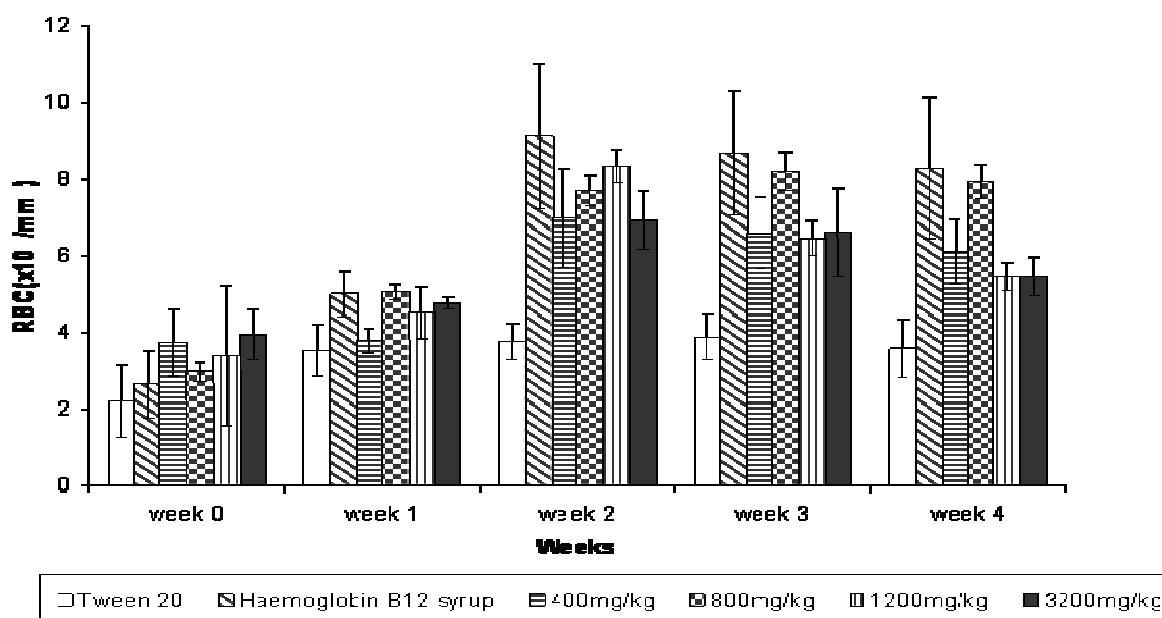


Figure 2. The effect of methanol leaf extract of *B. nitens* on red blood cell count in rats with phenylhydrazine-induced anaemia.

was progressive giving the highest effect on the second week of treatment. Under normal condition, the body can

generate new RBCs to replace the lost red cells; this will take much longer time as shown in this study. The re-

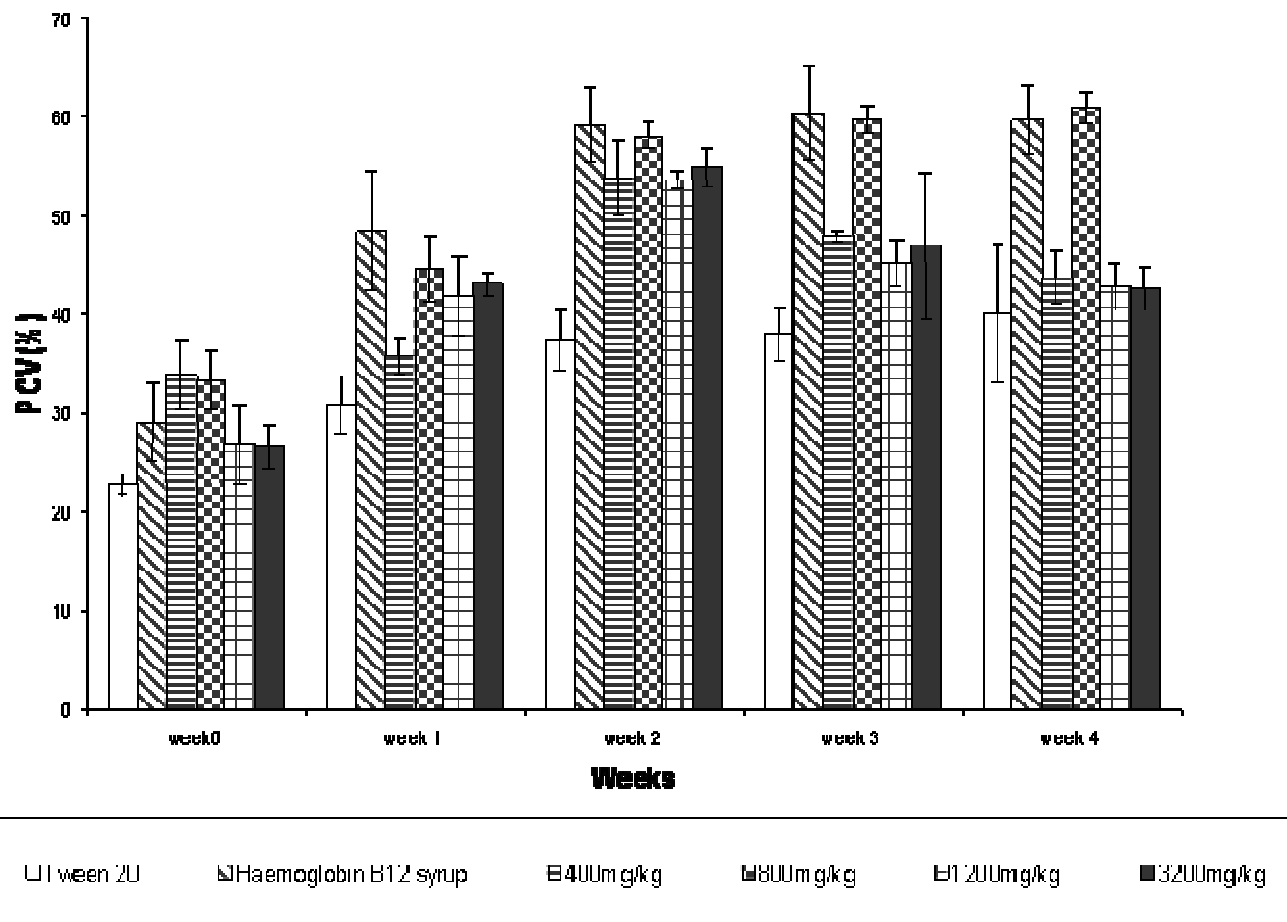


Figure 3. The effect of methanol leaf extract of *B. nitens* on PCV in rats with phenylhydrazine-induced anaemia.

covery time of two weeks for untreated rats has earlier been reported when rats were bled 20% of their total blood volume to induce haemorrhagic anaemia (Agbor and Odetola, 2001).

The increases in the haematological indices exhibited by *B. nitens* extract might not be unconnected with the vitamin and mineral contents of the leaves of *B. nitens*. These constituents are well known haemopoietic factors that have direct influence on the production of blood in the bone marrow. Most importantly, the leaf extract appears safe for use since the LD₅₀ of the methanol extract was greater than 5 g/kg.

In conclusion the extracts of *B. nitens* leaves reversed anaemia induced by phenylhydrazine model of anaemia similar to those induced by parasite such as *Plasmodium falciparum* (Diallo et al., 2008). The vitamin and mineral constituents of the leaf appear most likely as the active ingredients responsible for the haematinic effect of *B. nitens* leaves. This result supports at least partially the traditional use of *B. nitens* in the treatment of anaemia.

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