
**EFFECTS OF THE AQUEOUS EXTRACT OF *PAUSINYSTALIA YOHIMBE* (K. SCHUM PIERRE) STEM BARK ON THE RAT'S BONE MARROW:
A HAEMATOLOGICAL AND HISTOLOGICAL STUDY**

L. J. Hamidu¹, A.U Ekanem², H. A. Salami¹, S.H. Garba² and L.N. Nganso²

¹Departments of Human Physiology, University of Maiduguri, Borno State, Nigeria.

²Human Anatomy, University of Maiduguri, Borno State, Nigeria.

E-mail: saniwakawa@yahoo.co.uk

Abstract: This study investigated the effects of the aqueous stem bark extract of *Pausinystalia yohimbe* on the histology of the bone marrow and some haematological indices in rats. A total of 50 adult Wister albino rats divided into two batches (I and II) consisting of 25 rats each were used in this study. Rats in batch I were divided into 5 groups with group I serving as control while groups II, III, IV and V were administered 200 mg/kg of the extract for 7, 14, 21 and 28 days periods respectively. Batch II rats were treated similarly, but with a higher dose of 400mg/kg. At the end of the treatment periods haematological analysis and histological studies of bone marrow were carried out using standard methods. The results showed significant ($p < 0.05$) increase in packed cell volume (PCV) and red blood cell (RBC) counts at both doses. Post-treatment values of white blood cell (WBC) counts showed significant ($p < 0.05$) decrease in all the treatments except for the 14 days treatment group where there was marginal increase. Histological analysis of the bone marrow revealed that at the dose of 200mg/kg there was increased quantity of RBC, while at 400mg/kg there was appearance of fatty tissues and reduced quantity of RBCs, suggesting degenerative effects of the extract on bone marrow structures. The findings of this work corroborate the decrease RBCs and leucopenia reported by previous studies.

Keywords: *Pausinystalia yohimbe*, Rats, Bone Marrow, Degeneration.

INTRODUCTION

The stem bark of the tree, *Pausinystalia yohimbe* is used for many medicinal purposes, including its reputation as an aphrodisiac and stimulant in many parts of Africa, making the plant open for abuse (Abonnier, 2004). In Cameroon and the Northern parts of Nigeria, the bark of this plant is widely chewed to enhance erection (Akiniyi *et al.*, 1986). *Pausinystalia yohimbe* is also known as *corynanthe johimbe schum* and is native to the southern Cameroon, Gabon and French Congo of Africa. It belongs to the family, *Rubiaceae* and in Northern Nigeria; it is locally called “gai-gai” or “bura tashi”.

The longitudinally fissured bark of the trunk is considered to possess aphrodisiac and stimulant activities (Okujaku *et al.*, (2005). This bark contains the alkaloids yohimbine, mesoyohimbine, yohimbine as well as corynanthine; a closely related substance to yohimbine and asmalicine which have a vasodilatory effect on the coronary arteries (Veerdrager, 1978).

There are reports available in literature documenting damaging effects of high doses and long term use. Histological studies of spleen of rats administered 400mg/kg of *P. yohimbe* extract for 28 days showed hyperplasia and hypertrophy of splenic structures, enlargement of splenic nodules, arterioles and the presence of several venous sinusoids in the red pulp (Hamidu *et al.*, 2007). Leucopenia has also been reported in rats treated with doses of *P. yohimbe* extract for the period of 4 weeks (Hamidu, *et al.*, 2007). The reports of the effects of the extract on

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haematological parameters have not been consistent and more studies have been recommended (Akiniyi *et al.*, 1986 and Effraim *et al.*, 1998).

This present study was therefore designed to investigate the effect of varying doses of the extract on the histology of the bone marrow and some haematological parameters in rats.

MATERIALS AND METHODS

Animal Used

Fifty (50) Wister albino rats of both sexes weighing 160 to 205g and aged between 10 - 15 weeks were obtained from the central animal house of the College of Medical Sciences, University of Maiduguri. The rats were stabilized for one week in large well ventilated laboratory in the department of Human Physiology, University of Maiduguri and they were fed commercial feeds (ECWA Feeds, Maiduguri) and allowed clean water freely.

Plant Materials

Dry stem barks of *Pausinystalia yohimbe* were obtained from the South-Eastern part of Cameroon (Bertua region). The specimen was identified and authenticated by Dr. S. S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Nigeria. Herbarium specimen (Py001) was deposited at the Herbarium of the Department of Biological Sciences, University of Maiduguri, Nigeria.

Extraction

Dry bark of *P. yohimbe* was ground into fine powder using pestle and mortar. Two hundred (200g) grams of the powder was extracted with 1000ml of distilled water over a period of 6 hours using Soxhlet extractor.

The aqueous solution obtained was evaporated to dryness in oven. The pure extract weighed 26.05g and the weight of the residue was 151.76g. The pure extract obtained was then diluted at 5% (i.e. 5g of extract in 100ml distilled water). This provided the stock solution from which the required concentrations per animal was calculated and administered.

Experimental Protocol

This study was carried out in the Departments of Human Anatomy and Human Physiology, University of Maiduguri, Nigeria between January and October, 2008. The 50 Wister albino rats were divided into two batches, I and II irrespective of their sexes, and labeled for the purpose of identification.

Batch I consisted of 5 groups of 5 rats each. Rats in group I was designated as control and was given 10ml/kg of normal saline. Groups II, III, IV and V were experimental; and were given 200mg/kg B.W. aqueous extract of *P. yohimbe* orally by intubations intragastrically for period of 7, 14, 21 and 28 days, respectively.

Batch II was treated similarly, but with a higher dose of 400mg/kg. That is, Group I of batch II received 10ml/kg normal saline, Group II, 400mg/kg for 7 days, Group III, 400mg/kg for 14 days, Group IV, 400mg/kg for 21 days and Group V, 400mg/kg of extract for 28 days.

At the end of the treatment periods, that is on days 8, 15, 22 and 29, respectively blood samples were collected from the rats through the tail vein for haematological analysis.

Red Blood Cell (RBC) Count

The principle was based on counting the number of RBCs in a known small volume of accurately diluted blood. Blood was diluted exactly 1:200 with Hayem's solution (an isotonic red blood cells diluting third) in a red cell pipette and the red blood cells in a small volume of the diluted blood were counted in a counting chamber.

White Blood Cell (WBC) Count

This was to determine the number of WBCs per cubic millimeter of blood. The principle is similar to RBC except for some little details. Blood was diluted 1:20 with 1% glacial acetic acid with a dye (gentian violet). The acid destroys the RBC membranes so that they are not seen and to make WBCs prominent. The dye stains the white cell nuclei. The WBCs were counted using the entire squared area of the haemocytometer.

Packed Cell Volume (PCV) or Haematocrit

The haematocrits were determined using the micro-haematocrit method (using capillary tube). Blood was collected in heparinized capillary tube and centrifuged for 5 min. at 1500 rpm. The cells become packed at the bottom of the tube with the plasma on top. The haematocrit was then read directly using the microhaematocrit reader. The values were expressed as the percentage volume of the blood occupied by the red blood cells.

Histological Analysis of Bone Marrow

Following haematological analysis, the rats were anaesthetized and humanely sacrificed by cervical dislocation and segments of the sternum were removed and fixed in 10% formal-saline for 48 hours. Bone specimens obtained from the segments of the rats' sternum were first decalcified using nitric acid and then processed for paraffin sectioning. Sections were cut at 5 μ m with the rotary microtome and stained with Haematoxylen and Eosin (H&E).

Statistical Analysis

Data were summarized as mean \pm SEM. Differences between individual groups were assessed by the student's t-test. A P-value of less than or equal 0.05 was considered statistically significant (Woodson, 1987).

RESULTS AND DISCUSSION

Administration of 200 and 400 mg/kg the aqueous stem bark extract of *P. yohimbe* for 7 and 14 days pre- and post-treatment on the levels of Packed Cell Volume (PCV), Haemoglobin concentration (Hb), White Blood Cell (WBC) and Red Blood Cell (RBC) counts in rats are shown in table 1. Administration of the extract for 7 days showed increase in PCV values from 39.80 \pm 2.39 pre-treatment to 46.25 \pm 1.25 post-treatment in rats administered with 200mg/kg of the extract, while rats administered with 400mg/kg body weight showed an increase from 38.50 \pm 2.72 to 49.25 \pm 1.25. These increases were statistically significant ($p < 0.05$). The post-treated PCV values for both doses were also significantly higher than the values for the control group ($p < 0.05$). For Hb concentration, the rats given 200mg/kg B.W showed a decrease in mean values from 13.26 \pm 0.70 to 10.14 \pm 0.09, but at 400mg/kg B.W the Hb value increased significantly ($p < 0.05$) from 12.80 \pm 0.91 to 18.50 \pm 0.39. The mean RBC count showed significant increase at both dose levels. At 200mg/kg B.W, RBC count increased from 2.82 \pm 0.24 to 4.94 \pm 0.040 and at

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400mg/kg B.W, the increase was from 2.53 ± 0.13 to 7.05 ± 0.29 . However, the WBC count showed a decrease in mean values for both dose levels for this period of treatment. The decrease was however not significant.

Pre- and post-treatment for 14 days with the extract at 200mg/kg B.W. and 400mg/kg B.W. showed PCV levels to have increased significantly ($p < 0.05$) from 42.25 ± 1.25 to 51.75 ± 1.10 in rats given 200mg/kg B.W for 14 days at 400mg/kg BW. It showed an increase from 38.8 ± 0.85 to 47.60 ± 1.62 for Hb, at 200mg/kg B.W, there was an increase from 14.07 ± 0.41 to 19.62 ± 0.41 to 19.62 ± 0.64 . At 400mg/kg B.W., the Hb value decrease from 12.90 ± 0.27 to 10.46 ± 0.25 . The RBC count showed significant increases ($p < 0.05$) for both dose levels. The WBC count however showed decrease in mean values especially in the group administered 400mg/kg B.W from 9.36 ± 0.49 to 6.35 ± 0.60 (Table 1). The mean haematological parameters in rats treated for 21 and 28 days are presented in table 2. The mean values of all the parameters, except WBC count showed significant increases ($p < 0.05$) for both groups given 200mg/kg B.W and 400mg/kg B.W respectively. The PCV values for the groups given 200mg/kg B.W and 400mg/kg B.W, respectively showed slight increases, though not significant. The Hb values significantly ($p < 0.05$) decrease from 11.80 ± 0.2 to 9.12 ± 0.57 in groups given 200mg/kg B.W of extract and from 12.53 ± 0.28 to 8.73 ± 0.23 in rats treated with 400mg/kg B.W. Red blood cell count showed slight increases, but not statistically significant. For white blood cell count, there was no significant change in the values. The bone marrow study revealed increased quantity of RBC in the rats treated with 200mg/kg BW of extract for all periods of treatments.

Histomorphological observations indicate normal levels of erythrocytes, leucocytes and plasma cells in the bone marrow tissues of control rats (Fig.1). The rats that were administered with 200mg/kg B.W for 7 days and 21 days respectively showed bone marrow tissues with large numbers of erythrocytes and relatively few numbers of leucocytes (Fig.2 and 3). Proliferation very prominent fatty cells and gross reduction in the numbers of erythrocytes and leucocytes were the histopathological observations recorded in the bone marrow tissues of rats administered with 400mg/kg B.W of extract for 28 days (Fig. 4).

The presence of large number of reticulocytes observed from the Microscopic examination of experimental rats in the first 3 weeks of treatment might have accounted for the increased Hb, PCV and RBC count observed from the haematological analysis. It would be plausible to postulate that the extract stimulated the release of the reticulocytes to increase these parameters. This finding is consistent with earlier report (Effraim *et al.*, 1997). The aqueous extract of *P. yohimbe* has also been reported to cause increase in blood flow (Akiniyi *et al.*, 1986) and this has been associated to the vasodilatory actions of the extract on peripheral arteries (Effraim *et al.*, 1997).The extract has also been reported to cause increase in blood volume due to its ability to stimulate erythropoiesis (George and William, 1978). The mean corpuscular volume (MCV) for the data obtained in this study showed a lower MCV values compared to the control (data not shown) which is consistent with the fact that when reticulocytes number becomes elevated in the blood, the cells appear smaller than normal (Dacie, and Lewis, 1984).

Histological studies of bone marrow of the experimental rats showed the presence of large number of RBC [fig. 2] supporting that erythropoiesis was induced by the extract. However, at

higher dose (400mg/kg) and longer period of treatment (4 weeks), degeneration of structures in the bone marrow was observed, as evidenced by the presence of fatty tissues [fig. 4].

In conclusion, extract of *P. yohimbe* stem bark was found to stimulate erythropoiesis at lower doses of 200mg/kg and increased PCV, Hb and RBC count. This justifies its empirical use to treat erection disorder. However, it is the opinion of the authors that consumption of this plant material for long period constitutes a serious health risk; considering that in human, B-lymphocytes which are the antibody producing cells undergo early antigen - dependent maturation into immunocompetent cells in the bone marrow. Dysfunction of pluripotent stem cells or the various cell lines developing from it can result in immune deficiency disorders of varying expressions and severity (Stephen *et al.*, 1995).

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Table 1: Mean Haematological Values of Rats Before and After Treatment with Aqueous Extract of *P. yohimbe* for 7 and 14 Days Respectively.

Parameters	Experimental Period					
	Control	7 Days		Control	14 Days	
		200mg/kg)	400mg/kg		200mg/kg)	400mg/kg
PCV (%)						
Pre-treatment	40.20±0.49	39.80±2.39	38.50±2.27	41.10±0.41	42.25±1.25	38.80±0.85
Post-treatment	41.05±0.20	46.60±0.92*	49.25±1.25*	41.30±0.31	51.71±1.10	47.60±1.62*
Hb (g/d)						
Pre-treatment	12.30±2.10	13.26±0.79	12.80±0.91	13.70±0.10	14.07±0.41	12.90±0.27
Post-treatment	12.50±0.15	10.14±0.09	18.50±0.39*	12.75±1.15	19.02±0.64*	10.64±0.25
RBC (X10 ⁶ /mm ³)						
Pre-treatment	3.50±0.15	2.82±0.24	2.53±0.13	3.75±1.10	4.33±1.10	3.12±0.27
Post-treatment	4.10±0.15	4.94±0.40*	7.05±0.29*	4.20±0.80	10.33±1.00*	6.01±0.27*
WBC (X10 ³ /mm ³)						
Pre-treatment	3.41±0.40	5.40±0.94	5.60±0.45	4.20±0.90	5.11±0.31	5.00±0.50
Post-treatment	4.00±0.37	4.96±0.46	4.75±1.12	4.11±0.12	6.25±0.75	7.51±0.84

* p<0.05 experimental groups vs control group; N = Number of rats in each group

Table 2: Mean Haematological Values of Rats Before and After Treatment with Aqueous Extract of *P. yohimbe* for 21 and 28 Days Respectively.

Parameters	Experimental Period					
	Control	21 Days		Control	28 Days	
		200mg/kg	400mg/kg		200mg/kg	400mg/kg
PCV (%)						
Pre-treatment	40.00±0.41	47.20±0.85	44.00±1.50	42.00±0.10	35.50±0.60	37.60±0.81
Post-treatment	40.10±0.15	54.25±1.10*	52.30±1.44*	42.50±0.14	42.50±0.90	39.30±3.37
Hb (g/d)						
Pre-treatment	11.10±0.27	13.80±0.20	12.43±1.58	12.12±1.16	11.80±0.20	12.53±0.28
Post-treatment	12.12±0.41	20.87±0.17*	18.36±0.66*	13.00±1.12	1.12±0.59*	0.73±0.23*
RBC (X10 ⁶ /mm ³)						
Pre-treatment	4.00±0.13	5.60±0.84	7.25±0.68	5.00±0.15	3.48±0.57	3.03±0.40
Post-treatment	5.21±0.10	9.60±0.93	10.86±1.07	6.19±0.19	5.99±0.50	5.42±0.21
WBC (X10 ³ /mm ³)						
Pre-treatment	3.15±0.24	5.90±0.26	9.36±0.49	5.10±0.10	5.35±0.61	4.80±0.70
Post-treatment	3.16±0.96	4.60±0.30	6.35±0.60	5.12±1.12	5.27±0.35	6.36±0.40

* p<0.05 experimental groups vs control group; N = Number of rats in each group.

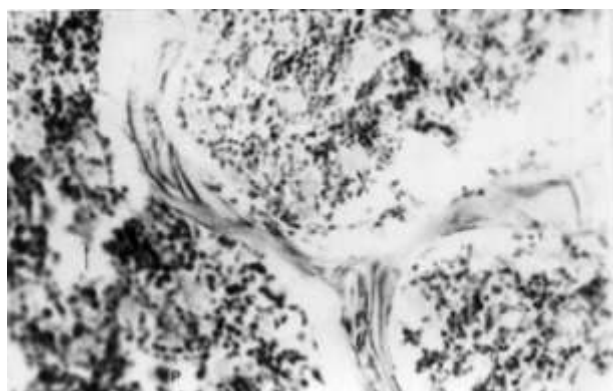


Fig. 1: Bone Marrow Section of a Control Rat Showing Normal Levels of Erythrocytes (Arrow) and Leucocytes (Dark Stained Cells) and Plasma Cells (P). H & E Stain. (Mag x 200).

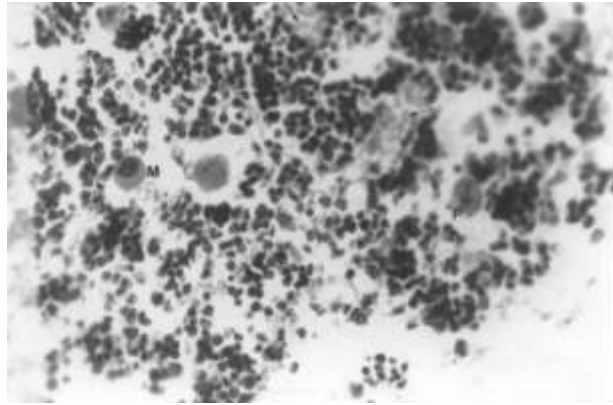


Fig. 2: Bone Marrow Section of a Rat Treated with 200 mgkg⁻¹ of the Extract for 7 Days Showing Large Numbers of Erythrocytes (Arrows) and Relatively Few Number of Leucocytes (Dark Stained Cells), Monocytes (M) and Plasma Cells (P). H & E Stain. (Mag x 400).

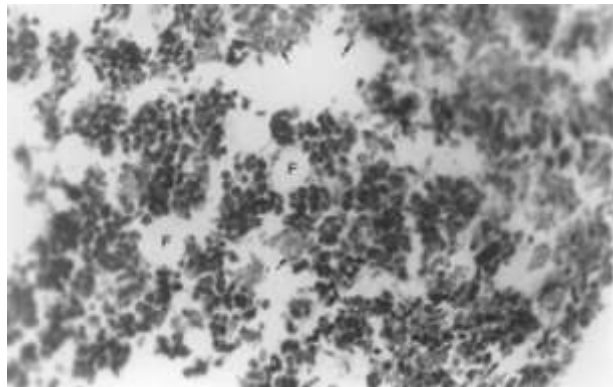


Fig. 3: Bone Marrow Section of a Rat Treated with 200 mgkg⁻¹ of the Extract for 21 Days Showing Large Numbers of Erythrocytes (Arrows) and Reduced Numbers of Leucocytes (Dark Stained Cells) and Fatty Cells (F). H & E Stain. (Mag x 400).

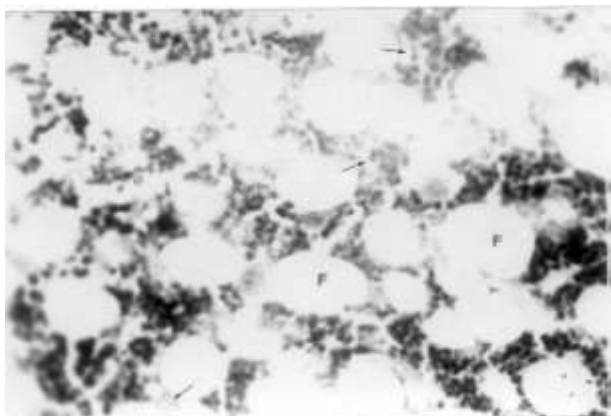


Fig. 4: Bone Marrow Section of a Rat Treated with 400 mgkg^{-1} of the Extract for 28 Days Showing a Large Number of Prominent Fatty Cells (F), Grossly Reduced Number of Erythrocytes (Arrows) and Leucocytes (Dark Stained Cells). H & E Stain. (Mag x 400).

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