AMERICAN JOURNAL OF PHARMTECH RESEARCH

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Haematopoiesis Stimulation by Aqueous Extract of *Parquentina nigrescens* in Wistar Albino Rats

Fola Tayo[1*], Moses O. Akiibinu[1]& Folorunso Abegunde[1]

1.Pharmaco-Herbal Development Laboratory, Famot HealthCare Ltd, Iyeri Estate, Ikorodu-Itoikin Road, Imota, Lagos State; Department of Chemistry & Biochemistry, College of Applied Sciences, Caleb University, Ikorodu-Itoikin Road, Imota, Lagos State, Nigeria.

ABSTRACT

Extract of Parquetina nigrescens is used traditionally to treat anaemia by Yoruba people of South Western Nigeria. There is no previous study on the stimulatory effects of the herb on lymphoid cells (i.e. CD4+ T-cells). This study determined the levels of products of bone marrow stimulation including CD4+ T-cell in Wistar rats after oral administration of aqueous extract of the leaf of P. nigrescens. Fifteen Wistar rats, divided into five groups were used for this study. Group A=untreated (controls), group B=treated with 0.008 g of extract/kg body weight (BW), group C= treated with 0.08 g of extract/ kg BW, group D= treated with 0.8g of extract/kg BW, group E= treated with 8.0g of extract/kg BW. Blood sample was collected from each rat through cardiac puncture for the determination of hematological indices (WBC, RBC, PCV, Hb, MCV, total lymphocyte and CD4+T-cells) using haemocytometer and flow cytometer respectively. Aqueous extracts of the leaf of P. nigrescens did not evoke any observable toxic effect. There were significantly ($p<0.05$) higher levels of CD4+ T-cells and WBC in groups D and E compared with controls. PCV increased significantly ($p<0.05$) in groups C and E, while RBC was significantly ($p<0.05$) higher only in group E. MCV increased significantly ($p<0.05$) in groups B, C and D compared with controls. Hb and lymphocyte levels increased significantly ($p<0.05$) in groups C and E compared with controls. The dose of extract correlated significantly with RBC ($r=0.660$, p-value=0.027) and CD4+T-cells ($r =0.812$, p-value =0.002). Aqueous extract of P. nigrescens at 0.8g/kg BW and 8.0g/kg BW may be beneficial to immune-compromised and anaemic individuals respectively

Keywords: *Parquentina nigriscence*, single dose, haemopoiesis.

*Corresponding Author Email: akiibinumoses@yahoo.com
Received 10 November 2019, Accepted 17 November 2019

Please cite this article as: Tayo F *et al.*, Haematopoiesis Stimulation by Aqueous Extract of Parquentina nigrescens in Wistar Albino Rats. American Journal of PharmTech Research 2019.

INTRODUCTION

Parquetina nigrescens or bullock is a herbaceous, perennial twine shrub. found in the region of equatorial West Africa. The plant grows commonly across the African regions, along Senegal to Nigeria, and also over the Congo basin down to south tropical Africa (Burkill, 1985). It is one of the plants commonly used for medicinal purposes in the tropical Africa (Sofowora *et al.,* 2013). It is woody at the base and as long as 7 to 8 meters in length. Microscopic examination shows that the leaf is hypostomatic and has non-glandular trichomes in the abaxial epidermal layer. The plant has vascular bundles, and other structures like collenchyma, sclerenchyma, prismatic calcium oxalate crystal, polygonal epidermal cells, straight anticlinal walls (Sopeyin & Ajayi, 2016), cardenoides, glycosides and alkaloids (Burkill, 1985; Marks *et al.,* 1995).

The leaves, roots and latex of *P. nigrescens* have been used by traditional medicine practitioners since antiquity (Owoyele *et al.,* 2011). Studies show that the aqueous extract has haematopoietic activity (Agbor & Odetola, 2005), hypoglycaemic potential, anti-oxidative properties, erythrocyte membrane stabilizing properties (Saba, Oyagbemi & Azeez, 2010) and also used in treating anaemia (van den Brook, 2005) in pregnancy. In addition, it has gastro-protective and anti-ulcer effects (Kayode & Yakubu,2017; Odetola *et al.,* 2006).

The effect(s) of *P. nigrescens* on the CD_{4+} T-cells in animal and/or man has not been documented. Since cellular immune deficiency is a serious risk factor associated with many tropical diseases such as pulmonary tuberculosis and malnutrition and HIV/AIDS, investigating the stimulatory effect of aqueous extract of *P. nigrescens* on erythropoiesis and the levels of CD_{4+} T-cells in particular provides a basis for this study. The study was therefore designed to bridge this gap in knowledge by determining the levels of mean cell volume (MCV), total white blood cell count (WBC), red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb), total lymphocyte (LYMP) and CD4 T-cells in Wister rats after increasing oral doses of Aqueous extract of *P. nigrescens.*

MATERIALS AND METHOD

Preparation of *Parquetina nigrescens* extract:

Fresh leaves of *P. nigrescens* were collected locally The plant was identified and authenticated in the Herbarium at the Department of Botany, University of Ibadan, Ibadan, Nigeria. Aqueous extract of the plant was prepared with 20g of fresh leaf in 1000mL of distilled water and grinded for 15 min in a grinding machine. The mixture was filtered and the filtrate (Aqueous extract) administered orally with an oral canulla.

Animals and Treatment:

Adult male (8) and female (7) Wister rats (150-250 g) were used for the study. They were obtained from the Animal House, Department of Pharmacology & Therapeutics, College of Medicine, University of Ibadan, Nigeria. They were kept in rat cages maintained under standard laboratory conditions. The animals were acclimatized for one week, fed on rat pellets purchased from Ladokun Feeds PLC, Ibadan, Oyo State, Nigeria and allowed free access to clean water. They were fasted overnight before the experiment was carried out. The study was approved by the Animal Welfare, Research and Ethics Committee of Caleb University Lagos, Nigeria. All conditions of animal use were also in agreement with the United States National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals, and in accordance with the recommendations of IASP (Zimmermann, 1983).

Acute toxicity study (Lalitha et al, 2012) was conducted. Wister rats were not allowed to eat overnight and then weighed prior to dosing. Increasing doses of the Aqueous extract of *P. nigrescens* was administered orally (0, 0.008, 0.08, 0.8 and 8.0g)/kg body weight. The extract was administered in a single dose by gavage using specially designed rat oral cannula. After the single dose treatments, the rats were observed for signs of toxicity for a period of 24 hours. Blood sample was collected from each Wister rat into EDTA bottle through cardiac puncture for the determination of WBC, RBC, PCV, HB, MCV, LYMP and CD_{4+} T-cells

The levels of MCV, WBC, RBC, PCV, Hb, NEUT, LYMP were determined using haemocytometer. The level of CD_{4+} T-cell was determined using flow cytometer.

Statistical analysis:

Statistical analysis was performed with Statistical Package for Social Sciences for Windows, version 21 (SPSS Inc., Chicago, USA). The data were expressed as mean ± SD and Student (t) test was used to test for the degrees of differences in the treated and control rats. Pearson correlation coefficient (r) was calculated. The changes were considered significant at p values ≤ 0.05.

RESULTS AND DISCUSSION

The results showed that there were no physical signs of toxicity in rats treated with the Aqueous extract of *P. nigrescens* (0, 0.008, 0.08, 0.8 and 8.0g)/kg body weight (Table 1). There were significantly ($p<0.05$) higher levels of CD_{4+} T-cells and WBC in Groups D and E compared with controls (Figures 1 & 3). LYMP count increased significantly ($p<0.05$) in Groups C and E compared with controls (Figure 2). PCV levels increased significantly ($p<0.05$) in Groups C and E compared with controls (Figure 5). RBC was significantly ($p<0.05$) higher in Group E compared

with controls (Figure 4). The Hb levels increased significantly ($p<0.05$) in Groups C and E compared with controls (Figure 6). MCV increased significantly ($p<0.05$) in Groups B, C and D compared with controls (Figure 7). As shown in Table 2, the dose of the administered doses of the extract of *P. nigrescens* correlated significantly with RBC ($r = 0.660$, p-value = 0.027) and CD_{4+} ($r = 0.812$, p-value = 0.002).

Table 1. Effect of Increasing Doses of Aqueous Extract of P. nigrescens on Various Physical Parameter of Wistar Albino Rats

S/N	Physical Parameter	Group observation A (No extract)	B (0.008g/kg bwt)	C (0.08g/kg bwt)	D (0.8g/kg bwt)	E (8.0g/kg bwt)
1.	Alertness	Very good	Very good	Very good	Very good	Very good
2.	Grooming	Normal	Normal	Normal	Normal	Normal
3.	Response	Very good	Very good	Very good	Very good	Very good
4.	Tremor	Nil	Nil	Nil	Nil	Nil
5.	Convulsion	Nil	Nil	Nil	Nil	Nil
6.	Gripping	Normal	Normal	Normal	Normal	Normal
7.	strength	Very good	Very good	Very good	Very good	Very good
8.	Response to food Pupils	Normal	Normal	Normal	Normal	Normal
9.	Urination	Normal	Normal	Normal	Normal	Normal
10.	Salivation	Normal	Normal	Normal	Normal	Normal
11	Hyperactivity	Normal	Normal	Normal	Normal	Normal
12.	Skin colour	Normal	Normal	Normal	Normal	Normal
13.	Corneal reflex	Normal	Normal	Normal	Normal	Normal
14	Response to Sound	Very good	Very good	Very good	Very good	Very good

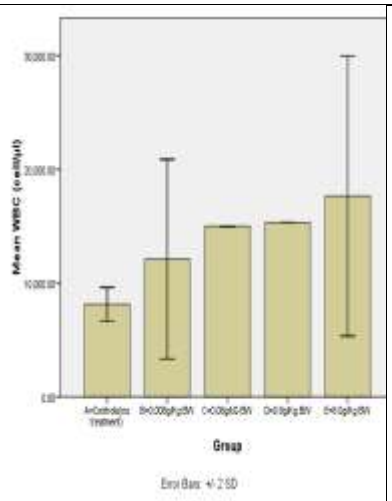

Figure 1: Levels of Total WBC in Wistar Rats Treated With Oral Doses of Aqueous Extract of P. Nigrescens

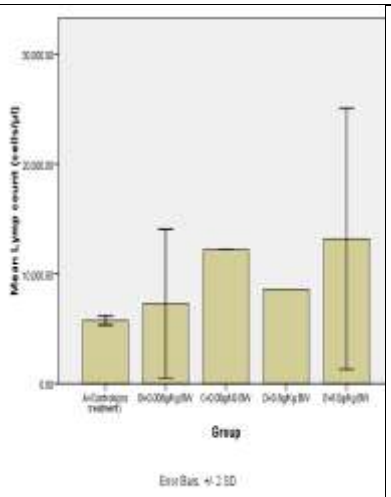

Figure 2: Levels of Total Lymphocytes in Wistar Rats Treated With Oral Doses of Aqueous Extract of P. Nigrescens

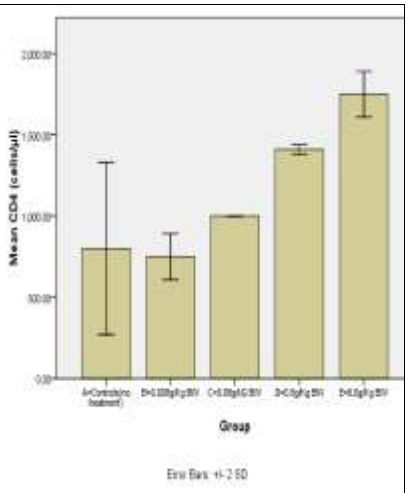

Figure 3: Levels of CD4+T-Cells in Wistar Rats Treated With Oral Doses of Aqueous Extract of P. Nigrescens

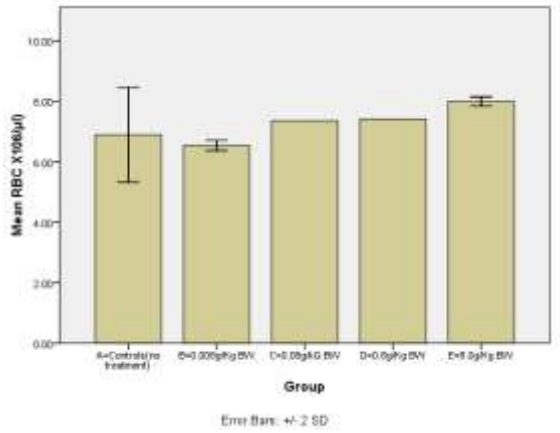

Figure 4: Levels of RBC in Wistar Rats Treated With Oral Doses of Aqueous Extract of *P. Nigrescens*

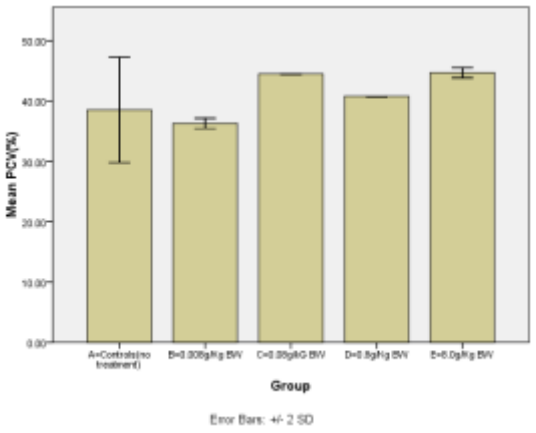

Figure 5: Levels of PCV in Wistar Rats Treated With Oral Doses of Aqueous Extract of *P. Nigrescens*

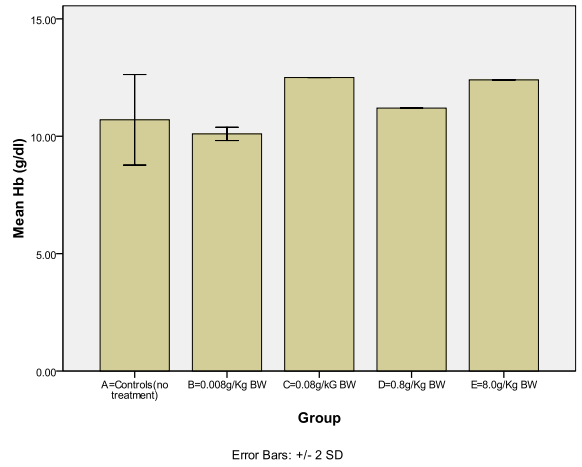

Figure 6: Levels Of Hb in Wistar Rats Treated With Oral Doses of Aqueous Extract of *P. Nigrescens*

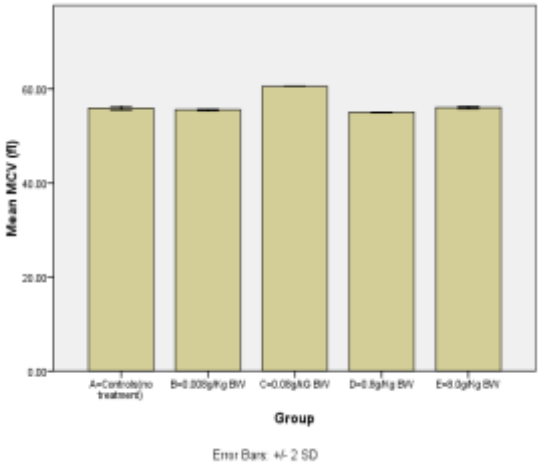

Figure 7: Levels of MCV in Wistar Rats Treated With Oral Doses of Aqueous Extract of *P. Nigrescens*

Table 2: Correlation of Doses, CD_{4+} T-cells, PCV, WBC, LYMP, RBC, Hb and MCV in Wister Rats Treated With Extract of Parquetina nigrescens

Parameters	r-values	p-values
Dose/CD4 T-cell	0.812	0.002 (S)
Dose/PCV	0.517	0.104(NS)
Dose/WBC	0.546	0.082(NS)
Dose/LYMP	0.553	0.078(NS)
Dose/RBC	0.660	0.027 (S)
Dose/HB	0.516	0.104(NS)
Dose/MCV	-0.162	0.635(NS)

Key: S = Significant; NS = Not Significant

DISCUSSION:

P. nigrescens is one of the medicinal plants used by traditional medicine practitioners in Tropical Africa. The plant has been identified to have broad therapeutic potentials. No toxic effect of *P. nigrescens* has been reported by previous workers (Nsiah et al., 2006) as well as in the present study. The plant extract was reported to improve sexual competence (Kayode & Yakubu, 2017), have antidiabetic properties (Saba et al, 2010) and haematinic potentials (Ukwubile et al, 2016). The increased levels of PCV, RBC and HB in the present study confirmed that *P. nigrescens* has stimulatory effects on the haemopoietic stem cells. This could be associated with the effects of micronutrients (Oliveria *et al*, 2018) in the plant. Antioxidant effect of the plant has been reported by Saba et al, 2010). Since high free radical load and oxidative stress has been associated with gelatinous transformation of the bone marrow and anaemia (Ghaffari, 2008), the high antioxidant potential of the extract of this plant might have neutralized the free radicals and enhanced the red blood cell production in the Wistar albino rats. In the present study, the RBC levels correlated significantly with the dose of the extract. Our findings therefore corroborate the report of Agbor and Odetola (2005) and Ukwubile *et al.* (2016) that erythrocytes indices increased in anaemic rats treated with *P. nigrescens* dose dependently. In addition, *P. nigrescens* also stabilized erythrocyte membrane (Saba *et al*, 2010).

White blood cells and their products protect the system against infectious agents and cancer cells. The CD_{4+} T-cell constituent of the white blood cells has critical roles to play in cellular immunity. Activated CD_{4+} T helper cell stimulates several other cells of the immune system for effective immune responses (*Parham, 2005*), while regulatory T-cells modulate the activities of other immune cells. Reduced levels of CD_{4+} T-cells have been associated with certain disease conditions like pulmonary tuberculosis, malnutrition and HIV, that are majorly tropical diseases. To our knowledge, the present study is the first to report the level of CD_{4+} T-cells in rats treated with aqueous extract of *P. nigrescens*. We also observed that the level of CD_{4+} T-cell correlated significantly with the dose of *P. nigrescens* administered to the rats. The aqueous extract of *P. nigrescens* induced the production of CD_{4+} T-cell best at 0.8g/kg body weight. The total WBC increased significantly at 0.8g/kg body weight. Although the pharmacokinetics of this extract in the bone marrow has not been established, the phytochemical and micronutrient properties of the plant extract could cause the re-activation of weak cells of the bone marrow and enhance the $CD4_{4+}$ T-cell count (Kaiser et al, 2006) in the treated groups. Akiibinu et al (2012) reported

significantly lower levels of micronutrients in symptomatic HIV/AIDS and tuberculosis patients. Kaiser et al (2006) stated that micronutrient supplementation can significantly improve CD_{4+} T-cell count. Therefore, restoration of these micronutrients could enhance both metabolic activities and haematopoiesis. This study may therefore hypothesize that aqueous extract of *P. nigrescens* could be used as adjuvant therapy in the management of diseases associated with a deficiency of CD_{4+}T-cell such as HIV/AIDS.

In conclusion, aqueous extract of P. nigrescens at 0.8g/kg body weight and 8.0g/kg body weight may be beneficial to immune-compromised and anaemic individuals respectively. Further investigation is in progress to elucidate other potential beneficial therapeutic effects of this plant.

AUTHORS' CONTRIBUTIONS:

FT designed the research, FT, FA and MOA did the analysis and all authors contributed and approved the final manuscript.

ACKNOWLEDGEMENTS:

The authors appreciate Mr.Oluwatosin Wale Ajao of Pharmaco-Herbal Development Laboratory, Famot HealthCare Ltd, Iyeri Estate, Ikorodu-Itoikin Road, Imota, Lagos State, Nigeria for the material and technical supports.

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