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Parquetina nigrescens reverses haemorrhagic and haemolytic anaemia with reduction of erythrocyte osmotic fragility in adult male Wistar rats

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Abstract

Anaemia is still a serious challenge in developing countries due to problems of haemo protozoan parasitic diseases, sickle cell anaemia and malnutrition that is almost always exacerbated by poverty. Thus, there is a need for the evaluation and development of local solutions towards the mitigation of anaemia. The present study was therefore designed to evaluate the haematinic potentials of methanol leaf extract of *Parquetina nigriscens* on experimentally-induced haemorrhagic and haemolytic anaemia in adult male Wistar rats.

Forty-five adult male Wistar rats used for the study were randomly assigned into 9 groups. Group A served as positive control without anaemia. Haemorrhagic anaemia was induced in groups B - E by withdrawal of 30% total blood volume of each rat, while haemolytic anaemia was induced in groups F to I by an intraperitoneal injection of Phenylhydrazine (40 mg/kg) for two days (Day 1 and 3). The rats were administered 25 or 50mg/kg *P. nigriscens* or $FeSO_4$ (2 mg/kg), while a group was untreated post-induction of anaemia. Blood samples were collected on day 8 for determination of haematological parameters and erythrocyte osmotic fragility.

Parquetina nigrescens significantly increased the PCV, RBC and haemoglobin values as well as erythrocyte indices (MCV, MCH and MCHC), which had been initially reduced by the acute haemorrhage and haemolysis. The extract also restored the leucocyte indices to values comparable to those of non-anaemic control rats. Finally, *P. nigrescens* increased erythrocyte osmotic resistance in both haemorrhagic and haemolytic anaemia-induced rats, an indicator of regeneration of RBC and increased circulation of new erythrocytes in circulation, which is a response to anaemia.

The study demonstrated that methanol leaf extract of *Parquetina nigrescens* extract possesses some haematinic effects and can be used to stimulate erythropoiesis in acute blood loss or convalescent cases of haemolysis diseases.

Keywords: Haemorrhage, haemolysis, anaemia, *Parquetina nigrescens*, rats

Introduction

One of the major challenges of the third world, underdeveloped or developing countries is poverty and its attendant problems. These problems include malnutrition, diseases, war, insurrection, drought and internal population displacement; all of which have been associated with nutritional diseases including

kwashiorkor and marasmus and anaemia in young subjects (Assis et al., 2004, Sassi et al., 2019). Many of the aforementioned conditions typify the current state of some regions in Nigeria, with insurrections and banditry in the North East; communal clashes in North Central and extreme poverty among the internally displaced people (Eme et al., 2018, Dumbili and Nnanwube, 2019). Besides poverty and malnutrition, anaemia is also a common condition in haemoprotozoan infections and haemolytic diseases such as malaria fever (Olukosi et al., 2018, Gari, 2018), tick fever (Dehhaghi et al., 2019), trypanosomiasis (Okoth et al., 2019), anaplasma and theileria infections (Jalali et al., 2018) as well as dengue (Abdullah et al., 2019) and rift valley fever (Bird et al., 2009) just to name a few.

Extreme poverty and malnutrition coupled with haemoprotozoan and other haemolytic infections have forced individuals in developing countries to depend on alternative sources of vitamins and haematinics due to their inability to afford orthodox medications. These alternatives are herbs and plant products that have been reported to possess haematinic properties. Plants such as *Telfairia occidentals* (Salman et al., 2018, Ajibade et al., 2018), *Spondia mombin* (Asuquo et al., 2013), *Hibiscus cannabis and H. sabdariffa* (Vasavi et al., 2019), *Asteracantha longifolia* (Dutta et al., 2014), *Sphenocentrum jollyanum* (Mbaka and Owolabi, 2011) and *Solanum nigrum* (Aduwamai et al., 2018) and many others have been scientifically proven to possess haematinic properties and correct natural and experimentally induced anaemia.

Parquetina nigriscens (family *Ascelpiadaceae*) is also traditionally used for the management of anaemia, but this practice has no scientific basis. The plant is a slender, glabrous-twining shrub that grows up to the tops of forest trees. The plant is present in low bushes in forests, savannah areas and transition forests in West African countries including Nigeria and Ghana. The plant has a high polyphenol content and has been extensively studied for its antioxidant potential against neurotoxicity, cardio-renal toxicity and apoptosis in rats (Ochigbo et al., 2017, Oyagbemi et al., 2018, Adeyomoye and Adewoye, 2018). Like many of the plants mentioned earlier, *Parquetina nigrescens* (known as *ewe ogbo* among the Yorubas, Southwest, Nigeria) has been suggested to possess haematinic and erythropoiesis-stimulating effects. For example, Saba et al. (2010) observed that methanol leaf extract of the plant corrected the normocytic normochromic anaemia associated with alloxan-induced diabetes in rats. However, the haematopoiesis stimulating effect on natural or induced anaemia has not been previously investigated.

The present study was therefore designed to evaluate the haematinic and haematopoietic activities of the methanol leaves extract of *P. nigrescens* using induced haemorrhagic and haemolytic anaemia model in the Wistar rats.

Materials and method

Plant materials and Preparation of extracts

Fresh leaves of *Parquetina nigrescens* were collected from the University of Ibadan, Nigeria. The leaves were air-dried at room temperature for two weeks after which they were oven-dried at 25^{o}C for 3 hours to constant weight. The leaves were macerated in ten litres of methanol for 96 hours. The extract was filtered out and concentrated

using rotary evaporator model RE400, Stuart Equipment, Staffordshire, UK. The remaining moisture was removed over a water bath set at 30°C.

Experimental Animals

A total of 45 adult male Wistar rats (120-150g) were used in this study. They were maintained at the Experimental Animal House of the Department of Veterinary Pharmacology and Toxicology, University of Ibadan in rat cages and fed *ad libitum* on commercial rat feed (Top Feed Nigeria Ltd). They were also allowed unrestricted access to clean drinking water.

Experimental Procedure

The rats were randomly and equally assigned into nine groups and acclimatized for one week. Group A served as the control group, while haemorrhagic anaemia was induced in Groups B, C, D, and E by collecting 30% of the total blood volume through the retro-orbital venous plexus according to the formula suggested by Lee and Blaufox (1985) which expresses the total blood volume in rats as (0.06 x BW + 0.77) ml. Haemolytic anaemia was induced in Groups F, G, H, and I via intraperitoneal injection of Phenylhydrazine at 40mg/kg for two days, (Days 1 and 3) according to Berger (2007) and Zangeneh et al. (2019). Phenylhydrazine and Ferrous sulphate were purchased from Chemos GmbH & Co. KG, Germany and May & Baker, Nigeria respectively. Phenylhydrazine was reconstituted in normal saline, while ferrous sulphate was dissolved in distilled water. The rats were then treated with the extract or ferrous sulphate as haematinic by gastric gavage for 8 days post-induction of anaemia as shown below.

Group A: Control group, non-anaemic.

Group B: Haemorrhagic anaemia group treated with methanol extract of *Parquetina nigrescens* administered orally at 25 mg/kg.

Group C: Haemorrhagic anaemia group treated with methanol extract of *Parquetina nigrescens* administered orally at 50 mg/kg.

Group D: Haemorrhagic anaemia group treated with ferrous sulphate, administered orally at 2 mg/kg.

Group E: Haemorrhagic anaemia group without treatment.

Group F: Haemolytic anaemia group treated with methanol extract of *Parquetina nigrescens* administered orally at 25 mg/kg.

Group G: Haemolytic anaemia group treated with methanol extract of *Parquetina nigrescens* administered orally at 50 mg/kg.

Group H: Haemolytic anaemia group treated with ferrous sulphate, administered orally at 2 mg/kg.

Group I: Haemolytic anaemia group without treatment.

Sample collection

The rats were treated for 8 days after which blood samples were collected into lithium-heparinized tubes for haematology and determination of erythrocytes osmotic fragility. Pack cell volume was determined by the microhaematocrit method, haemoglobin concentration by the cyanmethaemoglobin method, red blood cell, white blood cell and platelet counts using the improved Neubauer slide while differential neutrophils, lymphocytes, monocytes and eosinophils counts were determined in Giemsa stained blood smear. The osmotic fragility test was determined by standard protocol as previously described by Azeez et al. (2013). Anaemia was confirmed at 2 days post-induction by determining the PCV from drops of blood from the tail prick.

All experiment protocols complied with the University of Ibadan Ethics Committee on Research in Animals, as well as internationally accepted principles for laboratory animal use and care.

Data analysis

Values are expressed as mean ± S.D. "One-way ANOVA with Tukey's post-hoc test was also performed to compare the data between groups using GraphPad Prism version 7.0. A probability value of $P<0.05$ was considered significant at a 95% confidence interval.

Results

The baseline erythrocytic and leucocyte parameters of the rats before induction of haemorrhagic and haemolytic anaemia were similar across the various groups as shown in Tables 1 and 2, respectively. Three days post-induction of anaemia however, the PCV values were significantly lower in the groups than the pre-induction values (Table 3). After 8 days post-induction, there was a correction of the anaemia in the extract and haematinic-treated groups. A significant ($p<0.05$) dose-dependent increase in pack cell volume (PCV), red blood cell (RBC) and haemoglobin concentration (Hb) was observed in anaemic rats treated with *Parquetina nigriscens* extract at doses of 25 or 50mg/kg (Table 4).

In the haemorrhagic group, PCV in the extract-treated rats was 46.6±0.89% and 49.0±4.35%, while that of the control rats was 46.8±3.56%. The results from the extract-treated rats were comparable to those treated with $FeSO_4$ at 2mg/kg (47.2±3.63%), but significantly higher than the PCV values in the untreated group (40.75 ± 5.18%). A similar pattern showing restored PCV, RBC and Hb values was observed for red cell indices in the haemolytic anaemia model. The haemolytic untreated group also had higher MCHC than those treated with the extract. The result from the haemolytic group also showed a related pattern to what was observed in the haemorrhagic group. However, MCV obtained in haemolytic untreated rats was slightly lower than in anaemic rats treated with the extract or $FeSO_4$, although, non-significantly (Table 4).

A dose-related, but non-significant ($p>0.05$) decrease in leucocyte count was observed in the anaemic rats treated with *Parquetina nigriscens* extract at both dosages (25 and 50 mg/kg). For example, in the haemorrhagic group, leucocyte counts for the extract-treated rats were 3.12 ± 0.34 x 10^3/µL and 3.10 ± 0.80 x 10^3/µL, while that of the control rats was 3.55 ± 0.68 x 10^3/µl. The total WBC count from the extract-treated rats was also comparable to those treated with 2 mg/kg $FeSO_4$ (3.63 ± 1.10 x 10^3/µL), but significantly lower than the untreated haemorrhagic (5.11 ± 0.80 x 10^3 /µl) group (Table 5). The result from the haemolytic group also showed a dose-dependent decrease in leucocyte counts as observed in the haemorrhagic group. However, the leucocyte count of the $FeSO_4$-treated rats was significantly higher than that of the rats treated with the extract at the dose of 25 mg/kg. The leucocyte count of the haemolytic untreated group (7.32 ± 0.69 x10^3/µL) was also significantly ($p<0.05$) higher than the treated groups (4.83±2.01 and 3.70±1.91 at 25mg/kg and 50mg/kg, respectively) (Table 5).

In the haemorrhagic group, the platelet counts of the extract-treated rats at doses 25 or 50 mg/kg (1.04 ± 0.25 and 1.02 ± 0.13 x10^3/µL) and $FeSO_4$ treated rats (1.23 ± 0.13 x 10^5 /µL) was comparable to those of non-anaemic control but significantly ($p<0.05$) higher than

the untreated group (0.50 ± 0.09 x10^4/µl). Furthermore, the differential lymphocytes and neutrophil counts in the haemolytic untreated rats were significantly higher than those in the control and haemolytic rats treated with either of the extract dosages. However, monocyte and eosinophil counts were similar across the groups (Table 5).

The erythrocyte osmotic fragility of the anaemic rats – treated and untreated is shown in Fig. 1. It was generally observed that rats with induced haemorrhagic anaemia had comparable erythrocyte osmotic fragility that was comparable to or even lower than that of the non-anaemic control. Erythrocyte osmotic fragility was also lower in these rats compared to those with haemolytic anaemia, especially at 0.7% and 0.9% NaCl concentrations, although both categories had similar levels of anaemia (Table 3). At 0.7% NaCl concentration, haemorrhagic anaemic rats treated with 50mg/kg PN extract (Group C) and $FeSO_4$ (Group D) had lower ($P<0.05$) erythrocyte osmotic fragility (12.71% and 12.06%, respectively) than those of non-anaemic and anaemic controls (Control and HA only). They were also lower than the untreated haemorrhagic group (Group E – HA only) and any of the haemolytic anaemia group, whether treated with the extract, $FeSO_4$ or untreated (HL+PLNE25, HL+PNLE50, HL+$FeSO_4$ and HL only). Similarly, Group B rats with haemorrhagic anaemia treated with 25mg/kg PN extract had lower osmotic fragility than all the haemolytic anaemia groups, treated or untreated, but was similar to the non-anaemic control (Group A), HA+PNE50 (Group C) and HA+ $FeSO_4$ - (Group D). Also, groups C and D (with haemorrhagic anaemia treated with 50mg/kg PN extract and $FeSO_4$) had lower fragility than those of haemorrhagic untreated (Group E) as well as the haemolytic groups – treated or untreated (Figure 1).

Table 1: Baseline erythrocyte parameters of adult male rats before induction of haemolytic and haemorrhagic anaemia

	PCV (%)	HB (g/dl)	RBC (x10^6/µl)	MCV (fl)	MCH (ρg)	MCHC (g/dl)
GRP A (Control)	46.4±1.67	16.4±1.62	8.54±1.21	55.02±6.69	19.3±2.28	35.28±2.23
GRP B (HA + PNLE25)	47.0±3.80	15.7±1.06	7.89±0.52	59.51±1.35	19.97±0.48	33.5±0.69
GRP C (HA + PNLE50)	48.8±5.80	16.08±0.78	8.35±0.52	58.39±5.22	19.29±1.20	33.18±2.63
GRP D (HA + FeSO4)	49.6±5.22	16.66±1.90	8.36±0.91	59.36±1.93	19.95±1.57	33.61±2.23
GRP E (HA + Only)	45.2±1.48	14.72±0.63	7.38±0.16	61.30±2.86	19.95±0.56	32.59±1.65
GRP G (HL + PNLE25)	44.8±4.86	15.78±2.86	7.74±1.37	58.38±3.88	20.38±0.60	35.04±2.83
GRP F (HL + PNLE50)	49.0±2.64	17.1±1.89	8.60±0.81	57.14±2.76	19.86±0.86	34.83±2.24
GRP H (HL + FeSO4)	46.0±5.04	15.30±1.44	7.74±0.98	59.52±1.45	19.83±0.87	19.78±0.75
GRP I (HL Only)	47.2±1.30	13.44±1.37	7.26±0.66	65.50±6.82	18.64±2.45	28.47±2.83

Legend: HA = Haemorrhagic anaemia, HL = Haemolytic anaemia, PNLE50 = 50mg/kg *Parquetina nigriscens* methanol leaf extract, PNLE25 = 25mg/kg *Parquetina nigriscens* methanol leaf extract. Values are means ± SD of 5 rats per group

Table 2: Baseline leucocytes and platelet parameters of adult male rats before induction of haemolytic and haemorrhagic anaemia.

	WBC ($x10^3/\mu L$)	LYMPH ($x10^3/\mu L$)	NEUT ($x10^3/\mu L$)	MONO ($x10^3/\mu L$)	EOSINO ($x10^3/\mu L$)	PLATELET ($x10^5/\mu L$)
GRP A (Control)	4.05±0.38	2.96±0.22	0.98±0.15	0.073±0.019	0.049±0.016	1.11±0.077
GRP B (HA + PNLE25)	3.59±0.31	2.67±0.28	0.81±0.10	0.064±0.029	0.043±0.021	1.26±0.015
GRP C (HA + PNLE50)	4.02±0.16	2.74±0.27	1.17±0.092	0.056±0.021	0.056±0.021	1.10±0.010
GRP D (HA + FeSO4)	4.01±0.48	2.91±0.38	0.93±0.13	0.065±0.026	0.103±0.021	1.20±0.022
GRP E (HA + Only)	3.38±0.42	2.17±0.26	1.09±0.33	0.063±0.034	0.027±0.015	1.26±0.037
GRP G (HL + PNLE25)	3.52±0.80	2.51±0.72	0.89±0.13	0.058±0.024	0.057±0.025	1.27±0.017
GRP F (HL + PNLE50)	4.24±0.88	2.86±0.60	1.13±0.29	0.121±0.031	0.139±0.046	1.0±0.057
GRP H (HL + FeSO4)	3.66±0.79	2.59±0.65	0.96±0.24	0.059±0.027	0.051±0.022	12.42±0.35
GRP I (HL Only)	3.75±0.87	2.79±0.67	0.79±0.27	0.054±0.027	0.078±0.036	11.18±0.15

Legend: HA = Haemorrhagic anaemia, HL = Haemolytic anaemia, PNLE50 = 50mg/kg *Parquetina nigriscens* methanol leaf extract, PNLE25 = 25mg/kg *Parquetina nigriscens* methanol leaf extract, Values are means ± SD of 5 rats per group.

Table 3. Packed cell volume (PCV) of adult male Wistar rats, pre- and post-induction of haemorrhagic and haemolytic anaemia subsequently treated with methanol extract of *P. nigrescens* leaf extract

	Pre-induction PCV (%)	Post-induction PCV (%)	Post Treatment PCV (%)
GRP A (Control)	46.4 ± 1.67	47.00 ± 1.00	46.4 ± 1.67
GRP B (HA + PNLE25)	47.0 ± 3.80	36.10 ± 0.89*	46.6 ± 0.89
GRP C (HA + PNLE50)	48.8 ± 5.80	34.80 ± 1.64*	49.0 ± 4.35 *@
GRP D (HA + FeSO4)	49.6 ± 5.22	35.40 ± 1.14*	47.2 ± 3.63
GRP E (HA + Only)	45.2 ± 1.48	35.25 ± 1.25*	40.75 ± 5.18
GRP G (HL + PNLE25)	44.8 ± 4.86	37.20 ± 1.92*	48.6 ± 1.94
GRP F (HL + PNLE50)	49.0 ± 2.64	36.8 ± 0.83*	46.4 ±3.36
GRP H (HL + FeSO4)	46.0 ± 5.04	35.60 ± 2.07*	46.8 ± 1.78
GRP I (HL Only)	47.2 ± 1.30	37.00 ± 1.41*	43.7 ± 2.23

Significant difference compared to pre-induction (*) or post-induction (@) value in each row at P > 0.001. Values are means ± SD of 5 rats per group.

Table 4: Haematological parameters of anaemic male Wistar rats treated with *Parquetina nigriscens* extract

	PCV (%)	HB (g/dl)	RBC ($x10^6$/µL)	MCV (fl)	MCH (ρg)	MCHC (g/dl)
GRP A (Control)	46.4±1.67	15.50±1.38	7.67±0.76	61.10±1.61	20.2±0.2	33.0±0.50 #***
GRP B (HA + PNLE25)	46.6±0.89	15.60±0.3	7.67±0.2	60.70±1.28	20.4±0.39	33.6±0.34
GRP C (HA + PNLE50)	49.0±4.35 @*	16.40±1.31 @*	8.20±0.62 @*	59.50±2.61	20.0±0.7	33.6±0.36
GRP D (HA + FeSO4)	47.2±3.63	15.74±1.32	7.93±0.76	59.50±2.3	19.8±0.8	33.4±0.74
GRP E (HA Only)	40.8±5.18	13.60±1.47	6.76±0.98	60.45±1.40	20.2±0.93	33.4±0.74
GRP G (HL + PNLE25)	48.6±1.94	16.54±0.49	8.22±0.31	59.12±0.95	20.1±0.40	34.0±0.42#**
GRP F (HL + PNLE50)	46.4±3.36	15.52±1.27	7.71±0.72	60.28±1.62	20.2±0.69	33.4±0.74 #***
GRP H (HL + FeSO4)	46.8±1.78	16.10±0.64	7.88±0.33	59.40±1.44	20.4±0.80	34.4±0.62 #**
GRP I (HL Only)	43.7±2.23	16.85±1.74 $*	8.02±0.61	59.23±4.75	21.0±2.42	34.7±2.21

Legend

Significant difference compared to control (@), haemorrhagic untreated ($), and haemolytic untreated (**#**). * = p<0.05, ** = p<0.01, *** = p<0.001. HA = Haemorrhagic anaemia, HL = Haemolytic anaemia, PNLE50 = 50mg/kg *Parquetina nigriscens* methanol leaf extract, PNLE25 = 25mg/kg *Parquetina nigriscens* methanol leaf extract. Values are means ± SD of 5 rats per group.

Table 5: Leucocytes and platelet counts of anaemic male Wistar rats after treatment with *Parquetina nigriscens* extract

	WBC ($x10^3$/µL)	LYMPH ($x10^3$/µL)	NEUTRO ($x10^3$/µL)	MONO ($x10^3$/µL)	EOSINO ($x10^3$/µL)	PLATELET $x10^5$/µL
GRP A (Control)	3.55±0.68	2.56±0.56	0.82±0.11	0.07±0.02	0.05±0.016	1.03±0.19 $***
GRP B (HA + PNLE25)	3.12±0.34	2.21±0.14	0.80±0.04	0.08±0.01	0.04±0.021	1.04±0.25 $***
GRP C (HA + PNLE50)	3.10±0.80	2.26±0.62	0.70±0.13	0.09±0.05	0.06±0.021	1.02±0.13 $***
GRP D (HA + FeSO4)	3.63±1.10	2.64±0.88	0.87±0.21	0.05±0.02	0.10±0.021	1.23±0.13 $***
GRP E (HA Only)	5.11±0.80	3.63±0.61	1.34±0.23	0.09±0.03	0.03±0.015	0.50±0.09
GRP G (HL + PNLE25)	3.70±1.91 #**	2.78±1.40 #**	0.77±0.41 #**	0.06±0.03	0.06±0.025	0.88±0.19
GRP F (HL + PNLE50)	4.83±2.01	3.51±1.52	1.13±0.29	0.08±0.03	0.14±0.046	0.81±0.06
GRP H (HL + FeSO4)	3.05±0.47 #***	2.25±0.35 #***	0.69±0.14 #**	0.04±0.01	0.05±0.022	0.86±0.07
GRP I (HL Only)	7.32±0.69 @***	5.63±0.76 @***	1.54±0.20	0.09±0.04	0.08±0.036	0.78±0.13

Legend

Significant difference compared to control (@), haemorrhagic untreated ($), and haemolytic untreated (**#**). * = p<0.05, ** = p<0.01, *** = p<0.001. HA = Haemorrhagic anaemia, HL = Haemolytic anaemia, PNLE50 = 50mg/kg *Parquetina nigriscens* methanol leaf extract, PNLE25 = 25mg/kg *Parquetina nigriscens* methanol leaf extract. Values are means ± SD of 5 rats per group

Plate 1. *Parquetina nigriscens*

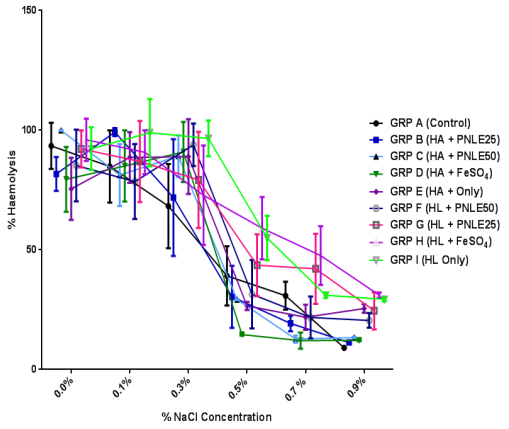

Figure 1: Osmotic fragility of anaemic rats treated with methanol extract of *Parquetina nigrescens* leaves for 7 days

Legend

HA = Haemorrhagic anaemia, HL = Haemolytic anaemia, PNLE50 = 50mg/kg *Parquetina nigriscens* methanol leaf extract, PNLE25 = 25mg/kg *Parquetina nigriscens* methanol leaf extract.

Discussion

Haemorrhagic and haemolytic anaemia are entrenched common conditions in almost all societies, whether developed, developing or underdeveloped. This assertion is given credence by many of the aetiologies of these conditions, ranging from haemorrhage due to trauma, surgery, blood-sucking parasites and haemolysis caused by haemoparasites and haemolytic diseases which occur in both humans and animals (Kauvar et al., 2006, Ardissino et al., 2016). The present study which investigated the haematinic properties of methanol leaf extract of *Parquetina nigriscens*

sought to solve the menace of anaemia in Africa, where the plant grows unhindered. The study showed that *P. nigrescens* reversed the haematological derangements observed post-induction of haemorrhagic and haemolytic anaemia in Wistar rats.

Anaemia is the decline of total circulating blood cells; packed cell volume, with a particular decline of red blood cells which results in a reduction in the oxygen-carrying capacity of the blood (Lopez *et al.*, 2016). This study showed that *P. nigrescens* restored packed cell volume, red blood cell counts, haemoglobin concentration, as well as red cell indices viz; MCV, MCH and MCHC levels to values similar to pre-induction levels. The extract also corrected the leucopenia associated with anaemia by restoring the total WBC, lymphocytes, neutrophil, eosinophil, monocytes and platelet counts to values similar to non-anaemic levels. The haematinic effect of the extract was similar to that of ferrous sulphate ($FeSO_4$), a standard haematinic used for the treatment of iron deficiency anaemia, which is one of the most common causes of anaemia in developing countries (Morton 2019).

This study confirmed that the extract of *Parquetina nigriscens* has significant haematinic and erythropoiesis-stimulating effects. It also corroborates and validates previous report by Saba et al. (2010), who

reported antidiabetic and haematinic effects of the *P. nigriscens* in alloxan-induced diabetes in rats, although their observations were on complications of alloxan-induced diabetes including anaemia. This study demonstrated that *P. nigriscens* extract could be recommended as a haematinic in all forms of anaemia, haemorrhagic or haemolytic to stimulate rapid erythropoiesis. The haematinic effect of *P. nigriscens* in this study is believed to be due to the high crude protein (40.96%), Vitamin B_{12} (Cyanocobalamin), Folic acid, Fe^{2+} and essential amino acids (Imaga et al., 2010). *Parquetina nigriscens* also contains significant quantities of antioxidants including flavonoids which may be responsible for its high free radical scavenging activities and anti-lipid peroxidation effects (Ayoola et al., 2011).

The result of erythrocyte osmotic fragility showed significantly lower erythrocyte osmotic fragility on extract or $FeSO_4$-treated anaemic rats while the untreated rats had higher erythrocyte osmotic fragility. This indicated regenerative anaemia with more immature cells, which are more resistant to osmotic lysis in the extract-treated rats. Erythrocyte osmotic resistance has been known to increase in cases of regenerative anaemia as a result of reticulocytosis and the presence of young immature red blood cells in circulation (Balan *et al.,* 2019; Shmukler *et al.*, 2020). This observation could also be a result of the presence of antioxidants in *P. nigriscens* extract. Ayoola et al. (2011) and Akinrinmade et al. (2016) reported significant antioxidant activities of *P. nigriscens* due to the presence of high flavonoids, essential amino acids vitamin B_{12} and iron content of the extract (Imaga et al., 2010).

Several epidemiological studies have shown the high prevalence of haemo protozoan parasite infections in man and animals in developing countries (Garcia et al., 2015, Drew et al., 2017). Thus, the haematinic effect of *P. nigriscens* observed in the present study is especially important in developing countries, including West African countries where subliminal haemolytic anaemia subsists because of haemo protozoan parasites including *Plasmodium falciparum* (one of the causative agents of malaria fever) and other blood parasites in man and animals (Moxon *et al.*, 2020).

Conclusion

Treatment with *P. nigriescens* extract increased haematological parameters and reduced erythrocyte osmotic fragility in anaemic rats. It could therefore be recommended for use, especially at 50mg/kg in cases of haemorrhagic and haemolytic anaemia. However, further fractionation and identification of the phytochemical constituents that might have complemented the activities of folic acid, vitamin B_{12} and iron in *P. nigriescens* extracts should be further investigated.

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