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Hematological studies of *Parquetina* nigrescens on haemorrhagic anaemic rats

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Summary
The effect of administration of aqueous extract of *Parquetina nigrescens* on haematological parameters was investigated in haemorrhagic anaemic rats from which about 30% of blood volume was removed through ocular vein under chloroform anesthesia. Anaemic rats were administered 400mg/kg, 800mg/kg and 1600mg/kg of *P. nigrescens* aqueous extract daily for four weeks.

Significant ($P < 0.05$) progressive and dose related increases were observed in red blood cell count (RBC, 10^6/ml), Haemoglobin (gm/dl), Haematocrit (Hct, %) and Reticulocyte (%) while a decrease in white blood cell count (WBC $10^3\mu l$) was observed in test rats treated with aqueous extract of *P. nigrescens* when compared with both normal and bled control rats.

Keywords: *Haematological parameters, haemorrhagic anaemia, wistar rats, parquetina nigrescens*

Résumé
L'effect de l'administration de l'extrait aqueux de *Paraquetina nigrescens* sur les parmetres haematologiques a ete inventorie chez des rats attenits d'anemie hemorragique, desquels environ 30% du volume de sany a ete enleve par la veine oculaire sous anesthesie au chloroforme. 400mg/dg. 800mg/kg et 1600mg/kg d'extrait aqueux de *P.nigrescens* ont ete administers aux rats anemies par jour pendant quatre semaines.

Une progression significative ($P < 0.05$) et l'augmentation en relation a la dose ont cte observees dans les decomptes des globules rouges (RBC, 10^6/ml), hemoglobine (gm/de), hematocrite (HCT, %) et reticulocyte (%) alors qu'une diminution en decompte des globules blancs (WBC $10^3\mu L$) a ete observee chez lec rats cobailles traits a l'extrait angneux de *P.nigrescens* lorsque la comparaison se foit a la fois avec les rats normaux et les rats de controles don't le sanrg a ete enleve.

Introduction

Anaemia is considered an important health problem affecting as many as 2.2 billion individuals worldwide, consisting about 30% of world's population [1,2]. It is prevalent in children [3,4], reflects socio-economic status [5,6] and it is the manifestation of many underlying diseases [7]. In the developing regions of the world, the most prevalent forms of anaemia are those caused by nutritional factors such as iron deficiency and vitamin deficiency [8]. Others are haemolytic anaemia as a result of persistent malarial infection [9] and haemorrhagic anaemia due to chronic haemorrhage from intestinal infestation with hookworm, liver flukes, coccidian and other external parasites [7, 10]. Sickle cell disease, a hereditary haemoglobinopathy, is common among dark skinned people. It is characterized by chronic haemolytic anaemia and recurrent sickle cell crises, which eventually cause organ dysfunction and frequent bacterial infections [11].

Although orthodox medications are readily available for the treatment of anaemia, most Africans still turn to herbal remedies for treatment of this condition. One of the medicinal plants commonly used in Nigeria is *Parquetina nigrescens*, a twiner with leathery glossy leaves. It is a popular herb for the preparation of herbal medications used for treatment of diverse diseases. The leaves and latex are used for the treatment of rickets, diarrhoea, skin lesions and other topical skin diseases [13, 14]. Decoction of the bark is given as a cardiac tonic, while the leaf and root decoction have been recommended for treatment of gonorrhoea and menstrual disorders. The root is also reputed for its anti-rrheumatic property [14]. The plant has been used as an ingredient in medications for insanity [15] and as an aphrodisiac [16].

The present study is designed to assess the reputed haematinic property of aqueous extract of leaves of *P. nigrescens* by investigating its effect on haematological parameters in haemorrhagic anaemic rats.

Materials and Methods

Plant Material: P. nigrescens, whole plant was collected from University of Ibadan Campus in April 1998 and authenticated at the herbarium of the Department of Botany and Microbiology, University of Ibadan where voucher specimen was deposited.

Extraction and Isolation: Shade-dried, powdered plant (560g) was soaked in about 3 litres of distilled water for 24 hours and boiled for 30 minutes. The mixture was then allowed to cool and filtered using a glass funnel plugged with glass wool. The residue obtained was further extracted twice as before. The resulting filtrate was then concentrated with a rotary evaporator to a thick brown syrup. This was further dried in an oven at 40°C. The yield was 269.7g/kg.

Animals: Twenty-five wistar albino rats weighing between 140g and 150g obtained from the animal house of the Primate Colony, Biochemistry Department, University of Ibadan were used. The animals were acclimatized for 10 days in the animal house of the Biochemistry Department before the experiment commmced. They were fed *ad. libitum* on standard rodent pellets obtained from Ladokun Feed Ltd, Mokola, Ibadan,

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Nigeria. The animals were also allowed free access to clean drinking water.

Haematological Studies: The rats were divided into 5 groups of 5 rats each and labeled A, B, C, D, E. Group A served as the normal control (unbled) and received vehicle (distilled water) only. Groups B, C, D and E were bled 30% of their total blood volume as described by Schalm *et al.* [17]. Group B served as the bled control and received vehicle only. Groups C, D and E were given, orally, graded doses of *P. nigrescens* (400, 800 and 1600mg/kg) respectively daily for 4 weeks. Blood was collected through the caudal vein 24 hours after bleeding to establish the baseline haematology values. Blood was also collected from the rats on days 7, 14, 21 and 28. The method described by Schalm *et al.* [17] was used for the determination of WBC, RBC, Haematocrit, and Haemoglobin while the method of Dacie and Lewis [18] was used for determination of reticulocyte. MCV, MCH and MCHC were calculated as described by Schalm *et al.* [17]. The total plasma protein was determined by the method of Cornell *et al.* [19].

Statistical Analysis: The results were expressed as means ± Standard Deviation. The significance of difference between means was tested by the Analysis of Variance (ANOVA) and results were regarded as significant at ($P < 0.05$).

Results

Table 1: Haematological Parameters of Rats 24hrs After bleeding

Parameters	Group A	Control group B	Group C	Group D	Group E
RBC[a](x10^6/µl)	7.16±0.25	5.78±0.9[a]	5.97±0.23[a]	5.585.58±0.12[a]	5.85±0.18[a]
Hb (g/dl///0	11.45±0.26	9.15±0.29[a]	9.350.21[a]	9.30±0.31[a]	9.23±0.61[a]
Hct (%)	41.25±1.29	35.00±1.22[a]	35.750.83[a]	35.00±1.41[a]	34.75±2.86[a]
Reticulocyte (%)	3.50±0.50	2.25±0.43[a]	2.500.50[a]	2.25±0.83[a]	2.00±0.00[a]
WBC (10^3/µl)	11200.00±748.00	14650.00±1211.40[b]	14525.00±1084.80[b]	15000.00±883.18[b]	400.00±839.64[b]
MCV (fl) +	57.69± 3.23	56.45±7.71	59.38±1.86	62.80±2.81	59.48±5.48
MCH (pg)	16.00±0.62	15.85±0.64	15.66±0.49	16.69±0.55	15.79±1.23
MCHC (%)	25.77±3.36	26.16±0.19	26.16±0.19	26.58±0.45	26.59±0.45

n = 5
a = *Significantly lower compared to unbled control rats Group A*
b = *Significantly higher compared to unbled control rats Group A*
$P < 0.05$
Group A = *unbled control*
Group B = *bled control*
Group C = *bled treated with 400mg/kg. P. nigrescens extract*
Group D = *bled treated with 800mg/kg P. nigrescens extract*
Group E = *bled treated with 1,600mg/kg P. nigrescens extract*

Table 1 presents the effect of bleeding on rats, twenty-four hours after bleeding. Results show that the mean values of RBC, HB, Hct and reticulocyte in the bled group, (Group B), are significantly lower ($P < 0.05$) by 19%, 20%, 15% and 30% respectively than values in the unbled control rats (Group A). There was also a significant ($P < 0.05$) increase of 30% in WBC count in the bled rats (Group B) compared to unbled control (Group A). The alterations in erythrocyte indices (MCV, MCH and MCHC) were however not significant.

The effect of bleeding and treatment with graded doses of *P. nigrescens* extract on bled rats for four weeks are presented in Figures 1–5. One week after bleeding, the RBC, Hct, Reticulocyte and Hb concentrations in the bled rats (Group B) were still significantly lower than those in the unbled control (Group A). Definite improvements noticed in week 2 progressed into week 3 when most blood parameters had returned to values within the normal range.

One week of treatment with extract of P. nigrescens resulted in 19%, 27% and 31% increase in RBC count of rats in groups C, D, and E treated with 400mg, 800,g and 1,600mg/kg (Fig. 1). This elevation was progressive and in spite of a drop between weeks 3 and 4, the RBC level was still much higher in week 4 than those in the unbled and bled controls (Groups A and B respectively).

Similar dose related increases were observed in the values of Hb (Fig. 2), Hct (Fig. 3), and Reticulocytes (Fig. 5) while dose related decreases were observed in the WBC count (Fig. 4).

Most of the blood parameters studied had reverted to values within normal values within one week of treatment with extract and progressed above normal values by week 4.

The red cell indices, MCV, MCH and MCHC are shown in Table 2. The elevation in MCV value noticeable in week 2 in the bled control (Group B) had returned to normal values by week 3. Treatment with extract reduced time of recovery of MCV to one week in groups C, D and E. The alterations in values of MCH and MCHC were however not significant.

Table 2: Effect of Parquetina nigrescens on the red blood cell indices, MCV, MCH and MCHC in haemorrhagic anaemic rats.

MCV (fl)

Groups	A	B	C	D	E
Week 0	57.69±3.23	56.45±7.71	59.39±1.86[a]	62.80±2.81[a]	59.48±5.48[a]
Week 1	58.13±1.21	62.42±0.40[a]	57.77±1.46	57.14±0.86	57.19±1.48
Week 2	57.82±1.33	59.71±0.24[a]	56.19±2.26	56.19±2.20	53.78±0.61[b]
Week 3	55.67±0.31	56.05±0.62	55.09±1.77	54.78±2.36	55.53±0.70
Week 4	57.60±2.45	59.51±7.53[a]	61.27±1.99[a]	60.96±1.27[a]	63.67±2.29[a]
MCH (pg.)					
Week 0	16.00±0.62	15.85(0.64	15.66±0.49	16.69±0.55	15.79±1.23
Week 1	16.07±0.46	16.38±0.29	16.04±0.58	15.61±0.39	15.73±0.33
Week 2	16.20±0.79	16.67±0.30	15.51±0.59	15.42±0.38	14.98±0.24
Week 3	15.77±0.78	15.43±0.33	15.19±0.43	15:09±0.39	14.67±0.17
Week 4	17.12±1.65	17.40±1.94	16.32±1.59	17.66±1.15	17.89±0.89
MCHC (%)					
Week 0	25.77±3.36	26.16±0.19	26.16±0.19	26.58(0.45	26.59±0.45
Week 1	27.64±0.43	26.04±0.43	27.76±0.54	27.39±0.19	27.49±0.15
Week 2	28.02±0.73	28.39±0.82	27.59±0.16	27.59±0.34	27.76±0.35
Week 3	27.59±0.43	27.37±0.17	27.59±0.18	27.82±0.11	27.66±0.38
Week 4	29.47±1.76	28.07±0.33	26.04±2.15	28.99±2.39	28.09±0.46

n = 5

a = *Significantly higher compared to unbled control group A*

b = *Significantly lower compared to unbled control group A*

Group A = unbled control. Group B = bled control

Groups C, D and E = Bled and Treated with P. nigrescens extract 400mg, 800mg and 1,600mg/kg respectively.

Fig. 1 EFFECT OF PARQUETINA NIGRESCENS ON RBC IN HAEMORRHAGIC ANAEMIC RATS

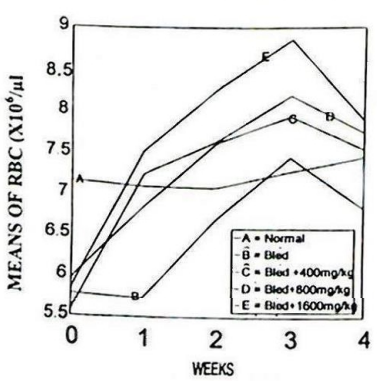


Fig. 2 EFFECT OF PARQUETINA NIGRESCENS ON HAEMOGLOBIN CONCENTRATION IN HAEMORRHAGIC ANAEMIC RATS

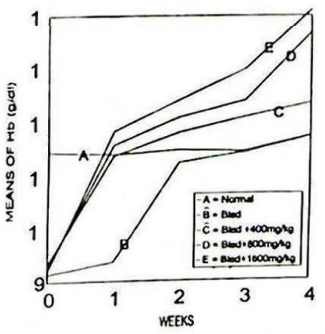


Fig. 3

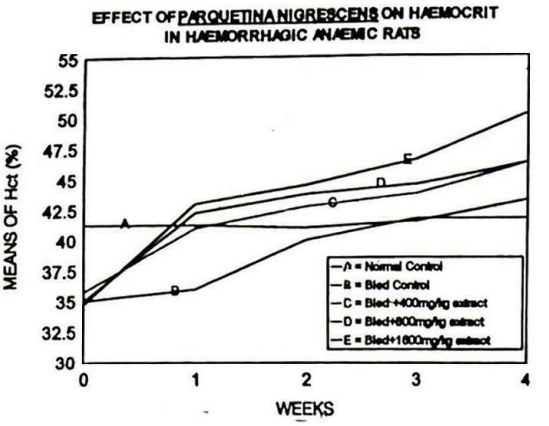


Fig. 4

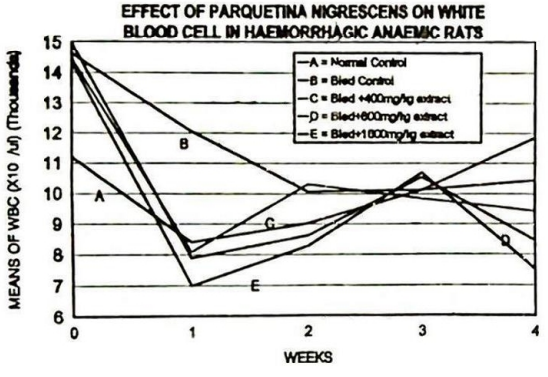
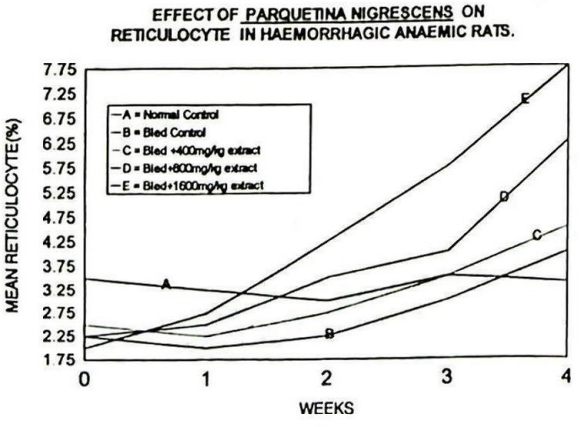


Fig. 5



Discussion

The results of this investigation showed that the body mechanism of bled rats responded to acute blood loss by rapid regeneration of RBC within 3 weeks. By the end of 4 weeks, the mean values of RBC, Hb, Hct and WBC in the bled control rats were already similar to those of the normal rats and within the normal range reported by the Canadian Council on Animal Care [20]. The recovery time of between 2 and 3 weeks is however longer than 1 week reported by Davis et al [21]. This difference could be due to differences in the ages of rats used for while Davis et al used young rats, mature rats were used in this study.

Administration of aqueous extract of *P. nigrescens* hastened the recovery of treated rats from effect of bleeding. Even the lowest administered dose of 400mg/kg reduced the recovery time of most blood parameters from about 3 weeks in the bled control to 1 week. In addition, the extent of recovery was dose related with the highest dose of 1600mg/kg effecting the highest change. Furthermore, the recovery was progressive such that after four weeks of continuous treatment, the RBC, Hb and Reticulocyte concentrations were higher in the treated rats than in the normal (unbled) control rats.

Bleeding often leads to leucocytosis [22]. This was confirmed in this study by significant increases in the WBC count of all bled rats (Groups B, C, D and E, Table 1), 24 hours after bleeding. Treatment with *P. nigrescens* however cleared the excess WBC from the blood within 1 week of treatment. Significant correlation with diagnostic values has been demonstrated between RBC, Hb, Hct and RBC indices (MCV, MCH, MCHC) in rats and humans [23, 24]. The observed elevation of MCV in bled rats by week 1 is indicative of anaemia due to acute blood loss. It is also indicative of microcytosis probably as a result of iron deficiency from acute blood loss while the reduced value of MCV in rats treated with the extract may be indicative of macrocytosis.

P. nigrescens water extract, in speeding up recovery from bleeding by increasing the levels of RBC, Hb and reticulocytes and by improving other red blood cell indices may have erythropoietic properties and can therefore be used as a haematinic, thus confirming its traditional use for the treatment of anaemia.

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