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## Effect of *Parquetina nigrescens* on Erythrocyte Indices and Serum Electrolytes of Rats Following Acute Blood Loss

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**Abstract:** The effect of graded doses of aqueous leaf extract of *Parquetina nigrescens* on erythrocyte indices and serum electrolytes was studied for two weeks on rats following acute blood loss. Red blood cell count, haemoglobin concentration, haematocrit, reticulocyte population and erythrocyte osmofragility were used as erythrocyte indices. The plasma electrolytes concentrations of sodium, potassium, calcium and bicarbonate were analysed. *Parquetina nigrescens* significantly ( $p < 0.05$ ) increased the erythrocyte indices which were initially reduced by bleeding except for erythrocyte osmofragility which instead decreased significantly ( $p < 0.05$ ). *Parquetina nigrescens* had no effect on serum electrolyte except for sodium ion concentration which increased significantly ( $p < 0.05$ ). The improved erythrocyte indices and reduced erythrocyte osmofragility are indications of erythropoietic potential of *Parquetina nigrescens*.

**Key words:** *Parquetina nigrescens*, blood loss, erythrocyte osmofragility, erythrocyte indices, plasma electrolytes

### INTRODUCTION

*Parquetina nigrescens* (periplocaceae) a shrub found in equatorial West Africa<sup>[1,2]</sup> has been in traditional medicine practice for centuries. The parts of the plant used for traditional medicine include the leaves, roots and the latex<sup>[3]</sup>. In Oyo state Nigeria, the leaves have been reputed for treatment of helminthiasis (intestinal worm) while the roots are reputed for use as antirheumatic purpose<sup>[4]</sup>. Over the years, *Parquetina nigrescens* has been used as an ingredient in the medications for insanity<sup>[5]</sup> and as an aphrodisiac in East Africa<sup>[6]</sup>. Decoction of the bark is given as a cardiactonic, while the leaf and root decoction have been recommended for the treatment of gonorrhoea and menstrual disorders<sup>[3]</sup>. While the whole plant is used to stupefy fish in Ghana and Liberia, the leaves and latex are used for the treatment of rickets, diarrhoea, skin lesions and tropical skin diseases<sup>[3,7]</sup>. The leaves of *P. nigrescens* have been used for the treatment of wound in Africa<sup>[1,2]</sup> and have sympathomimetic effect<sup>[8]</sup>.

The hydroxymethanolic extract of *P. nigrescens* reference solution has been shown to exert a stimulating and spasmogenic action on pregnant rats by increasing

the amplitude of spontaneous isometric contractions and a slight elevation of muscular basic tonus<sup>[9]</sup>. Hence having an oxytocin like effect which is characterised by an extra cellular influx of calcium responsible for the increase of the maximum isometric concentration amplitude<sup>[9]</sup>. *P. nigrescens* is also a constituent of a commercial herbal preparation (Jubi Formula) in Nigeria used in the treatment of anaemia in humans<sup>[10]</sup>. The Jubi Formula was shown to restore decreased haematocrit and haemoglobin concentration in *Trypanosoma brucei* induced anaemia<sup>[10]</sup>.

In our earlier study, we examine the antianaemic activity of aqueous extracts of *P. nigrescens* leaf on haemorrhagic anaemia induced in rats, which yielded a positive result<sup>[11]</sup>. The present study investigates the changes in erythrocyte indices in relation to osmofragility and serum electrolytes in rats treated with *Parquetina nigrescens* following acute blood loss in relation to the antianaemic activity earlier studied.

### MATERIALS AND METHODS

**Plant material:** *P. nigrescens*, whole plant was collected from the University of Ibadan campus in the month of April, 2004 and authenticated at the herbarium of the

Department of Botany and Microbiology, University of Ibadan.

**Preparation of extract:** The leaves were cleaned with tap water, air dried at room temperature and further extracted as earlier described by Agbor and Odettola<sup>[11]</sup>.

**Phytochemical screening:** Standard phytochemical screening procedures were used as described by Trease and Evans<sup>[12]</sup>. The presence of a bioactive compound was indicated by a colour change (qualitative tests).

**Animals:** Adult male Wister rats (160-180 g) and mice were obtained from the animal house of the Primate colony, Biochemistry Department University of Ibadan. The animals were acclimatized for 5 days prior to the start of the experiment.

The rats and mice were fed ad libitum on standard rodent pellets obtained from ladokum feed Ltd, Mokola, Ibadan Nigeria and were also allowed access to clean drinking water.

**Reagents:** All reagents used were of analytic grade obtained from BDH Company.

**Acute toxicity test:** Experimental design for the acute toxicity as earlier described by Agbor *et al.*<sup>[13]</sup> was adopted for this study while the method of Miller and Tainter<sup>[14]</sup> was applied in determining the LD<sub>50</sub> and LD<sub>100</sub>.

#### **Induction of haemorrhagic anaemia and treatments:**

Five rats were kept as normal control rats (Group 1); thirty rats were anesthetized with diethyl ether and bled (haemorrhagic anaemia) off 30% of their blood volume through the retro-orbital Venus plexus<sup>[15]</sup>. Twenty four hours after bleeding, the haemoglobin concentration of the bled rats was determined. Rats with haemoglobin concentration lower than 9.5 g dL<sup>-1</sup> were considered to be anaemic and hence randomly assigned to 4 Group of 5 rats each. Group 1 (normal control) and Group 2 (bled control) rats, were administered a daily oral single dose of distilled water while Group 3-5 (test) rats were administered daily oral single doses of 400, 800, 1600 mg *P. nigrescens* extract/kg body weight for a period of 2 weeks, respectively.

**Erythrocyte indices and electrolytes investigation:** Blood was collected from the caudal vein of the rats 24 h after bleeding and 2 weeks after treatment with *P. nigrescens* for determination of Red Blood Cell count (RBC),

Haemoglobin (Hb), Haematocrit (Hct) and Reticulocyte (Rct)<sup>[15-17]</sup>. At the end of the experiment, animals were anesthetized using diethyl ether and blood collected by cardiac puncture in to EDTA tubes was centrifuges at 3000 rpm for 15 min. The resulting plasma was used for the determination of osmofragility<sup>[18]</sup>, sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>) and bicarbonate (HCO<sub>3</sub><sup>-</sup>)<sup>[19]</sup>.

**Statistical analysis:** Experimental data were analysed by employing analysis of variance (ANOVA). Values were expressed as mean±SD.

## RESULTS

Table 1 presents the different bioactive constituents in the plant extract. Phytochemical screening of *P. nigrescens* revealed the presence of secondary metabolites such as glycosides, flavonoids, phenols, anthraquinones, saponins and tannins.

Bleeding resulted to a significant ( $p < 0.05$ ) decrease in the levels of the erythrocyte indices of albino rats (Group 2-5) as compared to the none bled rats (Group 1), confirming the presence of anaemia (Table 2).

A dose related and significant ( $p < 0.05$ ) increase in RBC, Hb, Hct and reticulocyte concentrations were observed in groups treated with plant extract (Group 3-5) when compared to the anaemic control group (Group 2). Erythrocyte indices of Group 5 animals even increased significantly ( $p < 0.05$ ) than the normal control rats (Group 1) (Table 3).

The mean serum electrolytes concentrations presented in Table 4 indicated decrease though not significant in the concentration of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup> in the bled control (Group 2) animals. Except for [Na<sup>+</sup>] which increased significantly ( $p < 0.05$ ) in response to treatment Group (4 and 5) all the other electrolytes were not affected by the treatment.

Bleeding induced a significant ( $p < 0.05$ ) decrease in erythrocyte osmofragility of the bled control rats (Group 2) when compared to the control (Fig. 1). This decrease was more significant ( $p < 0.05$ ) and dose related in groups treated with *P. nigrescens*. The osmofragility of bled control (Group 2) animals was significantly lower than that of unbled animals (Group 1) at [NaCl] of between 0.65 and 0.30%. Treatment with *P. nigrescens* induced further decreases in the erythrocyte osmofragility at [NaCl] of between 0.40 and 0.20%. At high [NaCl] there was no significant difference between the treated groups.

Table 1: Qualitative chemical analysis of *Parquetina nigrescens* leaf extract

Constituents	Name of test	Observation	Inference
Alkaloids	Mayer's test	No cream precipitate	Absent (-ve)
Glycosides	Fehlings solution	Brick-red precipitate	Present (+ve)
Cardiac glycosides	Salkowski test	Reddish brown colour	Present (+ve)
Flavonoids	Ammonium test	Yellow colouration	Present (+ve)
Phenols	Ferric chloride test	Red colour	Present (+ve)
Anthraquinones	Ammonium test	Red colour	Present (+ve)
Saponine	Frothing test	Persistent foam	Present (+ve)
Tannin	Ferric chloride test	Dark green colour	Present (+ve)

Table 2: Mean±SD of erythrocyte indices of albino rats 24 h after bleeding

Indices	Group 1	Group 2	Group 3	Group 4	Group 5
RBC ( $\times 10^6 \mu\text{L}^{-1}$ )	7.45±0.25	5.78±0.19 <sup>a</sup>	5.97±0.23 <sup>a</sup>	5.58±0.12 <sup>a</sup>	5.85±0.18 <sup>a</sup>
Hb (g dL <sup>-1</sup> )	11.45±0.26	9.15±0.29 <sup>a</sup>	9.35±0.21 <sup>a</sup>	9.30±0.31 <sup>a</sup>	9.23±0.61 <sup>a</sup>
Hct (%)	41.25±1.29	35.00±1.22 <sup>a</sup>	35.75±0.33 <sup>a</sup>	35.00±1.41 <sup>a</sup>	34.37±2.86 <sup>a</sup>
Reticulocyte (%)	3.25±0.50	2.25±0.43 <sup>a</sup>	2.50±0.50 <sup>a</sup>	2.25±0.83 <sup>a</sup>	2.00±0.00 <sup>a</sup>

<sup>a</sup>significantly lower compared to Group 1 p < 0.05, n = 5

Table 3: Mean±SD of erythrocyte indices after 2 weeks of experiment

Indices	Group 1	Group 2	Group 3	Group 4	Group 5
RBC ( $\times 10^6 \mu\text{L}^{-1}$ )	7.08±0.49	6.70±0.16	7.63±0.55 <sup>b</sup>	7.65±0.55 <sup>b</sup>	8.28±0.13 <sup>ab</sup>
Hb (g dL <sup>-1</sup> )	11.48±0.24	11.25±0.06	11.80±0.47	12.08±0.39 <sup>ab</sup>	12.40±0.18 <sup>ab</sup>
Hct (%)	41.00±1.83	40.00±0.82	42.75±1.50 <sup>b</sup>	43.75±0.96 <sup>ab</sup>	44.50±0.58 <sup>ab</sup>
Reticulocyte (%)	3.00±0.71	3.50±0.83	4.00±0.43 <sup>a</sup>	4.35±0.50 <sup>ab</sup>	4.50±0.43 <sup>ab</sup>

<sup>a</sup>significantly different compared to Group 1, <sup>b</sup>significantly different compared to Group 2 (p < 0.05), n = 5

Table 4: Mean±SD of electrolyte parameters after 2 weeks of experiment

Electrolytes	Group 1	Group 2	Group 3	Group 4	Group 5
Na <sup>+</sup> (mmol L <sup>-1</sup> )	164.25±4.72	159.25±3.59	163.75±4.03	177.00±5.49 <sup>ab</sup>	177.50±3.51 <sup>ab</sup>
K <sup>+</sup> (mmol L <sup>-1</sup> )	5.98±0.66	5.25±0.26	5.78±0.33	5.73±0.83	5.80±0.39
Ca <sup>2+</sup> (mg/100 mL)	8.55±0.66	8.38±0.09	8.60±0.08	8.65±0.13	8.48±0.17
HCO <sub>3</sub> <sup>-</sup> (mmol L <sup>-1</sup> )	24.75±0.50	23.00±0.82	22.75±1.26	24.25±0.51	23.75±0.50

<sup>a</sup>significantly different compared to Group 1, <sup>b</sup>significantly different compared to Group 2, (p < 0.05), n = 5

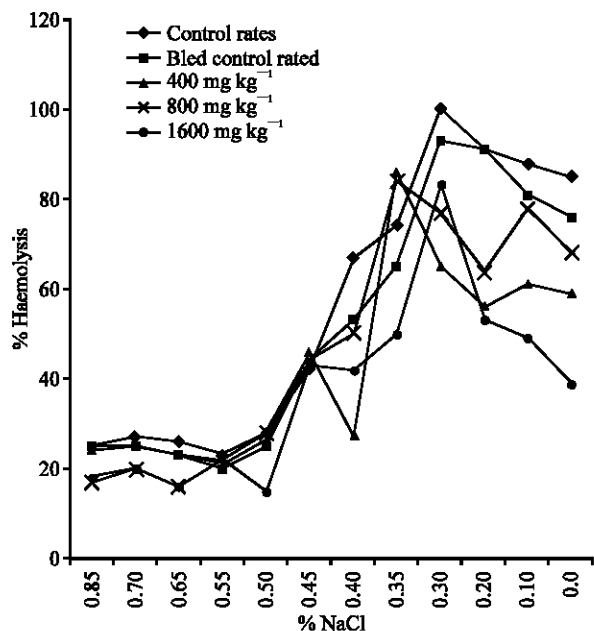


Fig. 1: Erythrocyte osmofragiligrams of rats after 2 weeks of experiment

## DISCUSSION

The phytochemical screening of *Parquetina nigrescens* revealed the different bioactive compounds it contains. Tannins<sup>[20,21]</sup> and flavonoids<sup>[22]</sup> have been implicated in the treatment of diarrhoea while flavonoids and phenolic compounds are known to have antioxidant properties. This is an indication of a possible application of *P. nigrescens* in the treatment of other disease conditions.

The aqueous extract of *P. nigrescens* was well tolerated by mice through the oral route of administration. The 100% mortality (LD<sub>100</sub>) dose of *P. nigrescens* was obtained at the administration of 10 g kg<sup>-1</sup> while the LD<sub>50</sub> was obtained to be 6.5 g kg<sup>-1</sup>. The considerable high LD<sub>50</sub> of 6.5 g kg<sup>-1</sup> obtained for *P. nigrescens* aqueous extract suggest that administration by oral route is relatively safe. Hayes<sup>[23]</sup> stated that doses for LD<sub>50</sub> higher than 5 g kg<sup>-1</sup> body weight are generally not considered as dose related toxicity.

Acute haemorrhagic anaemia often occurs in conditions such as trauma, surgical procedures, severe coagulation defects as in ulcerfarin poisoning and also from neoplasm<sup>[24]</sup>. Haemorrhagic anaemia may also be due

to chronic haemorrhage from intestinal infestation with hookworm, liver flukes, coccidian and other external parasites<sup>[25,26]</sup>. These often lead to loss of blood, low oxygen transportation and low energy production resulting to dizziness and coma. Bleeding of experimental animals resulted to a significant ( $p < 0.05$ ) decrease in the Hb, RBC, Hct and reticulocyte count. These decreases were reversed when rats were treated with *P. nigrescens* extract for 2 weeks. The increased Hct, Hb, RBC and reticulocyte in Table 3 as compared to Table 2 is an indication of erythropoiesis. These increases can also be seen to be dose related in Group 3-5 (Table 3) and significantly ( $p < 0.05$ ) higher when compared with the respective values in Group 2 (bled control) and Group 1 (normal control). Thus the treatment of albino rats with *Parquetina nigrescens* extract favour a speedy recovery of the test animals in Group 3-5 (Table 3). Similar results have earlier been obtained<sup>[11]</sup> where *P. nigrescens* was found to speed up recovering of anaemic rats. Thus, confirming the use of *P. nigrescens* in traditional medicine in the treatment of anaemia. Erah *et al.*<sup>[10]</sup> also reported an herbal preparation containing *P. nigrescens* was able to induce an increase in the haematocrit volume and haemoglobin concentrations in *Trypanosoma brucei* induced anaemia in rats.

Davis *et al.*<sup>[18]</sup> has reported increase in reticulocyte population tone responsible for increase osmotic resistance following acute blood loss in Wister rats. The reason being that after acute blood loss the body develops a defensive mechanism to prevent further loss of blood. Consequently, the erythrocytes become more osmoresistant (less fragile). Also, acute blood loss is usually followed by rapid regeneration of the red blood volume, with more reticulocytes in circulation. In our study, we obtained a dose related decrease in osmofragility (Fig. 1) which corresponds to the dose related increase reticulocyte population in the circulating blood of groups treated with aqueous extract of *P. nigrescens*. The osmofragility of bled control rats was significantly ( $p < 0.05$ ) lower than that of unbled control. However, the erythrocyte osmofragility of the animals treated with *P. nigrescens* were significantly lower than that of the bled control rats. The reason being that, there were more reticulocytes in the circulating blood of treated rats as compared to that of bled rats (Group 2). The decrease in the osmotic fragility of new erythrocytes developing in the recovery phase in rats following blood loss are in agreement with the observation previously reported by Davis *et al.*<sup>[18]</sup>.

The role of electrolytes in the human body is manifold. There is almost no metabolic process that is not dependent on or affected by electrolyte<sup>[27]</sup>. Thus abnormal

levels of electrolytes may result to a variety of disorders. Decreased plasma  $\text{Na}^+$  concentration is termed hyponatraemia and increased plasma  $\text{Na}^+$  concentration is termed hypernatraemia<sup>[28]</sup> which occurs less often than hyponatraemia. Since  $\text{Na}^+$  is the principal cation in the Extra Cellular Fluid (ECF) and by far the largest single contributor to plasma osmolality, hyponatraemia and hypoosmolality always co-exist in haemorrhagic anaemia. Though *P. nigrescens* reversed the hyponatraemic effect of bleeding in rats the other electrolytes remained unaltered. Thus *P. nigrescens* may reverse the defects associated with hyponatraemia. This may also account for the increased osmo-resistance of erythrocytes of rats treated with *P. nigrescens* extract.

From the above results it can be concluded that *P. nigrescens* leaf possesses an erythropoietic and an erythrocyte osmoresistant potential, coupled with the fact that they did not alter plasma electrolyte concentrations. *P. nigrescens* need to be further investigated for any toxicity and the pharmacokinetic profile of the administration using the active constituent as markers. The presence of different bioactive constituents suggested that this plant can be useful in the control of many other conditions.

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#### REFERENCES

1. Irvine, F.R., 1961. Woody Plants of Ghana, with Special Reference to their Uses. Oxford University Press, London.
2. Mabberly, D.J., 1987. The Plant-Book. A Portable Dictionary of the Higher Plants. Cambridge University Press, Cambridge.
3. Gill, L.S., 1992. Ethnomedical Uses of Plants in Nigeria. Uniben Press Publishers, pp: 180-181.
4. Adeyemi, S.O., 1994. Ethnobotanical study of the antirrhematic plants in part of Oyo, Ogun and Lagos States. Unpublished Work. University of Ibadan, pp: 46.
5. Iwu, M.M., 1993. Handbook of African Medicinal Plants. CRC Press Inc Florida, pp: 351.
6. Kokwaro, J.O., 1976. Medicinal Plants of East Africa. East Africa Literature Bureau, pp: 291.
7. Oliver, B., 1960. Medicinal Plants in Nigeria. University of Ibadan, Nigeria, pp: 18-21.

8. Datte, J.Y., A. Ziegler and J.P. Tillement, 1999. Sympathomimetic effects of *Parquetina nigrescens* (periplocaceae) extract in isolated portal vein smooth muscle. *Gen. Pharmacol.*, 32: 551-556.
9. Datte, J., A.M. Offoumon and O.M. Manda, 1996. Uterotonic effects of hydromethanolic extract of *P. nigrescens* on spontaneous contractile activity in the isolated muometrium of pregnant rats. *J. Ethnopharmacol.*, 53: 15-20.
10. Erah, O.P., C.C. Asonye and O.A. Okhamafe, 2003. Response of *Trypanosoma brucei-brucei* induced anaemia to a commercial herbal preparation. *AJB.*, 2: 307-311.
11. Agbor, A.G. and A.A. Odettola, 2001. Haematological studies of *Parquetina nigrescens* on aemorrhagic anaemic rats. *Af. J. Med. Med. Sci.*, 30: 105-109
12. Trease, G.E. and W.C. Evans, 1996. *Pharmacognosy*, 14th Edn., W.B. Saunders Company Ltd., London, pp: 194, 227, 245, 320, 343.
13. Agbor, A.G., E.J. Oben, O.B. Brahim and Y.J. Ngogang, 2004. Toxicity study of *Hibiscus cannabinus*. *J. Cam. Acad. Sci.*, 4: 27-32.
14. Miller, L.C. and M.L. Tainter, 1994. Estimation of the ED<sub>50</sub> and its error by means of logarithm-probit graph paper. *Proc. Soc. Exp. Biol. Med.*, 57: 261-264.
15. Schalm, O.W., N.C. Jain and E.J. Carrol, 1975. *Verterinary Haematology*. 3rd Edn., Philadelphia, USA, Lea Febiger, 42: 55-58.
16. Omotainse, S.O. and V.O. Anosa, 1992. Erythrocyte response to *Trypanosoma brucei* experimentally infected dogs. *Revue deevage et de medecine veterinaire des pays tropicaux*, 45: 279- 283.
17. Dacie, J.V. and M.S. Lewis, 1994. *Practical Haematology*. 8th Edn., Longman Singapore Publishers, pp: 52-56.
18. Davis, W.M., J.D. Bigelow and E.L. Alpen, 1954. Changes in red cell volume and osmotic fragility of erythrocytes in the rat following acute blood loss. *Am. J. Physiol.*, 178:17-22.
19. Toro, G. and P.G. Ackermann, 1975. *The Practical Clinical Chemistry*. 1st Edn., Little Brown and Co. Inc Boston, USA.
20. Carlo, D.C., N. Mascolo, A.A. Izzo and F. Capasso, 1994. Effect of quercetin on the gastrointestinal tract in rats and mice. *Phytother. Res.*, 8: 2-45.
21. Galvez, J., A. Zarzuelo, M.E. Crespo, M.P. Utrilla, J. Jemenez, C. Spiessens and P. de Witte, 1991. Antidiarrhoeic activity of scleroarya birrea back extract and its active tannin constituent in rats. *Phytother. Res.*, 5: 276-278.
22. Lutterodt, D. G., 1992. Inhibition of microlac-induced experimental diarrhoea with nacotic-like extracts of *Psidium guajava* leaf in rats. *J. Ethnopharmacol.*, 37: 151-157.
23. Hayes, 1987. Guidelines of Acute Oral toxicity Testing. In: *Principles and Methods of Toxicology*. 2nd Edn., Raven Press Ltd. New York, pp: 185.
24. Penney, R.H.C., 1978. *Practical haematology for the small animal clinician*. *J. Small Animals Practice*, 19: 479-492.
25. Sarro, D.I., T.W. Schilborn Van Venn and J.B. Adeyanju, 1979. The haemogram of dogs with intestinal parasites in Zaria, Nigeria. *J. Small Animals Practice*, 20: 243-247.
26. Nolan, T.J. and G. Smith, 1995. Times series analysis of the prevalence of endoparasitic infections in cats and dogs presented to veterinary teaching hospital. *Vet. Parasitol.*, 59: 87-89.
27. Tietz, N.W., 1987. *Fundamentals of Clinical Chemistry*. 3rd Edn., Published by W.B. Saunders and Company, London, pp: 676.
28. Whitby, L.H., I.W. Percy Fob and A.F. Smith, 1984. *Lecture Notes on Clinical Chemistry*. 4th Edn., Blackwell Scientific Publication Oxford, pp: 38.