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Hematological and biochemical studies on *Parquetina nigrescens* root extract in albino rats

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ABSTRACT

The haematological, lipid, and antioxidant effects of *Parquetina nigrescens* (PN) were investigated. 40 male wistar rats were grouped into 4 of 10 rats each. Group A: control; administered 10ml/kg of normal saline. The other groups were administered extract viz: Group B- 50mg/kg, Group C- 100mg/kg, Group D- 150mg/kg. After 21days, blood samples were collected for different analysis. The results revealed decreased WBC count at 50 mg/kg & 100mg/kg doses ($p < 0.05$), significant increase in RBC, PCV, & haemoglobin levels ($p < 0.05$) at 50mg/kg dose. There was also significant increase ($p < 0.05$) in total cholesterol (TC), LDL with no change in high density lipoprotein (HDL) at 100mg/kg dose while there was significant increase in the mean TC, TG, LDL as well as HDL at 150mg/kg doses. There was also significant increase ($p < 0.05$) in the level of SOD activity at 100mg/kg dose and a significant reduction in the GPx activity ($p < 0.05$) at 50mg/kg & 100mg/kg dose groups. The observations from this study reveal that PN possesses erythropoietic potentials at minimal dose which lends support to its folkloric use in the treatment of anaemia. It could also serve as a free radical scavenger as it possesses antioxidant activities. However caution should be taken in its use as it has potentials to increase LDL.

Keywords: *Parquetina nigrescens*, anaemia, white blood cells, superoxide dismutase, low-density lipoprotein

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INTRODUCTION

Medicinal plants are plants that possess therapeutic properties and despite the widespread use of modern medicine, herbal products are still in use in most developing countries of Africa and Asia for the management of ailments. A considerable percentage of medicinal plant identified the World over are from tropical Africa (Sofowora, 1993). *Parquetina nigrescens* happens to be one such medicinal plants. *Parquetina nigrescens*, also known as bullock is a shrub found in equatorial West Africa (Mabberly, 1987) has been in traditional medicine practice for centuries with its leaves, roots and latex all in use (Gill, 1992). It is perennial with twinning stem and woody base shortly tapering 10-15 cm long, 6-8 cm broad with a smooth long stem on the leaves. In Oyo State, Nigeria, the leaves have been reputed for treatment of helminthiasis (intestinal worm), while the roots are used for the management of rheumatism (Adeyemi, 1994). Over the years, *Parquetina nigrescens* has been used as an ingredient in the medications for insanity (Iwu, 1993), as well as an aphrodisiac in East Africa. Other uses include the decoction of the stem bark been given as cardiac tonic while the leaf and root decoction have been used for the treatment of gonorrhoea and menstrual disorders (Iwu, 1993; Sofowora, 1993; Odetola, 2006). *Parquetina nigrescens* is also a constituent of a commercial herbal preparation (Jubi formular) in Nigeria used in the treatment of anaemia in humans.

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Research has shown that oral ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters. These alterations could either be positive or negative (Ofuya & Ebong, 1996; Ajagbonna *et al.*, 1999). Antioxidants prevent or remove free radicals which can damage vital components of the cell. However, since reactive oxygen species do have useful functions in cells such as redox signalling, the function of antioxidant system is not to remove oxidants entirely but instead to keep them at an optimum level. Low levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases.

It has therefore become necessary to investigate the effect of aqueous root extract of *Parquetina nigrescens* on blood parameters such as haematological, lipid as well as antioxidant parameters. The study was thus carried out to provide data on the effect of aqueous root extract of *Parquetina nigrescens* on haematological, lipid parameters as well as its antioxidant properties and to verify claims by local medical practitioners of its use to influence growth and multiplication of blood cells.

MATERIALS AND METHODS

Plant material

The root of *Parquetina nigrescens* was collected from the metropolis of Ilorin, Nigeria and identified at FRIN, Ibadan, Oyo State with a voucher number 107128. The roots were air-dried at room temperature of $23 \pm 1^\circ\text{C}$ after which it was ground and sieved to fine powder. The fine powder (1kg) was extracted with 10 litres of distilled water by maceration. The macerated mixture was filtered and the filtrate was evaporated in a carefully regulated water bath (maintained at a temperature of 90°C) to yield 50.2g of dark solid extract. The extract was stored in a refrigerator pending the time for biological investigations.

Animals

Forty male wistar strain rats (200-240g, initial weight) were used in the study. The animals were housed in wire mesh cages at the Department of Physiology, University of Ilorin, Nigeria. The animals were allowed to acclimatize for two weeks before commencement of study. Food and water were provided ad libitum. Ethical approval was received from the College of Health Sciences University of Ilorin animal house committee.

Experimental Design

The experiment lasted for 21 days and they were thus divided into four groups. Group A were the control and were administered 10ml/kg of normal saline. The other groups (B, C, D) were the treatment groups. Groups B, C and D were administered 50, 100 and 150 mg/Kg body weight of the extract respectively. All animals were sacrificed on day 21 after which blood samples were used for the respective tests.

Biochemical and Hematological Analysis

Haematological Indices

The hematological parameters were evaluated with an automated hematological analyzer systemex KX-21 (Japan).

Lipid Profile Parameters

Lipid profile analysis was carried out using an Automated Analyzer (Roche, HITACHI).

Antioxidant Studies

Superoxide Dismutase estimation was carried out using the RANSOD kit (Randox, Crumlin, England). Whole blood samples were used. The kit contained mixed substrate (xanthine, 0.05 mmol/L, and I.N.T. 0.05 mmol/L), buffer (CAPS 40.00mmol/L, pH 10.2, EDTA, 0.94 mmol/L), xanthine oxidase standard (80U/L), sample diluents (5.40 U/L) and phosphate buffer (0.01mol/L, pH7.0) (50.00mL of 0.20 mol KH_2PO_4 +29.65 mL of 0.20 N NaOH made up to 1L, with distilled water). The percentage inhibition of each sample was used to obtain the SOD units from a standard curve of the reconstituted and diluted RANSOD kit.

Glutathione peroxidase (GPx) was measured as follows, 0.05ml of heparinised whole blood was diluted with 1ml of diluting agent incubated for 5 minutes and 1ml of double strength hemoglobin reagent was added (i.e 1 volume of haemoglobin reagent diluted with 24 volumes of redistilled water). It was mixed well and assayed within 20 minutes of adding the haemoglobin reagent.

Statistical Analysis

Data were recorded as Mean \pm standard error of the mean. Statistical difference between the means was determined by ANOVA. Any significant difference between means was assessed by both the Student's T-test and Duncan post HOC test. $P < 0.05$ was accepted as the significant level.

RESULTS AND DISCUSSION

The results of the hematological parameters (Table 1) showed that the mean RBC, PCV and Hb concentrations increased significantly ($p < 0.05$) at 50mg/kg of administration. The mean WBC count decreased significantly at 50mg/kg and 100mg/kg of administration. The Eosinophil count was decreased significantly at 50mg/kg of administration ($p < 0.05$). MCH, MCV, MCHC, neutrophil and lymphocytes did not show significant difference at all doses of administration. There was no significant difference in RBC, PCV, Hb concentrations as well as the eosinophil at 100mg/kg and 150mg/kg of administration. Literature has shown that oral ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters (Ajagbonna *et al.*, 1999; Ofuya & Ebong, 1996). These alterations can either be positive or negative. In this study, significant increases in RBC, Hb and PCV at 50mg/kg ($p < 0.05$) were observed. This may be due to the presence of erythropoietin-like principles in the extract which probably stimulated erythropoietin synthesis or release at minimal doses (50mg/kg). The active biological principles such as

Table 1: Effect of 21 days of oral administration of aqueous root extract of *Parquetina nigrescens* on haematological parameters in rats.

Parameters	GROUP A	GROUP B	GROUP C	GROUP D
RBC (106/ μ l)	5.84 \pm 0.22	6.72 \pm 0.18*	5.94 \pm 0.31	6.07 \pm 0.21
Hb(g/dl)	12.12 \pm 0.29	14.34 \pm 0.29*	12.62 \pm 0.40	12.66 \pm 0.15
PCV(%)	36.40 \pm 0.87	43.0 \pm 0.89*	37.80 \pm 1.24	38.0 \pm 0.44
MCH (pg)	17.6 \pm 0.24	18.0 \pm 0.70	18.0 \pm 0.31	17.20 \pm 0.20
MCV (fl)	62.80 \pm 0.97	64.40 \pm 1.58	64.40 \pm 1.96	62.80 \pm 2.35
MCHC	28.80 \pm 0.20	28.0 \pm 0.31	28.20 \pm 0.73	27.60 \pm 1.12
WBC	8.92 \pm 0.33	5.48 \pm 0.84*	5.52 \pm 1.27*	9.06 \pm 1.82
Neutrophil (%)	26.0 \pm 6.20	38.20 \pm 5.23	26.0 \pm 4.58	37.2 \pm 5.31
Eosinophil (%)	3.00 \pm 0.94	0.40 \pm 0.40*	1.00 \pm 0.63	1.20 \pm 0.49
Lymphocytes (%)	69.60 \pm 6.90	61.40 \pm 4.97	73.00 \pm 4.57	61.60 \pm 5.03

Hb, haemoglobin; pcv, packed cell volume; WBC, white blood cells, RBC, red blood cell; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; *represents significant difference at $p < 0.05$.

alkaloids, cardiac glycosides, saponins, tannins, terpenoids, phenols and steroids contained in the extract, may be responsible for its hematopoietic effects. This result agrees with the findings of (Agbor & Odetola, 2001) on the aqueous leaf extract of the *P. nigrescens* on erythrocyte indices. Red blood cell count, haemoglobin concentration, hematocrit, reticulocyte population, and erythrocyte osmofragility were used as erythrocyte indices. It was observed that the aqueous leaf extract of *Parquetina nigrescens* significantly ($p < 0.05$) increased the erythrocyte indices which were attributed to erythropoietic potential of *Parquetina nigrescens*. At higher doses, i.e. (100mg/kg and 150mg/kg), there were no significant changes in RBC, PCV and Hb when compared with the control ($p > 0.05$). This implies that the erythropoietic potential of aqueous extract of *Parquetina nigrescens* root is limited to a low dose of the extract. The findings also show that the aqueous root extract of *Parquetina nigrescens* may possess the ability to suppress immunity because its oral administration at 50mg/kg and 100mg/kg significantly decreased the WBC count when compared with the control ($p < 0.05$). Eosinophil count also decreased significantly at 50mg/kg when compared with the control. Neutrophils and lymphocytes were not significantly altered when compared with the control ($p > 0.05$). In consumption of the aqueous root extract of *Parquetina nigrescens*, care should be taken because of its effect on immunity. The erythropoietic potentials and immunity depression of the aqueous root extract of *Parquetina nigrescens* was only for the duration of the treatment. There was no significant difference in the recovery groups at all the doses when compared with the control (data not shown).

The lipid profile analysis showed that the mean total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were not significantly altered when compared with the control at 50 mg/kg. The 100 mg/kg dose significantly ($p < 0.05$) increased the mean TC and LDL. There were significant increases in TC, TG, LDL and HDL following the administration of 150 mg/kg dose of the extract. The effect of *Parquetina nigrescens* on lipid profile reveals that there were no significant changes observed in the total cholesterol (TC), triglyceride (TG), High-density lipoprotein (HDL) as well as the

Low-density lipoprotein (LDL) at the 50 mg/kg dose however, at the 100mg/kg of the dose, an increase in TC and LDL were observed. At the higher dose of 150mg/kg there was a significant increase in TC, TG, LDL and HDL when compared with the control ($p < 0.05$) (Table 2).

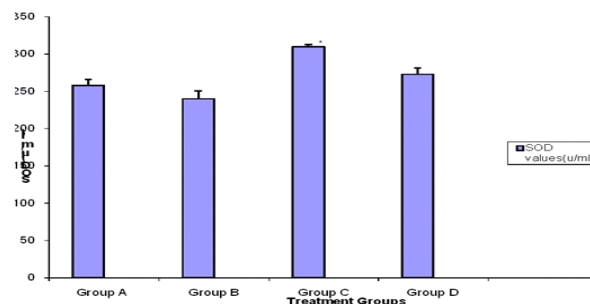
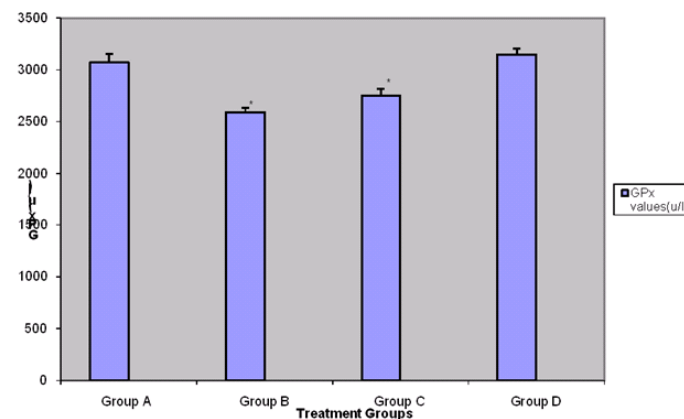
Table 2: Effect of 21 days of oral administration of aqueous root extract of *Parquetina nigrescens* on lipid parameters in rats.

Parameters (mmol/L)	GROUP A	GROUP B	GROUP C	GROUP D
TC	2.38 \pm 0.19	2.92 \pm 0.27	4.66 \pm 0.33*	6.84 \pm 0.84
TG	1.14 \pm 0.15	1.60 \pm 0.36	2.02 \pm 0.54	3.00 \pm 0.75*
HDL	0.22 \pm 0.04	0.48 \pm 0.12	0.73 \pm 0.25	1.50 \pm 0.138*
LDL	1.63 \pm 0.25	1.27 \pm 0.32	2.63 \pm 0.39	3.97 \pm 0.48*

TC-total cholesterol, TG-triglyceride, LDL- low-density lipoprotein, HDL- high density lipoprotein; *represents significant difference at $p < 0.05$.

Parquetina nigrescens may thus be effective at the 50-150 mg/kg doses. There have been reports on the lipid profile of various plants and some of which correlate with the present study (Ikeda and Sugano, 1998; Harword *et al.*, 1993, Lindequist *et al.*, 2005; Venkatesan *et al.*, 2003; Vinson *et al.*, 1998). Thus, alterations in the concentration of the various lipids can give useful information on the lipid metabolism and predisposition of the heart to atherosclerosis and its associated coronary heart diseases.

The result showed that there was a significant ($p < 0.05$) increase in the level of SOD activity in the group administered 100mg/kg orally when compared with the control group (Figure 1).

**Fig. 1** Effect of *Parquetina nigrescens* root extract on SOD activity.**Fig. 2** Effect of *Parquetina nigrescens* root extract on GPx.

Also the extract at 50mg/kg and 100mg/kg doses significantly ($p < 0.05$) reduced GPx activity and the decrease was

more in the 50 mg/kg dose compared to the 100mg/kg group. Meanwhile, there was an insignificant increase in the GPx activity of the group administered 150mg/kg (Figure 2).

The Superoxide dismutase (SOD) and Glutathione peroxidase (Gpx) properties were investigated because of their ability to work hand in hand. SOD catalyses the breakdown of superoxide, the most common free radical in the body into oxygen and hydrogen peroxide while GPx catalyses the breakdown of hydrogen peroxide to water. The study showed that SOD level was significantly ($p < 0.05$) increased at 100mg/kg dose of the extract. This could be due to the presence of secondary metabolites like phenol, tannin and saponins contained in *Parquetina nigrescens* while there was no significant change in the groups administered 50mg/kg and 100mg/kg doses of the extract. Whereas, there was a significant decrease in GPx activity in the rats treated with the 50mg/kg and 100mg/kg. There have been reports on antioxidant activities in various plants, some of which correlates with the present study. For instance, *Pelargonium reniforme* which is used locally for liver disorders, has strong antioxidant activities as a result of its tannin and flavonoid content (Fernandes *et al.*, 2004). *Mallotus oppositifolium*, a Nigerian plant rich in flavonoids has been said to possess antioxidant as well as anti-inflammatory activities (Farombi *et al.*, 2001).

CONCLUSION

We conclude that the aqueous root extract of *Parquetina nigrescens* possesses erythropoietic potentials at minimal dose and the overall results lend support to the folkloric use of the aqueous root extract of *Parquetina nigrescens* in the treatment of anaemia.

Parquetina nigrescens can be used as a radical scavenger as it possesses antioxidant activities which can help in the prevention as well as cure of illnesses associated with oxidative stress. However, since the effect of the extract was dose dependent, caution has to be taken in its use.

Parquetina nigrescens needs to be further investigated for any toxicity and pharmacokinetic profile of the administration using the active constituents as markers. However, the isolation of the active principles in the extract and elucidation of their mechanisms of action would constitute further studies.

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CONFLICT OF INTEREST

We wish to say there is no conflict of interest of any sort.

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