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Erythropoietic potential of Parquetina nigrescens in cephalosporin-induced anaemia model

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ABSTRACT

Parquetina nigrescens is a folklore plant in Africa, particularly Nigeria, where its consumption is believed to stimulate red blood cells production. On this basis, the erythropoietic potential of the plant was evaluated in Cephalosporin-induced anaemia model, using Wistar rats as experimental subjects. Thirty-two male rats were randomly assigned to four groups ($n = 8$). Group 1 animals served as control, and experimental anaemia was induced in other groups of animals via oral administration of cephalosporin (10 mg/kg BW) for a period of seven days. Animals in groups III and IV were treated orally with aqueous extract of *P. nigrescens* at respective dosage of 250 and 500 mg/kg BW, twice daily for a period of 10 days; while group II animals were left untreated. All animals were thereafter fasted overnight and sacrificed by cervical decapitation. Blood was collected via the retro-orbital sinus and used for biochemical analyses. The results obtained showed that cephalosporin effectively induced anaemia as evidenced by significant changes in erythropoietic indices of the untreated anaemic animals. Treatment of anaemic animals with *P. nigrescens* particularly at a dosage of 250 mg/kg BW significantly ($P < 0.05$) boosted the levels of RBC (35.8%), Hb (25.2%), PCV (39.4%), cobalt (70.9%), vitamin C (82.6%), and concomitantly decreased erythropoietin level (18%) relative to untreated anaemic animals. The observations made in this study support the local use of *Parquetina nigrescens* as blood tonic and therapy for anaemia. The botanical may therefore be a useful supplement for patients placed on antibiotics which are often associated with haemolysis.

1. Introduction

Anaemia is a health condition characterized by significant decline in red blood cells or haemoglobin counts, with an attendant effect of decreased capacity of the blood to transport oxygen round body tissues. The abnormal low counts of haemoglobin and circulating red blood cells may results from decreased blood cell production or increased red blood cell lyses. Anaemia is arguably the most prevailing blood disorder, posing a critical health risk to at least one third of the global populace. It remains a major public health concern in several developing and under-developed countries. People of all ages, particularly infants, young women of child bearing age and the elderly are affected by the condition [1]. According to Smith [2], the disease affects different aspects of life; it lowers psychomotor skills, mental performance and resistance to infections of the sufferers.

Anaemia has also been associated with decreased red blood cell-life span, impaired iron metabolism and altered levels of erythropoietin.

Erythropoietin is the principal hormone that regulates erythropoiesis (a complex, multi-step process of blood production) and its transcription is mediated by hypoxia inducible factor-1(HIF-1). In addition to erythropoietin, the erythropoietic machinery requires a constant supply of certain minerals (iron, copper) and vitamins (A, B_{12}, B_6, C, E, folic acid, riboflavin and nicotinic acid). Inadequate supply of any of these constituents has the propensity to result in anaemia [3].

Varieties of drugs commonly used by people have dreaded side effects. For instance, a couple of conventional drugs, particularly antibiotics have been associated with lyses of red blood cells. This is

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worrisome for obvious reasons, recognizing the critical role of red blood cells in the body, especially in the area of tissues oxygenation. This has caused a huge concern about the frequent or prolonged use of drugs with haemolytic side effects even when it is clear that such use is necessary. Interestingly, *Parquetina nigrescens* which is commonly known as flax and locally referred to as *Ewe Ọgbọ* among the Yoruba-speaking tribes in Nigeria, is believed to stimulate and enhance blood production. Previous studies have substantiated the medicinal relevance of the plant in some other therapeutic applications. For instance, Owoyele et al. [4] substantiated the use of Exclude *Parquetina nigrescens* leaves and roots for treatment of helminthiasis and rheumatism respectively. The leaves and stems of *Parquetina nigrescens* are used as indigenous herbal remedy in folklore medicine for the management of anaemic conditions. In some parts of Nigeria, water extract of the plant leaves is taken to increases packed cell volume (PCV) in anaemic individuals.

Cephalosporins are commonly used antibiotics and like other classes of antibiotics, they have been associated with lyses of red blood cells [5]. The present study therefore sought to assess the locally acclaimed erythropoietic properties of *Parquetina nigrescens* in cephalosporin-induced anaemia model, as a probable alternative therapy for anaemia and viable supplement for patients placed on antibiotics against infections.

2. Material and methods

2.1. Collection and management of animals

Adult male Wistar rats of average body weight ranging from 150 to 180 g were used for the study. The animals were purchased from the animal breeding unit, University College Hospital (UCH), Ibadan, Nigeria. The handling and care of the animals were carried out according to the guidelines of the National Academy of Science published by the National Research Council [6]. The procedure was approved by the Ethics Committee on animal care and use in research, Faculty of Basic Medical & Applied Sciences, Lead City University, Ibadan, Nigeria. The animals were kept in plastic cages, subjected to natural photoperiod of 12 h light and 12 h dark cycle in a well ventilated and hygienic rat house. They were provided rats pellets (Top feeds) and water *ad libitum* under suitable conditions of temperature and humidity. Animal acclimatization was done for a period of two weeks prior to commencement of the study. All animal experiments were carried out without anesthesia during the study.

2.2. Collection and authentication of Parquetina nigrescens leaves

Fresh leaves of *Parquetina nigrescens* were obtained from Olodo area of Ibadan, Oyo state (South-western region of Nigeria), in the month of January 2019. Identification and authentication of the leaves were carried out by a curator botanist, Mr Donatus Esimekhuai at the Department of Botany, University of Ibadan, Nigeria, where a specimen was deposited and assigned a voucher number, UIH-22820.

2.3. Preparation of Parquetina nigrescens leaf extract

The leaves were removed from the stem, washed with water to remove debris and soil particles, air dried and grounded into powder using an electric blender. Six hundred and ten (610) gram of the powdered plant sample was macerated in 2500 mL of distilled water at room temperature. The mixture was allowed to stand for 48 h and stirred intermittently with a glass rod to facilitate extraction. The mixture was then filtered with a muslin cloth (maximum pore size 2 mm). The resulting filtrate was further filtered through Whatman filter paper (No. 42) and subsequently reduced in volume with a rotary evaporator at 40 °C. Final elimination of solvent and drying were done using a regulated water bath at 40 °C. The crude extract obtained was placed in appropriately labelled air-tight bottle and stored at 4 °C.

2.4. Induction of anaemia with cephalosporin in experimental rats

Anaemia was (po) induced in test animals using a second generation Cephalosporin; Cefuroxime. Cefuroxime was ground into powder and dissolved in distilled water to obtain a dosage of 10 mg/kg body weight (BW) at a volume of 1 mL. The drug was orally administered twice daily at 8 h interval for a period of 7 days.

Test animals were randomly assigned to groups (II, III, and IV) as shown below, while the control rats constituted the first group (group I). Each group contained eight rats ($n = 8$). Groups III and IV animals were respectively treated with *P. nigrescens* aqueous extract at a dosage of 250 and 500 mg/kg BW while group II animals were left untreated as shown in Table 1. Extract administration was done orally, twice daily at 8-h interval for a period of 10 days and all animals were allowed equal access to normal laboratory chow and water *adlibitum* throughout the study. At the end of treatments, the animals were fasted overnight (12 h), sacrificed by cervical decapitation and blood sample was collected via the retro orbital sinus of the eye by ocular puncture into plain and EDTA tubes for biochemical analyses.

2.5. Estimation of haematological parameters

Blood samples (2 mL) were collected into EDTA bottles for estimation of haematological parameters. A complete blood count and differential count was performed on the blood sample using Sysmex KX-21 N, an automated 3-part differential haematology analyser. The machine automatically dilutes whole blood sample in the CBC/differential mode and enumerates white blood cells, red blood cells, haemoglobin concentration, packed cell volume, platelets, lymphocytes and neutrophils.

2.6. Estimation of vitamin C content

Vitamin C content in the blood serum was estimated using Folin-Ciocalteu method as described by Olgun *et al.* [7]. Serial dilutions of pure ascorbic acid of 1 mg/mL was used to obtain a standard calibration curve for the estimation of vitamin C in the serum samples.

2.7. Estimation of vitamin B_2 content

HPLC method was used for the determination of serum vitamin B_2 as described by Liu *et al.* [8]. Briefly, serum samples were pretreated at room temperature on a C18 reversed phase chromatographic column, methanol plus phosphate buffer was used for mobile phase, vitamin B_2 contents of serum samples eluted from a

Table 1
Experimental design and treatments.

Experimental groups	Treatment/Dose	Nomenclature
Group I	Served as normal control	NC
Group II	Anaemic rats left untreated	DC
Group III	Anaemic rats treated with plant extract (250 mg/kg body weight)	D1
Group IV	Anaemic rats treated with plant extract (500 mg/kg body weight)	D2

Sep-park C18 column were detected with a photodiode array detector.

2.8. Estimation of minerals

The Atomic emission spectrophotometry (AES) method was used for estimation of minerals in the serum. The principle is Atomisation, Excitation and ionisation. Samples were prepared in a Class 100 clean hood to prevent contamination by atmospheric particulates. Digestion was performed by microwave methods using the Discovery SPD. Three clean test tubes were respectively labelled blank, standard and test. To each of the tubes 10 mL of distilled water was added. To the second tube (standard), 100 μL of standard was added and to the third tube (test) 100 μL of the sample was added. The solutions in the tube were then mixed gently and aspirated into an Atomic Emission Spectrophotometer (AES). The concentration of the analyte (mineral) is automatically displayed on the machine readout.

2.9. Estimation of erythropoietin

Erythropoietin was estimated by the use of end point colorimetric diagnostic kit (Randox Laboratories Limited, England). Briefly, 1 mL of the colour reagent (chromogen) was added to each of three clean test tubes respectively labelled blank, standard and test. 10 μL of the standard solution from the kit was added to the tube labelled (standard), and 10 μL of sample was added to the third tube labelled as test. The solutions in the tubes were mixed gently and incubated at 37 °C for 15 min and the absorbance was read in a spectrophotometer at a wavelength of 500 nm. The concentration of the standard was obtained from the kit manual and erythropoietin concentration of was calculated as follows:

$$\frac{Optical\ density\ of\ test\ (OD)}{Optical\ density\ of\ s\tan dard} \times Concentration\ of\ s\tan dard$$

3. Statistical analysis

Data analysis was performed using statistical software, Prism graphpad, version 6.4. The statistical significance of difference between groups was analysed using the one-way analysis of variance (ANOVA) followed by independent-sample *t*-test. The level of significance was set at $p < 0.05$. The results were presented as the mean ± SD or mean ± SEM.

4. Results

4.1. Effect of Parquetina nigrescens on Hb, RBC and PCV in cephalosporin-induced anaemic animals

Compared to the control animals, induction of anaemia in experimental animals caused significant decrease in the levels of most erythropoietic parameters. For instance, the red blood cell count (RBC), haemoglobin concentration (Hb) and packed cell volume (PCV) were significantly ($P < 0.05$) reduced by 37.93, 36.5 and 16.7% respectively. Conversely, treatment of anaemic animals with *Parquetina nigrescens* at a dosage of 250 mg/kg BW boosted the levels of RBC, Hb and PCV by 35.8, 25.2 and 39.4% respectively and treatment with the extract at a dosage of 500 mg/kg BW increased the levels of RBC, Hb and PCV by 42.4, 13.3 and 33.9% respectively (Table 2).

4.2. Effect of Parquetina nigrescens on the vitamin C and B_2 contents of cephalosporin-induced anaemic animals

As shown in Table 3, vitamins B_2 and C contents were respectively reduced by 7.7 and 60.4% in untreated anaemic animals compared to the control animals (group I). Surprisingly, oral administration of *Parquetina nigrescens* at a dosage of 250 and 500 mg/kg BW caused further reduction in vitamin B_2 content by 20.8 and 12.5% respectively. Conversely, the plant extract caused a significant increase in vitamin C content, with higher increase observed at 250 mg/kg BW dose (82.6%) compared to 500 mg/kg BW dose (50.2%).

4.3. Effect of Parquetina nigrescens on the mineral content of experimental rats

Table 4 shows the effects of *P. nigrescens* extract on the mineral contents of experimental animals. Compared to the control

Table 3
Effect of *Parquetina nigrescens* on *vitamin C and B_2 contents* in cephalosporin-induced anaemic animals.

Parameter	Group I	Group II	Group III	Group IV
Vitamin C (mg/mL)	53.9 ± 2.7	^{a}21.3 ± 3.1	^{b}38.9 ± 4.8	32.0 ± 3.2
Vitamin B_2 (mg/mL)	2.6 ± 0.3	^{a}2.4.± 0.2	^{b}1.9 ± 1.1	2.1 ± 0.5

Values are expressed as mean of eight rats ± standard error of mean. a = Significant when compared to normal control, b = Significant when compared to disease control, c = Significant when compared to treatment at 250 mg/kg BW.

Table 2
Effect of *Parquetina nigrescens* on *Hb, RBC and PCV* in cephalosporin-induced anaemic animals.

Parameters	Group I	Group II	Group III	Group IV
Hb (mg/dL)	16.20 ± 1.3	^{a} 13.50 ± 2.1	^{b} 16.90 ± 1.1	15.30 ± 2.4
RBC (x10^3/μL)	8.54 ± 1.2	^{a} 5.3.00 ± 2.1	^{b} 7.2.00 ± 1.9	7.10 ± 2.0
PCV(%)	52.00 ± 2.4	^{a}33.00 ± 3.7	^{b}46.00 ± 1.8	47.00 ± 2.2
WBC (x10^3/μL)	17.45 ± 3.4	^{a}19.20 ± 2.2	^{b} 16.57 ± 4.4	17.13 ± 3.4
Platelets (x10^3/μl)	13.98 ± 4.6	12.34 ± 1.1	11.68 ± 1.4	12.44 ± 2.6
Lymphocytes (%)	74.00 ± 2.8	76.00 ± 2.3	72.00 ± 3.1	74.00 ± 2.1
Neutrophils (%)	20.00 ± 1.8	18.00 ± 1.9	14.00 ± 3.2	17.00 ± 2.5

Values are expressed as mean of eight rats ± standard error of mean. a = Significant when compared to normal control (Group I), b = Significant when compared to disease control (Group II), c = Significant when compared to treatment at 500 mg/kg BW (Group IV).

Table 4
Effect of *Parquetina nigrescens* on the mineral content of experimental rats.

Parameter	Group I	Group II	Group III	Group IV
Copper (mmol/L)	71 ± 3.4	[a]53 ± 3.7	[b]46 ± 1.8	47 ± 2.2
Zinc (mmol/L)	64 ± 6.3	[a]61.5 ± 4.1	[b]55 ± 3.1	60 ± 5.4
Iron (mmol/L)	140 ± 4.1	128 ± 5.8	136 ± 0.7	131 ± 2.5
Cobalt (mmol/L)	43 ± 1.2	31 ± 1.1	53 ± 2.6	39 ± 1.5

Values are expressed as mean of eight rats ± standard error of mean. a = Significant when compared to normal control, b = Significant when compared to disease control, c = Significant when compared to treatment at 250 mg/kg BW.

animals, cephalosporin-induced anaemia caused significant ($P < 0.05$) decrease in the serum levels of the minerals analysed (iron, copper, zinc and cobalt). However, treatment of anaemic animals with *Parquetina nigrescens* at 250 and 500 mg/kg BW was only able to boost the levels of iron (6.25 & 2.3% respectively) and cobalt (70.9 & 25.8% respectively) compared to untreated anaemic animals.

4.4. Effect of Parquetina nigrescens on erythropoietin concentration in cephalosporin-induced anaemic animals

The erythropoietin content of the untreated anaemic rats was significantly ($P<0.05$) increased by 34.6% compared to the control animals. However, co-administration of *Parquetina nigrescens* curtailed the increase in erythropoietin content by 46.4% at a dosage of 250 mg/kg BW and 44.8% at a dosage of 500 mg/kg BW (Fig. 1).

5. Discussion

Antibiotics are powerful medicines that fight certain infections and can save lives when used properly. They either stop bacteria from reproducing (bacteriostatic) or destroy them (bactericidal), and are usually taken for a period between 7 and 10 days or more [9]. However, their clinical usage is often associated with red blood cell lyses [10]. This is obviously a cause for concern, knowing the extreme significance of red blood cells in the overall functioning of the body system. In light of the above, this study sought to assess a botanical (*Parquetina nigrescens*) with locally acclaimed erythropoietic properties as a probable alternative therapy for anaemia, and viable supplement for patients placed on antibiotics against infections.

Cephalosporin (Cefuroxime) is an antibiotic that belong to a class known as beta-lactams which shares similar mechanism of action and structure with penicillin. Its choice as model antibiotics in this study was informed by its common clinical usage and ability to interact with erythrocyte membrane to form hapten-red blood cell complex. This complex does not only stimulate antibodies' production but also triggers the body's immune system to destroying its own red blood cells, with possible attendant anaemic condition [11]. Cephalosporin also inhibits ATP synthesis in red blood cells under hypoxia accompanied with inhibition of catalase and glutathione reductase enzymes (Harrison and Bratcher 2008) and by these effects, red blood cells become more susceptible to haemolysis. In light of this, repeated administration of cephalosporin causes reduction in red blood cells which can lead to anaemic condition.

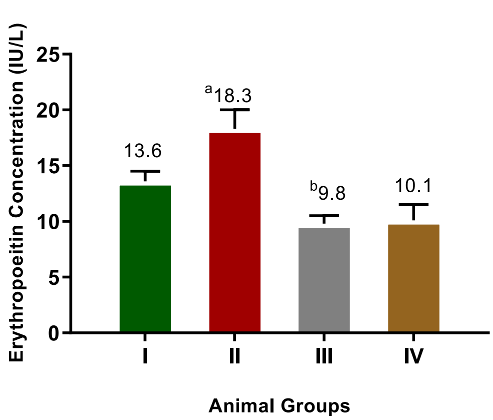

Fig. 1. The effect of *Parquetina nigrescens* on erythropoietin content in Cephalosporin-induced anaemic animals.

In support of this, previous studies have shown that cephalosporin causes immune haemolytic anaemia [12]. This postulation is consistent with the results obtained in this study in which cephalosporin administration to experimental animals at a normal dosage of 10 mg/kg BW caused significant ($P<0.05$) reduction in erythropoietic indices including red blood cells (RBC), packed cell volume (PCV), haemoglobin (Hb), iron (Fe), copper (Cu), cobalt (Co), zinc (Zn), vitamins B_2 and C. The hallmark of anaemia is marked decrease in red blood cell count, which invariably leads to low PCV. Minerals like copper, zinc and cobalt, and vitamins including B_2 and C are also known to play important roles in red blood cells formation. Hence, in anaemic condition, their levels in the blood are likely to be compromised as observed in this study.

Haemoglobin and iron are also decreased because they are conjugate component of RBC. Besides, during anaemia, the incorporation of haemoglobin into red blood cells is compromised [13] This disruption increases the deficiency of the enzyme glucose-6-phosphate dehydrogenase, whose function is critical in Hexose monophosphate pathway which generates reduced nicotinamide dinucleotide phosphate (NADPH) required for the maintenance of normal red blood cell morphology and osmotic fragility [14]. Moreover, there is empirical evidence that cephalosporins inhibit the activity of the glutathione enzymes which are integral part of the antioxidant defence system [15].

Conversely, significant increases in white blood cells and erythropoietin were observed in the untreated cephalosporin-exposed animals when compared to the control group. The latter (white blood cells) are integral components of the body immune and defensive grid (Kul et al., 2017)For this reason, the increase in white blood cells noted in the experimental animals following cephalosporin administration may be suggestive of the ability of the drug to trigger the body's immunological response. On the other hand, blood level of erythropoietin is inversely proportional to tissue oxygenation and is normally increased under anaemic cellular condition [17]. This arguably explains the increase in erythropoietin content noted in cephalosporin-induced anaemic animals in this study.

The ability of *Parquetina nigrescens* extract to improve the blood levels of most of the erythropoietic markers (RBC, PCV, Hb, iron, cobalt, vitamin C and erythropoietin) in the treated-anaemic animals is of great pharmacological significance, considering the important roles of these molecules in the process of erythropoiesis. For instance, Iron is an essential metal micronutrient and is distributed mainly within erythrocytes. It is a component of heme-containing proteins; haemoglobin, myoglobin and cytochromes. The distribution of body iron stores shows the importance of iron to red blood cell production. The production of functional erythrocytes therefore requires the timely delivery of sufficient iron to erythroid precursors [18]. Erythropoiesis involves the close interaction of iron and erythropoietin which is the key protein that drives erythropoiesis [19] Cobalt on the other hand plays an important role in the synthesis of vitamin B_{12} [19] by the rumen bacteria which in turn is required for the normal production of erythrocytes [20]. Vitamin C plays an important role in the kinetics of iron metabolism and the utilization of iron for the formation of red blood cells [21]. In several trials with haemodialysis patients, intravenous vitamin C decreased erythropoietin requirements in erythropoietin-resistant patients [22] indicating that it supports haemoglobin production and formation of new red blood cells.

Previous studies on the phytochemical screening of *Parquetina nigrescens* revealed that the plant contains bioactive phytoconstituents including saponins, tannins, flavonoids, alkaloids, cardiac glycosides, terpenoids, phenols and sterols which may be responsible for its haematopoietic effects [23]. Orally administered flavonoids have been observed to prevent pulmonary haemorrhage and inhibit vascular permeability [24]. Saponins and alkaloids have been reported to possess anti-anaemic potentials [25]. Saponin containing herbs have been successfully used to vitalize blood circulation [26]. On this note, the erythropoietic properties exhibited by *Parquetina nigrescens* in the present study might be associated with its phytoconstituents, which are well known haematopoietic factors that have direct or indirect influence on the production of red blood cells. Considering the level of influence of the plant extract on the various evaluated markers, the study suggests that P. *nigrescens* promotes erythropoesis mainly through its ability to improve the synthesis or blood availability of vitamin C, cobalt (Co) and hemoglobin (Hb).

5.1. Conclusion

The outcome of this study suggests that consumption of the leaves of *Parquetina nigrescens* particularly during prolonged use of antibiotics or any other anaemia-inducing drugs may be useful in maintaining physiologically healthy red blood cells count and packed cell volume (PCV). More importantly, it apparently showed that *Parquetina nigrescens* possesses erythropoietic potential and thus, lends credence to its use as therapy for anaemia in folklore medicine.

CRediT authorship contribution statement

O.M. Ighodaro: Conceptualization, Supervision, Writing - original draft, Methodology. **F.O. Asejeje:** Methodology, Writing - review & editing. **A.M. Adeosun:** Methodology, Software, Formal analysis. **T.S. Ujomu:** Investigation, Supervision. **F.C. Adesina:** Validation, Resources. **K.T. Bolaji:** Investigation, Resources, Writing - original draft.

Declaration of competing interest

The authors unanimously declare that there is no conflict of interest concerning this article.

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