SHST
Horticulture and Forestry Society of Transylvania

Awoniyi AJ *et al.* (2022)
Notulae Scientia Biologicae
Volume 14, Issue 3, Article number 11275
DOI:10.15835/nsb14311275
Research Article

NSB
Notulae Scientia Biologicae

Parquetina nigrescens leaf infusion: a food-based approach for the management of diet-induced iron deficiency in weanling rats

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Abstract

Micronutrients are vitamins and minerals needed by the body in minute amount and whose impact on the body's health is vital. Hence, deficiency in any one of them could cause severe and even life-threatening conditions. Deficiency in iron is the most common micronutrient deficiency and is prevalent in children and pregnant women. The infused extracts of *Parquetina nigrescens* (PN) leaf were used in the management of iron deficiency in weanling rats to provide a food-based approach strategy that is readily accessible, affordable, and acceptable to the low-income population who are more susceptible to micronutrient deficiencies. Diet-induced iron-deficient weanling rats (55.0 ± 10.0) g was administered infused extracts (PN, PN+ ginger PN+ lemongrass, PN+ milk) of PN at 1000 mg/kg body weight for 28 days. The haemoglobin concentration of rats administered PN extract was significantly ($p < 0.05$) increased compared to the control. Serum protein assay indicated administration of PN extract reduced significantly ($p < 0.05$) concentrations of Divalent Metal Transporter 1, transferrin, ferroportin, and increased hepcidin and ferritin concentrations. Higher concentrations of flavonoids in the PN extract are proposed to support its haematopoietic activity alongside its ascorbic acid content. The infused extract of PN showed better haematopoietic activity when taken alone than when additives were added.

Keywords: anaemia; infusion; iron deficiency; micronutrient; *Parquetina nigrescens*
Abbreviations: *Parquetina nigrescens* (PN)

Introduction

Micronutrient deficiencies, including iron deficiency have been associated with food insecurity which is one of the most important issues of global concern. According to the Food and Agricultural Organization of the United Nations, a person is food insecure when he/she lacks regular access to enough safe and nutritious food for normal growth and development and active healthy life (FAO, 2021). There are multiple challenges to food insecurity one of which is micronutrient malnutrition or 'hidden hunger' which results from inadequate intake of micronutrients in the regular diet. Moderate or severe food insecurity (based on the Food Insecurity

Received: 11 May 2022. Received in revised form: 10 Jun 2021. Accepted: 04 Aug 2022. Published online: 23 Sep 2022.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Experience Scale) at the global level has been slowly on the rise, from 22.6% in 2014 to 26.6% in 2019, then in 2020, it rose nearly as much as in the previous five years combined, to 30.4 % majorly due to COVID-19 pandemic. (FAO, 2021). Many of the countries in South Sahara Africa and South Asia are hot spots where this prevalence is high (WHO, 2008).

Hidden hunger can contribute to various forms of malnutrition/deficiency and can have serious consequences for health and well-being. Iron deficiency is the most common micronutrient deficiency in the world, it results from inadequate diet and it occurs mostly among pregnant women and children under the age of five (WHO, 2008). This micronutrient deficiency occurs when the store of iron in the body has been depleted; this can lead to a reduction in haemoglobin concentration and affect the metabolic activities of iron-dependent enzymes (Abbaspour *et al*., 2014). Therefore, there is a need for the proper management of iron deficiency.

In Nigeria and many developing countries, the use of medicinal plants is an age-long approach to the treatment of many diseases mainly because it is accessible, acceptable, and affordable for many (Iyare and Obaji, 2014). It has also been employed for alleviating micronutrient deficiencies like iron deficiency (Oladiji *et al*., 2007). *Parquetina nigrescens* (PN) is a shrub commonly found growing in equatorial West Africa; not cultivated, but as a secondary forest. The plant has been used in traditional medicine practice for centuries with its leaves, roots, and latex all in use (Owoyele *et al*., 2011). It has been reported that PN leaves have antibacterial, antimicrobial, haematopoietic and gastrointestinal protective properties (Agbor and Odetola, 2001; Odetola *et al*., 2006; Makanjuola *et al*., 2010). As it has been known that the biological activities of plants have been attributed to the chemical compounds in them, studies have shown that PN leaves contain alkaloids, saponins, flavonoids, cardiac glycosides, steroids, tannins, phlobatannins, cardenolides, phenolics, anthraqinones and titerpenes. (Sopeyin and Ajayi, 2016; Kayode and Yakubu, 2017). These chemical compounds can work synergetically in the plant to exert its biological action as suggested by Adeyemi *et al*. (2018). Although PN has been reported to possess haematopoietic activities, increasing erythrocytes indices in anaemic rats on a dose basis (Agbor and Odetola, 2001; Agbor and Odetola, 2005), however, the infused extract of its leaves has not been used for the management of iron deficiency anaemia in weanling rats, and the mechanism by which its anti-anaemic action is carried out has not been studied.

This study is therefore focused on determining the haematopoietic effect of the infused leaf extract of *Parquetina nigrescens* on anaemic models. Hence, providing a food-based approach sourced from a plant considered as waste, for the alleviation of iron deficiency in the resource-poor population.

Materials and Methods

Plant materials and extract preparation

Parquetina nigrescens leaves were harvested from Flower Garden Area (8029'28" N 4034'51" E) Ilorin, Nigeria, and were identified and authenticated at the Department of the Plant Biology University of Ilorin, Ilorin Nigeria where the voucher number UILH/001/980 was issued. The leaves were air-dried for 2 weeks after which it was pulverized using a Kenwood BL 440 electric blender. The additives (ginger and lemongrass) were added individually in a ratio of 1:4 to 100 g of the pulverized plant sample; mixed thoroughly and infused with 1.5 litres of boiling water (100 °C) for 10 minutes. The infusion was filtered using a Buchner funnel and concentrated in a water bath at 40 °C. A calculated amount of the resulting extract was reconstituted in distilled water to give 1000 mg/kg body weight (Awoniyi, 2019).

Chemicals, assay kits and reagents

Hepcidin, Ferroportin, and Divalent Metal Transporter 1 ELISA kits were products of Elabscience Biotechnology Co., Ltd. U.S.A. Ferritin ELISA kit was a product of Calbiotech, Inc. California U.S.A., while Transferrin kit was a product of Fortress diagnostics Ltd. U.K. All other chemicals were of analytical grade.

Other materials

Zingiber officinale (Ginger) was obtained from Oja Oba Market Ilorin Nigeria. *Cymbopogon citratus* (Lemongrass) was obtained from Flower Garden G.R.A Ilorin Nigeria. Milk was a product of Friesland Campina WAMCO Nigeria PLC.

Feed components

The locusts' bean seed, maize, and rice bran were obtained from Ganmo Market, Ilorin, Nigeria. The vitamin mix was a product of TAV VETERINARIA, S.L., Barcelona, Spain, and the vegetable oil was a product of Grand Cereals Limited, Jos, Nigeria while sucrose was a product of Dangote flours and mills in Nigeria.

Secondary metabolites screening

The secondary metabolites present in the extracts of *P. nigrescens* were quantitatively determined using the methods of Harborne (1998); Jagadish *et al.* (2009); Obadoni and Ochuko (2001); Olajire and Azeez (2011) and Trease and Evans (1989).

Determination of ascorbic acid content

The method described by Kyaw (1978) was used in the determination of the ascorbic acid content of the extracts. The principle was based on phosphomolybdate being stoichiometrically reduced by ascorbic acid in the presence of inorganic phosphorous to give a characteristic molybdenum blue colour which absorbs light at 660 nm.

Diet formulation

The modified method of Modupe and Oladiji (2016) was used for the diet formulation. The locust bean seed was de-hulled, ground, and then autoclaved to remove anti-nutrients. The maize was ground into powdery form to serve as corn starch. All the components of the diet as shown in Table 1 were thoroughly mixed, made into pellets to ensure good handling by the animals, and oven-dried at 50 °C. The dried feeds were packed into air-tight polythene bags and stored in the freezer to prevent rancidity, auto-oxidation of the oil, and microbial contamination.

Animal experiment ethics

The animal study was carried out using an appropriate procedure following standard guidelines for animal experiments and approved by the Ethical Review Committee of the University of Ilorin, Ilorin, Nigeria with approval number UERC/ASN/2017/916.

Animal grouping

Thirty weanling Wistar rats of both sexes with a mean weight of (55.0 ± 10.0) g were obtained from the animal house of the Central Research Laboratories of the University of Ilorin, Ilorin Nigeria. The rats were housed in well ventilated standard housing conditions (28-31 °C, 12h light-12h dark cycle) and allowed free access to water and diets, they were acclimatized to breeding conditions for 7 days with normal rat chow and afterward fasted for 12 hours (food except the water was removed) before a change in diet. The rats were divided

into two groups (A and B) based on body weight. Group A consisted of 5 rats fed with an iron sufficient (IS) diet for 6 weeks while rats in group B were 25 and were fed an iron-deficient (ID) diet for 6 weeks to induce an iron deficiency state.

Haematological indices of rats in each group were determined after 6 weeks of exposing the rats in different groups to their respective diet. This was done by drawing blood from the orbital sinus of the rats using a heparinized capillary tube. Rats in group B that had haemoglobin concentration and red blood cell count significantly ($p < 0.05$) less than that of rats in group A (iron sufficient) were considered to be iron deficient.

Table 1. Composition of formulated diet (g/kg)

Feed components	Iron sufficient (g/kg)	Iron deficient(g/kg)
Cornstarch	40	40
Locust bean seed	750	750
Soyoil	40	40
Rice bran	20	20
Sucrose	100	100
*Vitamin mix	10	10
**Mineral mix	30	30
Methionine	5	5
Lysine	5	5
$FeSO_4.7H_2O$	35.06 mg/kg	-

Soybean oil: polyunsaturated fatty acids (58%), monounsaturated fatty acids (29%), saturated fatty acids (13%)
*Vitamin mix (per kg diet): Thiamine hydrochloride, 6mg; pyridoxine hydrochloride, 7mg; nicotinic acid, 30mg; calcium panthothenate, 16mg; folic acid, 2mg; biotin, 0.2mg; cyanocobalamin, 0.01mg; retinol palmitate, 4000IU; cholecalciferol, 1000IU; α-tocopherol acetate, 50IU; menadione, 0.05mg; choline chloride, 2g
**Mineral mix (g/kg diet): $CoCl2{\cdot}6H_2O$ (0.001), $CuSO_4{\cdot}5H_2O$ (0.079), $MnSO_4{\cdot}7H_2O$ (0.178), KI (0.032), NaCl (3.573), $ZnCO_3$ (1.60), $CaSO_4$ (11.61), $MgSO4{\cdot}7H_2O$ (2.292), K_2HPO_4 (10.559).
Source: Modupe and Oladiji (2016)

Extract administration

The rats in group A were fed with an iron sufficient diet and orally administered with 0.5ml of the vehicle (distilled water) on daily basis for 28 days designated as IScontrol. The rats in group B were further grouped into five (5) with five (5) rats in each group as follows:

B1- Iron deficient rats orally administered on daily basis for 28 days with 0.5ml of the vehicle (distilled water) designated as ID.

B2- Iron deficient rats orally administered on daily basis for 28 days with 0.5ml 1000mg/kg body weight of infused leaf extract of *Parquetina nigrescens* designated as PN

B3- Iron deficient rats orally administered on daily basis for 28 days with 0.5ml 1000mg/kg body weight of infused leaf extract of *Parquetina nigrescens* + ginger designated PN+ ginger.

B4- Iron deficient rats orally administered on daily basis for 28 days with 0.5ml 1000mg/kg body weight of infused leaf extract of *Parquetina nigrescens* + lemongrass designated PN+ lemongrass.

B5- Iron deficient rats orally administered on daily basis for 28 days with 0.5ml 1000mg/kg body weight of infused leaf extract of *Parquetina nigrescens* + milk designated PN+ milk. Milk was added to the concentrated extract in ratio 1:4 at the point of administration.

Collection of blood and preparation of serum

After terminating extract administration, the rats were subjected to fasting for 12 h and anesthetized with diethyl ether. Blood was sampled from the jugular vein and collected in an EDTA anticoagulant. Serum was separated by centrifugation at 3000×g for 15mins at 4 °C after standing at room temperature for 30 min

(Yakubu *et al*., 2005). The supernatant was collected into dry, clean sample bottles and stored at -18 °C until used for analysis.

Determination of haematological parameters

The Sysmex KX 21N haematology analyzer was used to determine the haematological parameters. Haemoglobin was released during red blood cell lysis, converted to red methemoglobin, and read photometrically at 555nm.

Selected serum protein assay

The procedures as instructed on the kit's protocols were followed for assay of Divalent Metal Transporter 1, ferroportin, hepcidin, transferrin, and ferritin.

Statistical analysis

SPSS (Statistical Package for Social Sciences, Inc., Chicago, IL, USA) version 20.0 was used. Values obtained were expressed as mean ± standard error of the mean (SEM). The values were subjected to Analysis of Variance (ANOVA) and Duncan's Multiple Range Test, which was used to determine statistical significance. Differences were considered significant at $p<0.05$.

Results

Secondary metabolites of infused extracts

The results obtained for the quantitative determination of some secondary metabolites of the studied plant extracts are shown in Table 2. Plants contain various ranges of chemical components referred to as secondary metabolites which are proven to have biological activity. The PN extract had a significantly ($p < 0.05$) higher concentration of most (phenols, flavonoid, terpenoids, steroids, and tannin) of the secondary metabolites analysed when compared to other extracts. Also, concentrations of saponin and cardiac glycosides were highest in the PN+ ginger extract while values of terpenoids, alkaloids, and phlobatannins were similar in the PN+ ginger and PN+ lemongrass extracts.

Table 2. Concentration of selected secondary metabolites of infused extracts of *Parquetina nigrescens* leaf (mg/L)

Samples	Phenols	Saponin	Flavonoids	Cardiac glycosides	Terpenoids	Steroids	Tannins	Alkaloids	Phloba-tannins
PN	2.0 ± 0.01^{c}	2.37 ± 0.01^{a}	2.27 ± 0.01^{c}	3.05 ± 0.01^{a}	0.51± 0.01^{b}	0.42 ± 0.01^{c}	0.89 ± 0.01^{c}	2.68 ± 0.05^{a}	0.32 ± 0.01^{a}
PN + ginger	0.95 ± 0.01^{b}	8.4 0± 0.02^{c}	0.91 ± 0.01^{b}	5.56± 0.07^{c}	0.09 ± 0.01^{a}	0.30 ± 0.01^{b}	0.44 ± 0.01^{b}	15.05± 0.37^{b}	1.41± 0.01^{b}
PN + lemongrass	0.31 ± 0.01^{a}	4.22± 0.06^{b}	0.67 ± 0.01^{a}	4.17± 0.01^{b}	0.10 ± 0.01^{a}	0.22 ± 0.01^{a}	0.36 ± 0.02^{a}	12.99 ± 0.86^{b}	1.17± 0.01^{b}

Values are means of 3 determinations ± SEM. Values with different superscripts down the column are significantly ($p < 0.05$) different. PN: *P. nigrescens*

Ascorbic acid content

The results in Table 3 are to investigate the possible effect of the ascorbic acid content of the plant on iron absorption. As seen in Table 3, appreciable amounts of ascorbic acid are seen in all the extracts studied with the PN+ ginger extract having the highest concentration and PN+ milk the lowest. Concentrations of PN and PN+ lemongrass are statistically not different from each other.

Table 3. Ascorbic acid content of infused extracts of *Parquetina nigrescens* leaf with additives

Groups	Ascorbic acid (µg/g)
PN	22.96 ± 3.70^{b}
PN + ginger	72.59 ± 0.74^{c}
PN + lemongrass	24.55 ± 0.12^{b}
PN + milk	2.37 ± 0.04^{a}

Values are means of 3 determinations ± SEM. Values with different superscripts down the column are significantly ($p < 0.05$) different. **PN:** *Parquetina nigrescens*

Growth pattern of rats

The weights of rats in the iron-deficient group were lower than rats in the iron-sufficient group (Figure 1). The growth pattern of rats administered with PN and PN + ginger extracts increased progressively throughout extract administration while rats in the PN + lemongrass group experienced fluctuations in weight throughout treatment. Animals administered the PN + milk extract increased in weight gradually until the 4th week when there was a 13% reduction, but this was reversed by a 16% increase by the 5th week.

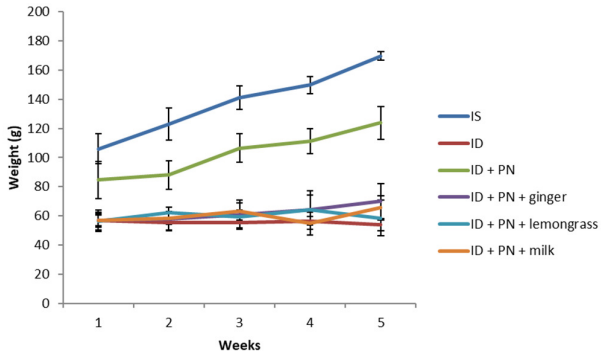


Figure 1. Growth pattern of rats following oral administration of 1000mg/kg body weight infused extracts of *P. nigrescens* with additives for 28 days

Values are means of 3 determinations ± SEM. **IS**: Iron Sufficient Rats; **ID**: Iron Deficient Rats; **PN**: *Parquetina nigrescens*

Haematological indices of rats

The haematological indices of rats fed with the various extracts are shown in Table 4. Intake of the PN extract significantly ($p < 0.05$) increased the HGB concentration of the iron-deficient rats. Conversely, there was no significant difference in HGB concentrations of rats fed with other extracts. The PN group had concentrations of HCT, MCV, MCH, and MCHC that compared well with the IS group. Administration of the PN+ ginger extract resulted in significantly higher concentrations of HCT and MCV in iron-deficient rats.

Table 4. Haematological indices of rats following oral administration of 1000 mg/kg body weight infused extracts of *Parquetina nigrescens* leaf with additives for 28 days

Groups	WBC (x10^{5}/µL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
IS	12.20 ± 1.02^{a}	10.90 ± 0.09^{c}	33.23 ± 0.67^{a}	47.67 ± 0.56^{a}	15.67 ± 0.35^{b}	32.90 ± 0.65^{b}
ID	19.70 ± 1.50^{abc}	8.95 ± 0.05^{a}	44.45 ± 0.05^{b}	61.10 ± 1.50^{bc}	13.30 ± 0.20^{a}	21.80 ± 0.20^{a}
PN	13.00 ± 1.46^{a}	10.43 ± 0.64^{bc}	34.80 ± 1.13^{a}	48.57 ± 1.68^{a}	14.50 ±0.32^{ab}	30.0 ± 1.50^{b}
PN + ginger	14.20 ±8.30^{ab}	8.60 ± 0.40^{a}	42.15± 1.85^{bc}	68.60 ± 7.30^{c}	14.00 ±1.45^{ab}	20.40 ± 0.10^{a}
PN + lemongrass	24.00 ± 1.00^{bc}	9.59 ± 0.01^{abc}	41.60± 0.01^{bc}	59.43 ± 0.03b^{c}	13.71± 0.01^{ab}	23.11 ± 0.01^{a}
PN + milk	25.50 ± 0.50^{c}	9.10 ± 0.40^{ab}	39.80 ± 1.80^{b}	58.15 ± 2.05^{b}	13.30 ± 0.50^{a}	22.85 ±0.05^{a}

Values are means of 3 determinations ± SEM. Values with different superscripts down the column are significantly ($p < 0.05$) different. **IS:** Iron Sufficient Rats; **ID**: Iron Deficient Rats; PN: *Parquetinanigrescens;* **WBC:** White Blood Cell; **HGB:** Haemoglobin; **HCT:** Haematocrit; **MCV:** Mean Corpuscular Volume; **MCH:** Mean Cell Haemoglobin; **MCHC:** Mean Cell Haemoglobin Cell.

Selected serum protein

Concentrations of divalent metal transporter 1 (DMT1), ferroportin, and transferrin of the iron-deficient rats were significantly ($p < 0.05$) reduced during the administration of the PN extract, while those of hepcidin and ferritin were increased (Table 5). Administration of PN+ ginger and PN+ lemongrass extracts resulted in a decrease in the concentrations of DMTI of the iron-deficient rats and subsequent elevation of their hepcidin concentrations. Intake of the PN+ milk extracts gave a decrease in transferrin concentration and an increase in hepcidin concentration. Concentrations of ferritin were significantly ($p < 0.05$) increased in iron-deficient rats administered PN and PN + lemongrass extracts.

Table 5. Concentrations of selected serum proteins following oral administration of 1000 mg/kg body weight infused extracts of *Parquetina nigrescens* leaf with additives for 28 days

Groups	DMT1 (ng/ml)	Ferroportin (ng/ml)	Hepcidin (pg/ml)	Transferrin (g/L)	Ferritin (ng/ml)
IS	5.18 ± 0.03^{a}	12.65 ± 0.55^{a}	904.17 ± 55.12^{bc}	33.58 ± 1.08^{a}	25.00 ± 2.87^{c}
ID	7.35 ± 0.45^{b}	16.80 ± 0.15^{b}	457.50 ± 42.50^{a}	53.92 ± 1.08^{bc}	16.33 ± 1.33^{ab}
PN	5.63 ± 0.01^{a}	11.87 ± 0.78^{a}	1400.00 ± 100.00^{d}	33.75 ± 4.75^{a}	27.00 ± 1.15^{c}
PN + ginger	4.65 ± 1.35^{a}	13.95 ± 0.05^{ab}	737.50 ± 37.50^{b}	44.25 ± 0.75^{ab}	12.50 ± 2.50^{a}
PN + lemongrass	4.95 ± 0.05^{a}	15.15 ± 2.85^{ab}	1058.33 ± 68.21^{c}	60.75 ± 0.75^{c}	47.75 ± 2.75^{d}
PN + milk	5.88 ± 0.58^{ab}	15.00 ± 1.20^{ab}	1010.00 ± 10.00^{c}	39.00 ± 5.50^{a}	21.00 ± 1.00^{bc}

Values are means of three determinations ± SEM. Values with different superscripts down the column are significantly ($p < 0.05$) different. **IS**: Iron Sufficient Rats; **ID**: Iron Deficient Rats; **PN**: *P. nigrescens*

Discussion

A few of the secondary metabolites present in the extracts studied are suggestive of their antioxidant properties as it has been reported by (Saha *et al*., 2018) to be essential for iron uptake and absorption. The PN extract with significantly ($p < 0.05$) higher concentration of most metabolites such as phenols, flavonoid, steroids, and tannins have been shown to possess better hematopoietic activity than extracts of PN + ginger and PN + lemongrass. This activity may be attributed mainly to the higher concentration of flavonoids in the extract just as it has been reported by (Raja *et al*., 2011; Iyare*et al*., 2015) that the hematopoietic properties of plants could be attributed to the presence of flavonoids.

All extracts of the plant contained ascorbic acid (Table 3). This can be an indicator that intake of the extracts may enhance absorption of iron, as previously reported by (Teucherl *et al*., 2004) that ascorbic acid is the most effective non-haem iron enhancer. The exceptionally high concentration of ascorbic acid in PN + ginger extract could be due to the previous reports of high concentrations of ascorbic acid in ginger (Kumari and Gupta, 2016). Therefore, the presence of ascorbic acid in the extracts may enhance the absorption of iron.

The result in Figure 1 showed that iron-deficient rats experienced reduction in growth rate. This is in agreement with previous studies (Fernandes *et al*., 1997; Clark, 2008; Oladiji *et al*., 2007) where weight loss was indicated as one of the features of iron deficiency. This reduction in growth rate in iron deficient rats has been attributed to reduction in disaccharidase activities in the last stage of carbohydrate digestion. (Lanzkowsky *et al*., 1982; Fernandes *et al*., 1997; Modupe and Oladiji 2016). It was observed that on administration of the PN extract, the growth pattern of the rats improved indicating the extract can help to increase growth rate in iron deficiency condition.

Earlier studies stated that iron deficiency in the body limits the synthesis of haemoglobin (HGB) and decreases the production of red blood cells (RBC) in the bone marrow resulting in anaemia (Nagababu *et al*., 2008; Oladiji *et al*., 2007). Therefore, decreased concentration of HGB in the iron-deficient rats which was

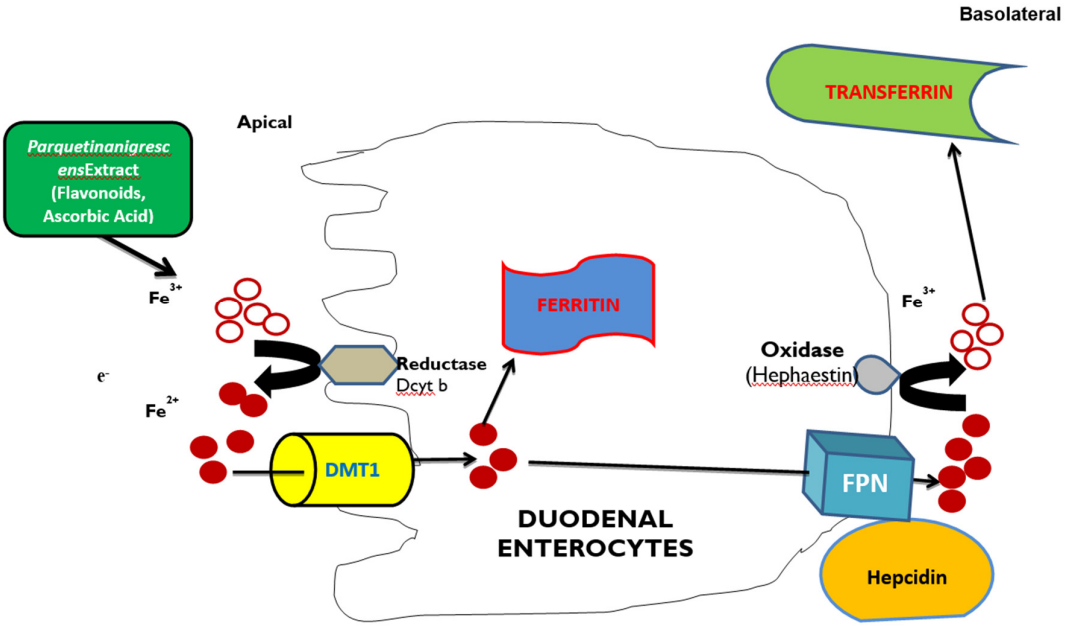
significantly ($p < 0.05$) increased on the administration of the PN extract (Table 4) was an indication that the extract was able to ameliorate the iron deficiency state in the rats.

Divalent Metal Transporter 1 (DMT1) is an intestinal iron transporter that is produced more in an iron-deficient state (Barisani, 2004; Shawki*et al*., 2015). Therefore, significant ($p < 0.05$) increase in concentration of DMTI (Table 5) in the iron-deficient rats indicates an iron deficiency state; this condition was ameliorated with the administration of the PN, PN + ginger, and PN + lemongrass by the observed reduction in the concentration of DMT1.

Ferroportin is the sole iron exporter in mammals and it functions to release iron from the enterocytes (Frazer and Anderson, 2014), while transferrin is the principal iron transport protein in the plasma. Its concentration increases iron deficiency to maximize utilisation of available iron (Kelly *et al*., 2017). Hepcidin is a major regulator of intestinal iron absorption and iron recycling from macrophages (Nemeth *et al*., 2004). In epithelial cells, hepcidin acts on the efflux of iron through direct interaction with ferroportin at the cell surface, leading to internalization and degradation of the ferroportin, thereby blocking blood uptake of iron (Ganz, 2005; Delaby*et al*., 2005). In this study, the ferroportin and transferrin concentrations increased significantly ($p < 0.05$) in the iron-deficient rats and the corresponding reduction in the concentration of hepcidin as shown in Table 5; demonstrates that the rats were in a state of iron deficiency. This condition was observed to improve on the administration of the PN extract, indicating iron could have been exported to the bloodstream by ferroportin, and transported within the blood by transferrin, hence a reduction in their concentrations. The resultant surge observed in the hepcidin concentration of the PN group could be due to the need to internalize and degrade ferroportin to prevent further iron uptake until the body requires iron again.

Ferritin is the intracellular storage form of iron, with small amounts of it secreted in the serum (Kelly *et al*., 2017; WHO, 2011). The concentration of the serum ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation; a low serum ferritin value therefore reflects depleted iron stores (WHO, 2011). In this study, the observed low serum ferritin concentration of the iron-deficient rats (Table 5) suggests the iron stores were depleted. However, upon administration of the PN and PN + lemongrass extracts, the ferritin level was elevated, indicating that intake of these extracts can help improve the availability of iron and hence ameliorate iron deficiency.

In this study, animals administered the PN extract seem to have better activity than other groups, demonstrating better haematologic activity and growth in iron deficiency condition. Flavonoids have been reported to be responsible for the hematopoietic properties exhibited by some medicinal plants (Iyare*et al*., 2015; Raja *et al*., 2011), and have also been implicated as an electron donor for duodenal cytochrome *b* like that of ascorbate by observing the reduction of extracellular Fe(III) to Fe(II) (Vlachodimitropoulos *et al*., 2010). Ascorbic acid has been reported to enhance the absorption of iron (Cook and Reddy, 2001; Sharp, 2010). Therefore, the presence of flavonoids and ascorbic acid in this extract could be responsible for the significantly ($p < 0.05$) higher concentrations of haemoglobin in rats administered the PN extract compared to other extracts. Also, the iron transport proteins: DMT1, ferroportin, ferritin, transferrin, and hepcidin in rats administered PN extract, were able to transport and store iron well than other extracts. Based on these findings, a proposed mechanism by which PN ameliorate iron deficiency is depicted in Scheme 1.



Scheme 1. Proposed mechanism of action of *P. nigrescens* leaf extract
DMT1: Divalent Metal Transporter 1; **FPN:** Ferroportin

It is proposed that flavonoids in the PN extract serve as an electron donor for duodenal cytochrome *b*. This electron is used to convert extracellular Fe^{3+} to Fe^{2+}, which is then transported into the small intestine by divalent metal transporter 1 (DMTI) and some of theFe^{2+}stored as ferritin in the cytoplasm and the remaining exported out of the cell by ferroportin. As a result of iron sufficiency, the concentration of hepcidin rose to block ferroportin and maintain iron homeostasis.

Conclusions

The infused extracts of *P. nigrescens* possess haematopoietic properties, with the PN extract demonstrating a better activity than PN+ ginger, PN + lemongrass, and PN + milk extracts. Hence, *P. nigrescens* infused extract possess greater haematopoietic activity when taken without additives. Also, flavonoid and ascorbic acid concentrations of the PN extracts are proposed to enhance its haematopoietic activity.

Authors' Contributions

Conceptualization and supervision of this study was done by Prof. Oladiji A. T. The methodology was jointly carried out with Dr. Abidakun O. M. The initial draft of the manuscript was written by me while Prof. Oladiji A.T. and Dr. Abidakun O.M. reviewed and edited the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The animal study was carried out using an appropriate procedure following standard guidelines for animal experiments and approved by the Ethical Review Committee of the University of Ilorin, Ilorin, Nigeria with approval number UERC/ASN/2017/916.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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