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EFFECT OF AQUEOUS LEAF EXTRACT OF *JUSTICIA CARNEA* ON GEOPHAGY-INDUCED IRON DEFICIENCY ANEMIA IN WISTAR RATS.

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Abstract

Iron is an important mineral for many physiological processes especially erythropoiesis and hematopoiesis. Its deficiency can have detrimental effects, thus, iron deficiency anemia. This research was carried out to determine the effect of aqueous extract of *J. carnea* on geophagy induced iron deficiency anemia (IDA) in Wistar rats. The animals were divided into six groups consisting of two control groups (Normal and untreated) and 4 treatment groups. Iron deficiency anaemia was induced in the treatment groups by feeding them with 30% edible clay for 49 days. The extracts and standard drug (Chemiron) were then orally administered to the various groups after confirmation of IDA for 28 days. The animals were sacrificed by anaesthetizing with chloroform and their blood samples drawn via cardiac puncture. Hematological indices were assayed using auto hematology analyzer. The results obtained showed that in the extract treatment groups, there was a dose dependent decrease in serum TIBC level (987.02±333.06, 1268.99±329.45, 1216.61±42.95 for 200,400,600mg/kg respectively) and increase in MCV (52.73±0.78, 53.80±0.67, 53.55±2.25 for 200,400,600mg/kg respectively). There were slight increases in red blood cell count (RBC), from 6.53±0.43 to 7.45±0.54 in the group treated with 200mg/kg of *Justicia carnea* extract; packed cell volume (PCV) from 35.67±0.88 to 39.23±2.54 in the group treated with 200mg/kg of *J. carnea* extract and hemoglobin count (Hb) from 11.57±0.22 to 12.77±0.67 in the group treated with 200mg/kg of *J.carnea* extract in the treatment and standard drug groups when compared to the control. These differences were significant ($p<0.05$). These findings suggest that aqueous extract of *Justicia carnea* could be a potential candidate for the management of iron deficiency anemia. Further research should be carried out with novel techniques to isolate and purify the component(s) of this raw extract that has the potential to increase systemic iron transport and storage.

Keywords: Geophagy, Anemia, Aqueous leave extract, *Justicia carnea*.

Introduction

Traditional medicine practitioners have long relied on plants as a primary source of therapeutic agents. According to estimates, approximately 70-80% of the global populations depend on herbal remedies for their fundamental healthcare needs (Adenuga *et al*., 2020). Many edible plants possess numerous health benefits outside normal nutritional properties commonly attributed to their nutraceutical properties (Hunter and Fletcher, 2002). A good number of medicinal plants have been traditionally employed in alleviating anemic conditions. Some of such plants include; *Telfeiraocc identalis, Combretumdolic hopetalum, Psorospermum ferbrifugum, Jatropha curcas, Flacourtia flavenscens, Brillantasia nitens* and *Justicia carnea* (Dina *et al*., 2006).

Anaemia can be defined as a reduction in the number of circulating red blood cells (RBCs) (Kumar *et al*., 2020) or as a condition in which the number of erythrocytes (and subsequently their ability to carry oxygen) is insufficient to meet physiological needs (WHO, 2011). Anemia is characterized by a decrease in the haemoglobin (Hb) level in the blood (generally less than 13.5 g/dL in men and 12.5 g/dL in women), which results in a reduction in carrying capacity of oxygen. Diseases that decrease Hb production (e.g., iron deficiency, B12, or folate deficiencies) or accelerate its destruction are often the result of a defect in the structure of Hb (Rhodes and Varacallo, 2020). Anaemia is a major public health problem. It affects people of all ages, especially pregnant women and children. According to statistics, globally, anemia affects 41.8% of pregnant women and 47.4% of pre-school children (Mclean *et al*., 2009). Moreover, it has negative effects on health and development, including neonatal and perinatal mortality, low birth weight (Figueiredo *et al*., 2018), premature birth (Haider *et al*., 2013; Rahman *et al*., 2016), and developmental delays of the children (McCann *et al*., 2007). Clinically, it is characterized by pallor, fatigue, dizziness, difficulty breathing, dyspnea and weakness (Hillman *et al*., 2001). In the absence of effective management, anaemia can promote decreased cognitive ability, weakened immune system, and increased mortality. Anemia is equally characterized by an elevated level of erythropoietin in response to decreased Hb and generally reflects a loss of erythrocytes, caused by bleeding or hemolysis. Bone marrow responds appropriately to a low erythrocyte mass by increasing reticulocyte production.

Classification of anemia according to erythrocyte morphology is more useful in medical practice. Microcytic anaemia is the type of anemia in which circulating RBCs are smaller than usual. The most common cause of this type of anemia is a decrease in the body's iron reserves, which can have several causes, such as decreased iron in the diet; reduced absorption of iron in the intestine; acute and chronic blood loss; and increased iron demand in certain situations, such as pregnancy and recovery after a major trauma or surgery (Coralia *et al*., 2021).

Iron-deficiency anemia (IDA) is one of the most common type of anaemia. It typically results from insufficient iron intake, poor gastrointestinal absorption, or overt or occult blood loss. Iron deficiency is a state in which reduction of iron stores precedes development of overt iron-deficiency anemia (Besarab and Hemmerish, 2018). One of the major causes of IDA is the consumption of food low in iron or very low absorbable iron. Also, this can be initiated by eating foods rich in iron chelators. With this sustained increase in iron chelation,

there will be depletion in the iron stores. In Africa, one of the major causes of IDA is the practice of pica (act of craving and chewing of substances that have no nutritional value, such as clay) especially geophagy. Pica describes a behavior of craving and subsequent purposeful ingestion of non-food substances (Young, 2010). Pica was documented as early as 400 B.C. by Hippocrates and continues to be practiced today. Pica is generally considered to be a chronic behavior (> 1month) (Njiru *et al*., 2011). There are three commonly described forms of pica, corresponding to three most frequently consumed non-food substances: geophagy- ingestion of earth (soil, clay or baked clay), amylophagy- ingestion of raw starch and pagophagy- ingestion of ice (Lopez *et al*., 2007).

Overall, geophagy occurs most often, especially among pregnant women and children, although the prevalence of pica and the non-food substances consumed vary geographically. In Africa for example, geophagy is the most common (Al-Rmalli *et al*., 2010).

Several studies in both developing and non-developed countries, have demonstrated that anemia and low hemoglobin concentration are commonly found in pregnant women who practiced geophagy. However a causal relationship remains unclear as studies of iron supplementation among children with anemia and geophagy failed to stop soil ingestion (Thihalolipaven *et al*., 2012). A large majority of women who reported craving for the non-food substances cited an affinity towards the substance's taste, odour and texture as reasons for their ingestion. This is to say that the practice of geophagy ingestion is most likely driven by more complex interplay of multifactorial etiologies that warrant further investigation (Corbett *et al*., 2003).

Women are at high risk of iron deficiency mainly due to menstrual flow and having a diet with low iron bioavailability or unbalanced and inadequate micronutrient intake (Horvath *et al*., 2014). Effects of iron deficiency can be detrimental, for example health concerns such as memory disorders, anemia during pregnancy and lactation (Perez *et al*., 2009)

In Nigeria, studies shows that the decoction of *Justicia carnea* plant leaf is used as a blood supplement and for the management of anemia. *Justicia carnea* commonly known in Nigeria as:''blood root'' (Oladele and Elem, 2018), "hospital too far" (Udedi *et al*., 2020), "ogwuobara" in Igbo, 'Oso-afia in Ogba/Egbema/Ndoni (Oladele and Elem, 2018), in Brazil and South America as:"Jacobinia", "Pink jacobinia" Cardinal's guard","Brazilian plume", "Pine-bur begonia" andflamingo flower" (Orjiakor *et al*., 2019) is a medicinal plant with varying potentials .

The use of medicinal plants, such as *Justicia carnea* (Plate 1), in the treatment of various diseases dated back thousands of years (Parsaeimehr *et al*., 2017; Akinyemi *et al*., 2018). Notably, *Justicia* is the largest genus within the Acanthaceae family, comprising approximately six hundred species (Kitadi *et al*., 2019; Correa and Alcantara, 2012). Several species of *Justicia* are widely used in folk medicine for the treatment of inflammation, respiratory, and gastro intestinal disorder (Correa, 2012). Most of the medicinal properties exhibited by plant extracts such as antimicrobial, antioxidant, hypocholesterolemic and anti-cancerous are associated with their bioactive constituents mainly phenols and flavonoids (Uroko *et al*., 2017). It has also been reported to be rich in both macronutrients and trace elements of which calcium and iron are in high concentration (Faiza *et al*., 2013).

The traditional use of *Justicia carnea* as a blood tonic is deeply rooted in the Nigeria folklore, with many indigenous communities relying on this plant to treat various blood

related disorders (Asogwa *et al*., 2020; Alkitimelin *et al*., 2021). However the Lack of scientific validation and standardization of *Justicia carnea* extracts poses significant concerns regarding their safety and efficacy. To address this concerns, it is essential to conduct comprehensive scientific investigation into the hematological effect of *Justicia carnea.* Thus this study investigated the effect of the aqueous leaf extracts of *J. carnea* on hematological indices of geophagy induced iron deficiency anaemic wistar rats.



Plate 1: Picture of *Justicia carnea* leaves. (Photo by the researcher).

Materials and Methods

Sample collection

The leaves of *Justicea carnea* were collected from Mr. Fidelis Asogwa's compound at Elugwu Idah, Ibeku Opi, in Nsukka Local government Area, Enugu state, Nigeria. The plant sample was taken to the department of Botany herbarium, Nnamdi Azikiwe University Awka, where it was identified and authenticated by, Dr. Iroka Chisom, a taxonomist in the department, a voucher specimen was deposited, and a voucher number NAUH 34 was assigned to it.

The edible clay (kaoline) was purchased from a local vendor at Eke Awka market, Awka South Local government Area, Anambra State.

Preparation of *Justicia carnea* and edible clay sample

The leaves were separated from the stalk using hands and rinsed with deionized water, dried in a filter basket and then were allowed to dry at room temperature in the drying room of the department of Applied Biochemistry, Nnamdi Azikiwe University Awka for seven (7) days. Then the leaves were grinded into fine powder and store in an air tight container until further use.

The edible clay was dried at 60^{o}C in an oven for one hour. After drying, it was ground into fine powder using mortar and pestle, and the fine powder stored in an airtight container till further use.

Extraction of Phytochemicals in the Leaf of *Justicia Carnea*

The phytochemicals in the leaf of *Justicia carnea* was extracted using the method of Barros *et al.* (2007). One kilogram (1kg) of the ground leaf sample was weighed and transferred into a 12L transparent bucket and 10L of deionized water was added to it. This was allowed to stand for 24hrs at room temperature after which it was filtered using three layers of muslin cloth. The filtrate was further filtered using Whatman paper (No.4). This was then concentrated using water bath at 60^{o}C. The slurry obtained was placed in an airtight container and stored in refrigerator at 4^{o}C till further use.

Purchase of Experimental Animals

A total of eighteen (18) wistar rats were purchased from Mr. Onyewuchi's Animal farm, Department of Applied Biochemistry, Nnamdi Azikiwe University Awka and was acclimatized for 7 days during which they were fed with growers marsh and distilled water ad libitum.

Ethical clearance

Ethical clearance was obtained from the Animal research ethics Committee, Nnamdi Azikiwe University, Awka with Reference No:NAU/AREC/2025/0018

Determination of LD_{50} of the extract

This was done by adopting the method of Lorke, 1983.

Induction of Iron deficiency Anaemia

Iron deficiency was induced by geophagy according to the report by Geissler *et al*. (2002). Thirty healthy wistar rats were fed with 30% edible clay (30% clay and 70% grower's marsh) and water for 49 days. After the 49days, blood was collected from four (4) randomly selected rats and their RBCs, MCV, PCV and hemoglobin was assayed to establish the induction of iron deficiency anaemia. The Iron deficient anaemic rats were grouped and used for the study.

Grouping of the animals

The rats were divided into six groups of six animals each. Negative control group: This group received only food and water

a. Untreated group: This group of animals has iron deficiency anaemia but not treated.
b. 200mg/kg extract group: This group has iron deficiency anaemia and was treated with the extract at a dose of 200mg/kg.
c. 400mg/kg extract group: This group has iron deficiency anaemia and was treated with the extract at a dose of 400mg/kg.
d. 600mg/kg extract group: This group has iron deficiency anaemia and was treated with the extract at a dosage of 600mg/kg.
e. Baseline (Not induced): This group is normal group that received water and growers marsh only
f. Standard drug group (Positive control): This group has iron deficiency anaemia and was treated with a standard drug (Chemiron syrup), 10mg/kg.

Sacrifice of the animals and blood collection

The animals were anaesthetized with chloroform and blood collected via cardiac puncture. The samples for hematological analysis were placed in EDTA bottles while those for iron and total iron binding capacity (TIBC) was collected in a plain container and allowed to coagulate. The coagulated blood was centrifuged for 15mins at

4000rpm and the serum was carefully collected using Pasteur pipette.

Hematological Analyses
The red blood cell, the white blood cell, the mean corpuscular volume, hemoglobin and packed cell volume were analyzed using autohematology analyzer (Incubio iCell-820) according to the method described by Asogwa *et al*. (2024).

Serum iron determination
This was carried out by adopting the method of Jung and Parekh, (1970) with little modification. A teco diagnostics serum iron/UIBC kit was used. To 0.2ml of serum, deionised water (blank) and Fe standard (500µg of ferrous chloride in hydroxylamine hydrochloride), 1ml of Fe buffer reagent (acetate buffer containing 220mM hydroxylamine hydrochloride, pH 4.5 with surfactant) was added. The absorbance of this mixture was measured at 560nm immediately and recorded as A_1. This was followed by addition of 20µl Fe color reagent (16.6mM ferrozine in hydroxylamine hydrochloride). All the tubes were placed in a heating water bat at 37^{o}C for 10 minutes. The absorbance was measured at 560nm and recorded as A_2. The serum iron concentration was calculated thus:

$$\frac{A2sample - A1sample}{A2standard - A1standard} \times Concentrationofstan$$

Where Concentration of standard=500µg/dl.

Unsaturated iron binding capacity (UIBC)
This was carried out by adopting the method of Jung and Parekh, (1970) with little modification. A teco serum iron/UIBC kit was used. To 1ml of UIBC buffer reagent containing a mixture of 0.5M Tris buffer, pH 8.0 with surfactant, and sodium azide as preservative, 0.25ml of sample, Fe standard and 0.5ml of deionised water (blank) were added in the respective test tubes. This was followed by the second addition of 0.25ml of the Fe standard in the sample and standard tubes. The absorbance of this mixture was measured at 560nm and recorded as A_1. To measure A_2, the Fe color reagent (16.6mM ferrozine in hydroxylamine hydrochloride) was added. The mixture was heated in a water bath for 10minutes at 37^{o}C. The absorbance (A_2) was thus measured and the unsaturated iron binding capacity was measured thus:

$$Concentration\ od\ standard - \frac{A2sample - A1sample}{A2standard - A1standard} \times Concentration\ of\ standard$$

Where concentration of standard=500µg/dl.

Total iron binding capacity (TIBC). This was carried out by adopting the method of Jung and Parekh, (1970) with little modification. The total iron binding capacity (TIBC) was determined by summing the serum iron value and the UIBC value.

Statistical Analysis
The results obtained were analyzed using One-way ANOVA through Statistical Product and Service Solutions (SPSS) version 22, and significant mean differences were separated with Tukey's Post-hoc test. A P-value of less than 0.05 was deemed statistically significant.

Results
Results of the LD_{50} of the aqueous leaf extract of *Justicia carnea*.
Table 1 shows the acute toxicity test (LD_{50}) results of the aqueous leaf extract of *Justicia carnea*. There was no mortality when the extract was administered to the experimental animals at all doses (up to 5000 mg/kg).

Table 1: Acute toxicity test (LD_{50}) of the aqueous leaf extract of *Justicia carnea*.

Phases	Serial	Groups	Number of	Dosage	Mortality	Observed

	number		animals			side effects
Phase 1	1	Group I	3	100mg/kg	0/3	Nil
	2	Group II	3	500mg/kg	0/3	Nil
	3	Group III	3	1000mg/kg	0/3	Nil
Phase 2	4	Group IV	1	1500mg/kg	0/1	Nil
	5	Group V	1	2000mg/kg	0/1	Nil
	6	Group VI	1	2250mg/kg	0/1	Nil
	7	Group VII	1	2500mg/kg	0/1	Nil
	8	Group VIII	1	2750mg/kg	0/1	Nil
	9	Group IX	1	3000mg/kg	0/1	Nil
	10	Group X	1	3500mg/kg	0/1	Nil
	11	Group XI	1	4500mg/kg	0/1	Nil
	12	Group XII	1	5000mg/kg	0/1	Nil

TIBC and Serum Iron levels

Geophagy caused a significant ($p < 0.05$) reduction in serum ion from 444.11±8.47 µg/dl in the normal control to 84.03±5.11 µg/dl in the Geophagy induced iron deficiency anaemic rat. This confirmed IDA. After 3 weeks of extracts and drug administration, the untreated group had the least serum iron level (95.07±19.39 µg/dl) while the standard drug group had the highest level (1450.70±464.24 µg/dl). The increase in serum iron in the extract groups was also concentration dependent (A linear increase in serum iron with increase in extract concentration). Also, Geophagy caused a significant increase in TIBC level from 744.11±40.01 µg/dl in the normal group to 1327.11±30.43 µg/dl in the anaemic group. After the treatment, the standard drug group and the extract groups showed a reduced TIBC levels when compared with the untreated group. The difference in TIBC levels between the groups was not statistically significant ($p>0.05$) as shown in Table 2.

Table 2. Average serum iron levels and TIBC in the various groups after the experimental period

Groups	Serum iron (µg/dl)	TIBC (µg/dl)
Normal group	404.93±57.25	688.27±210.75
200mg/kg aq. Extract	552.82±143.85	987.02±333.06
400mg/kg aq. Extract	739.44±207.04	1268.99±329.45
600mg/kg aq. Extract	773.47±132.25	1216.61±42.95
Untreated	95.07±19.39*	1563.55±780.04*
Standard drug	1450.70±464.24	772.60±182.28

*Significantly different

The result of the hematological analyses is shown in Table 3. After induction, the baseline (induced) group had an RBC level of $5.34±2.01×10^{12}$/L while the baseline (not induced) group had an RBC of $6.77±2.11×10^{12}$/L (Fig. 1). After the experimental period, the 200mg/kg extract group and the standard drug group had the highest RBC count ($7.45±5.37×10^{12}$) and $6.90±2.07×10^{11}$ respectively while the 400mg/kg extract group and untreated group had the least ($5.40±4.79×10^{12}$ and

6.53±4.29×10^{12}/L respectively). There was a significant difference in RBC count when all the groups were compared ($p<0.05$) with the significant difference occurring when the 400mg/kg extract group was compared with the 200mg/kg extract group showing the potency of this extract to increase RBC at a lower concentration. From the result, it can be seen that geophagy did not affect the hemoglobin level. This shows that Hb level may not be a perfect marker for IDA with the slight difference in Hb level. The results after the experimental period followed the same trend with the RBC level with the 200mg/kg extract group and standard drug having the highest Hb level while the untreated and 400mg/kg extract group had the least. This also shows the linear relationship (perfect positive correlation) between RBC, Hb and even PCV levels. The difference in Hb level was significant when all the groups were compared ($p<0.05$) especially when the 400mg/kg group was compared with the 200mg/kg group and the standard drug group. After the induction period, the geophagy induced IDA anemia group had a PCV level of 36.50±0.22% while the control group (Baseline (not induced)) had a PCV level of 37.22±0.41%, thus a slight reduction due to IDA induction. After treatment with various doses of the extracts and standard drug, the 200mg/kg group and the standard drug group had the highest PCV level while the untreated and 400mg/kg extract group had the least (Appendix 3a) just like in Hb and RBC. This difference in PCV levels between the groups was significant ($p<0.05$) with this difference more significant when the PCV level of the 400mg/kg extract group was compared with that of the standard drug and 200mg/kg groups (since it had the lowest PCV level). The difference in MCV levels between the groups was not statistically significant ($p>0.05$), thus, the similar average MCV levels in all the groups with the untreated group having the highest MCV level (54.90±2.40 fL) and the 200mg/kg group having the least (52.73±0.78 fL). The control group had the highest TWBC count (1.86±0.25×10^{9}/L) while the 200mg/kg extract group had the least (1.07±0.19×10^{9}/L). The difference in TWBC count was not statistically significant when all the groups were compared ($p>0.05$).

Table 3: The average hematological indices of the various groups after the experimental survey

Groups	RBC (x10^{12}/L)	Hb (g/dl)	PCV (%)	MCV(fL)	WBC (x10^{10}/L)
Control	6.34±0.98	11.60±0.29	34.50±0.60	54.37±0.18	1.42±0.20
200mg/kg aq. Ex	7.45±0.54*	12.77±0.67	39.23±2.54	52.73±0.78	1.07±0.19
400mg/kg aq. Ex	5.40±0.48	9.50±0.90	29.03±2.69	53.80±0.67	1.47±0.19
600mg/kg aq. Ex	6.77±0.21	12.10±0.10	36.20±0.40	53.55±2.25	1.47±0.75
Untreated	6.53±0.43	11.57±0.22	35.67±0.88	44.90±2.40*	1.86±0.25
Standard drug	6.90±0.21	12.16±0.27	37.54±1.26	54.44±1.01	1.67±0.2

*Significantly different

Discussion

One of the challenges of herbal medicine is the dosage. Excessive intake sometimes leads to toxicity. The acute toxicity test of the aqueous extract *J. carnea* leaves in Table 1 shows that no deaths or adverse reactions occurred in the rats after 24 h of administration of the extract, even when 5000 mg/kg body weight of the extract was administered. This findings indicate a significant level of tolerance and safety associated with the aqueous extract, likely

due to the absence of toxic components that could elicit negative reactions at the administered doses (Orjiakor *et al.,* 2019). Furthermore, this suggests that the leaves are generally safe for consumption by both humans and animals; however, it is important to note that excessive intake of the aqueous extract may lead to chronic toxicity, as no substance can be deemed entirely safe.

Serum iron is a laboratory assessment that quantifies the concentration of iron present in the bloodstream, specifically focusing on the iron that is bound to transferrin as well as the fraction that circulates freely. The result in Table 2 show that geophagy causes significant decrease in serum iron. This may be attributed to the high level of calcium in edible clay (Ogah and Ikelle, 2015). Several studies has shown that high level of calcium inhibit iron absorption (Hallberg *et al.,* 1991). Treatment with leaf extract of *Justicia carnea* led to increase in serum iron from 95.07±19.39 in the untreated anaemic rat to 773.47±132.25 in the group treated with 600mg/kg body weight of *Justicia carnea* leaf extract. This was comparable with that of chemiron, a standard drug. *Justicia carnea* has been reported to be rich in iron (Asogwa *et al*., 2020).

The Total Iron-Binding Capacity (TIBC) test evaluates the capacity of blood to bind and transport iron throughout the body. In instances of iron deficiency anemia, one typically observes low iron levels accompanied by elevated TIBC. As indicated in Table 1, the TIBC values for the untreated group were significantly higher than those of the groups treated with plant extracts and standard drug ($p < 0.05$). It is important to note that a reduced TIBC may also suggest potential liver damage (Asogwa *et al*., 2024). A study conducted by Zwain (2020) investigating the biological effects of various herbs on anemia in rats found that the administration of cumin, ginger, and saffron extracts resulted in a decrease in TIBC among iron-deficient anemic rats, while simultaneously increasing their ferritin levels. This finding aligns with the results of the current study.

The red blood cell count depicts the number of red blood cells per liter of blood. There was a reduction in RBC count after the induction of iron deficiency anaemia. This reduction may be attributed to geophagy. Numerous cross-sectional studies have reported that geophagy has a positive correlation with anaemia and iron deficiency (Geissler *et al,* 2002). The significant increase ($p<0.05$) in RBC count in the group treated with 200mg/kg of *Justicia carnea* and standard drug group shows the potency of the extract to improve erythropoiesis at a lower concentration and the standard drug's efficacy as a blood booster. This could be attributed to the abundance macro and micro nutrient compositions of these plant and their rich antioxidant molecules. This result is in agreement with what was reported by Asogwa *et al*. (2024) that administration of boiled extract of *Justicia carnea* leaf on Asunra®-induced iron deficiency anaemic rat restored the red blood cell count. The slight reduction in RBC in the untreated group may be attributed to lower concentration of transport iron bound to hemoglobin, thus leading to destruction of RBCs. It has been reported that iron deficiency in the body limits the synthesis of heme which in turn limits the synthesis of hemoglobin and decreases the production of red blood cells in the bone marrow (Nagababu *et al.,* 2008).

The results from the Hb and PCV analysis followed the same trend as that in RBC because these three parameters are highly dependent on the availability of transport iron. Many animal studies have clearly shown that calcium interferes with dietary iron absorption and that addition of calcium to diet may even lead to iron deficiency

(Hallberg *et al.,* 1991). A study by Ogah and Ikelle (2015) on the determination of the amount of some heavy metals in edible clay of Enyigba village in Abakaliki, Ebonyi state, Nigeria, reported that edible clay was highly abundant in calcium (249.00±1.73 mg/g) than any other mineral. A reduction in RBC level due to excessive calcium in the diet of the untreated group may have restricted the availability of iron in transport to the hemoglobin, thus a lower PCV too.

Mean corpuscular volume (MCV) depicts the average volume or size of a red blood cell. A low MCV is a biomarker for IDA. Iron deficiency causes microcytic anemia. A person usually develops an iron deficiency due to an underlying health condition of factors such as diet and medications. A low MCV level suggest microcytic anemia. This is the type of anemia in which sizes of red blood cells are smaller than usual. A higher MCV shows red blood cells larger than usual which results in macrocytic anemia (Asogwa *et al.,* 2024). Megaloblastic anemia is a type of macrocytic anaemia. The decrease in MCV in the untreated group suggests that geophagy may have caused microcytic anaemia in the animal (Faruqi *et al.*, 2024). Administration of aqueous extract of *Justicia carnea* leaf was able to reverse the MCV from 44.90fl in the untreated group to 53.80fl in the group treated with 400mg/kg of the extract. This was not significant when compared with the group treated with Chemiron (a standard drug).

The initial elevation in the white blood cell (WBC) count may be attributed to the anemia induced in the rats. The immune system of the animals might have interpreted the anemia as a potential consequence of an infection or disease, leading to an increased production of white blood cells to combat such threats. It is well-documented that WBC counts can rise swiftly in response to the invasion of pathogens, as the body's physiological mechanisms typically react by enhancing its defense systems (Eyong *et al.,* 2004). The anemia-related loss of blood could be perceived by the animal's immune defenses as indicative of an underlying infection or disease, prompting an increase in WBC production to address the perceived threat. Notably, after the treatment, an increase in the WBC count was recorded, although this change was not statistically significant ($p > 0.05$).

Conclusion

The results obtained in this study has shown that the aqueous extract of *J.carnea* is safe for consumption as a herbal supplement or for administration as a drug. The extract also showed high potential in ameliorating iron deficiency anaemia. Thus, the leaf of *Justicia carnea* may be a potential candidate as an affordable alternative for the management of iron deficiency anaemia. Further research should be carried out to isolate and purify the particular component of this extract that has this potential to increase systemic transport iron and storage iron.

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