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Formulation of Nutraceutical from the Leaf Extracts of *Justicia carnea, Ficus capensis* and *Mucuna pruriens* for the Management of Iron Deficiency Anaemia

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Iron deficiency anemia (IDA) is a global health issue, particularly in low-income populations where access to conventional treatments is limited. This study evaluated the efficacy of a herbal teabag formulated with *Justicia carnea, Ficus capensis*, and *Mucuna pruriens* in improving hematological and biochemical markers of IDA in Wistar rats, showing results comparable to standard ferrous sulfate therapy. The results obtained showed that the teabag significantly increased the rbc from

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3.58±0.61 x 10^{12}/L in the negative control to 5.77±0.65 x 10^{2}/L and 5.64±0.73 x 10^{12}L in the groups treated with 2ml and 4ml/kg decoction of the teabag respectively. The hemoglobin increased from 6.51±1.61mg/dl in the negative control to 9.31±1.61 and 7.97±1.38mg/dl in the groups treated with 2ml and 4ml/kg decoction of the teabag respectively. These values were not significantly difference from the normal control and the group treated with ferrous sulfate ($p > 0.05$). The mean corpuscular volume also increased from 72.38±20.63fl in the negative to 91.07±11.72 and 90.68±8.20fl in the groups treated with 2ml and 4ml/kg decoction of the teabag respectively. The ferritin, serum iron and pack cell volume of the iron deficiency anemic rats were also significantly ($p<0.05$) improved on treatment with the decoction of the formulated tea bag. When compared with that of the group treated with the standard drug, ferrous sulphate, there was no significant difference. The total iron binding capacity (TIBC) was also significantly decreased on treatment with the teabag decoction. The findings highlight the potential of this cost-effective herbal formulation as an alternative treatment for IDA, warranting further research for clinical application.

Keywords: Nutraceutical; leaf extracts; Mucuna pruriens, Iron supplementation, antioxidant properties.

1. INTRODUCTION

Iron deficiency occurs when the body does not have enough of the mineral iron that enable the red blood cell haemoglobin to carry oxygen. As a result of this, iron deficiency may lead to tiredness and shortness of breath. Without enough iron, the body cannot produce enough hemoglobin. This low level of iron in the body leads to abnormally low level of red blood cells; this is called iron deficiency anemia. In children under two years, iron deficiency can cause significant and irreversible effect in brain development [1]. Defects in brain development can lead to negative consequences on learning and school performance later in life. The ability of one to perceive, think and gain understanding is termed cognition; a child's cognitive development can be affected if the mother is iron deficient during her last trimester of pregnancy.

In many developing countries, iron deficiency anemia is aggravated by worm infestations, malaria and other infectious diseases such as HIV and tuberculosis. The prevalence of iron deficiency anemia among preschool children in Nigeria is estimated at 69% [2]. According to WHO [1], there are 614 million women and 280 million children globally who suffer from iron deficiency anemia.

"Pregnancy is one of the more important periods in life when increased micronutrients, and macronutrients are most needed by the body; both for the health and well-being of the mother and for the growing fetus and newborn child" [3]. "In developing countries about 50% pregnant woman and about 40% of preschool children are estimated to be anemic" [4]. "Globally, approximately two billion people, the majority women and young children, are affected, by micronutrient deficiencies, with even higher rates during pregnancy" [5].

When iron stores are depleted, it leads to reduced production of hemoglobin and circulating red blood cells, resulting in anemia. The primary goal of treatment is to replenish the body's iron stores and provide relief from symptoms. If left untreated, iron deficiency can have negative consequences such as neurodevelopmental delays in children and adverse pregnancy outcomes for expectant mothers. Women of child-bearing age, who experience monthly menstruation and pregnancy, are particularly at risk of developing anemia [6]. The elderly population is also prone to iron-poor diets and slow gastrointestinal blood loss due to gastritis or underlying malignancies. Patients with chronic kidney disease or undergoing hemodialysis often experience iron deficiency as their kidneys cannot produce enough erythropoietin, further worsening the anemia. Additionally, individuals with malabsorptive conditions like Whipple disease, small intestinal bacterial overgrowth (SIBO), celiac disease, or pernicious anemia struggle to effectively digest dietary iron.

"Iron supplementation plays a crucial role in replenishing iron stores, promoting erythropoiesis, and facilitating oxygen transport throughout the body. Divalent metal transporter 1 (DMT1) is responsible for transporting iron across the cell membrane, where it is stored as ferritin in macrophages" [7]. "This stored iron is later converted into an absorbable Fe^{2+} ion, bound by transferrin, and distributed to different parts of the body, including the bone marrow for red blood cell synthesis. Ultimately, iron combines with porphyrin and globin chains to create hemoglobin, which carries oxygen from the lungs to various organs" [7].

1.1 Treatment of Iron Deficiency Anaemia

"Treatment for iron deficiency anemia involves taking iron supplements to boost the low levels of iron in the body" [8]. "This is usually effective, and the condition rarely causes long-term problems. It will need to be monitored every few months to check the treatment is working and the iron levels have returned to normal. The underlying cause will need to be treated so that anemia will not develop again. Increasing the amount of iron in diet may also be recommended.

The World Health Organization (WHO) recommends daily or intermittent iron and folic acid supplementation as a public health intervention for adult women and adolescent girls living in settings where anemia is highly prevalent. In the postpartum period, iron and folic acid supplementation may also reduce the risk of anemia by improving iron status of the mother, including in settings where malaria is endemic" [1].

Iron supplementation is necessary for individuals with iron-deficient conditions caused by factors like iron deficiency anemia, iron deficiency without anemia, nutritional deficiency, malabsorption, chronic inflammatory state, blood loss, or increased iron requirements. Iron is a vital mineral essential for overall well-being [6].

Iron supplementation can be administered orally or intravenously, and iron-fortified foods can also help manage and treat iron deficiency.

Oral iron supplementation is typically the preferred initial treatment for individuals with IDA (Shersten *et al*., 2007). Transfusion may be indicated for patients of any age suffering from iron deficiency anemia (IDA) who report symptoms such as fatigue or dyspnea during physical activity. Additionally, transfusion is considered for asymptomatic cardiac patients whose hemoglobin levels fall below 10 g per dL (100 g per L). The absorption of iron can significantly differ depending on dietary factors and other variables. The bone marrow's response to iron is capped at 20 mg of elemental iron per day. Patients undergoing iron therapy may have an increase in hemoglobin levels of approximately 1 g per dL (10 g per L) every two to three weeks; however, it may take up to four months for iron stores to normalize after hemoglobin levels have improved. A dose of 325 mg of ferrous sulfate delivers 65 mg of elemental iron, while 325 mg of ferrous gluconate provides 38 mg of elemental iron [9]. It is advisable to avoid sustained-release iron formulations as initial treatment, as they diminish the amount of iron available for absorption by the duodenal villi.

The absorption of elemental iron in the gastrointestinal tract is improved in an acidic gastric environment, which can be achieved by concurrently consuming ascorbic acid (vitamin C) [10]. While iron absorption is more effective when taken on an empty stomach, this approach may lead to gastrointestinal discomfort associated with iron supplementation. It is important to balance increased patient compliance with the potential for reduced absorption. Consumption of foods high in tannates (such as tea) or phytates (like bran and cereal), as well as medications that elevate gastric pH (including antacids, proton pump inhibitors, and histamine H2 blockers), can hinder absorption and should be minimized when possible. Some individuals may experience challenges in iron absorption due to inadequate dissolution of the coating; therefore, a liquid iron formulation may be more suitable for these patients. Additionally, the use of laxatives, stool softeners, and ensuring sufficient fluid intake can help mitigate the constipating effects associated with oral iron therapy.

Justicia carnea hooker is called *˝Ulogwu di anya*" in some parts of Abia state, Nigeria where the boiled extract of the leaf is used for the treatment and prevention of anemia. "Pregnant women, nursing mothers, people with sickle cell anemia and patients suffering from malaria, typhoid and hepatitis also take the boiled extracts of the leaves. The boiled leaves extracts are obtained by boiling a mixture of the leaves in water for a period of 10 to 15 minutes with the resultant appearance of a blood-like colour solution" [3]. "This extract can be taken when hot or it can be allowed to cool before taking. It is used to treat anemia in many parts of Nigeria most especially in Abia and Cross River States of Nigeria. The plant, according to Pius" [11], is used as a treatment of anemia in Congo by Jehovah's Witnesses, well known for their refusal of blood transfusion. "Phytochemical screening of the plant revealed the presence of alkaloids and polyphenols such as flavonoids, tannins, leucoanthocyanins, quinones and anthocyanins" [11].

Ficus capensis, locally called *"akokoro"* or '*akpulu*' in igbo, "*uwaryara"* in hausa, "*opoto*" in Yoruba, "*rimabichehi*" in Fulani and "*obada*" in Edo, belongs to the family Moraceae and has been considered an underutilized plant [12]. "The

leaves of this plant have been found to be abundant in dry season as a result of the plant's resilience, adaptation and tolerance to adverse climatic conditions, making it a good substitute to help with the cases of reduced consumption of green leafy vegetables experienced in the dry seasons. It is one of the plants used in traditional medicine in Nigeria, for treating various diseases and promotes vascular health" [12]. "The leaves of *F. capensis* are commonly used as a vegetable in foods with a substantial blood boosting effect, and possess the ability to prevent the sickling of red blood cells" [12]. "In Nigeria, decoctions and aqueous extract of *F. capensis* are said to be used traditionally in the treatment of anemia, tuberculosis, pains, convulsions and wounds. Oral administration of aqueous extract of *F. capensis* has be reported to increase haemoglobin concentration, packed cell volume and red blood cells of albino rats" [12].

Mucuna pruriens is a vegetable plant native to tropical and subtropical regions in Africa, South America, and Asia. It is part of the fabaceae family and is one of many species of *Mucuna.* "It is commonly referred to as velvet bean or cowhage" [13]. "The plant is notorious for its extreme itches and skin irritation, particularly the young foliage and seed pods. When in contact with the skin, it produces severe irritations and many medium-sized red swollen areas on the skin that are actively itchy" [14]. "It has been traditionally used in Ayurvedic medicine for the treatment of a wide range of ailments including anemia. In the south-eastern geopolitical zone of Nigeria *M. pruriens* is popularly known as '*agbala*'and '*karara*' in the Hausa language speaking region" [14]. "The leaf is squeezed with water and drank to treat anemia. Others boil the leaf in water and drink it for the treatment of anemia.

Herbal teas in the form of teabags are extensively enjoyed around the world, serving not only as remedies for various health issues but also as a form of nourishment" [15]. Typically available in sachets or bulk, these teas are prepared just prior to consumption and can consist of a single herb (monoherbal) or a combination of several herbs (polyherbal), intended for oral aqueous preparations through methods such as infusion, decoction, or maceration. The characteristics of herbal teas, including their color, clarity, and aroma, vary based on the specific constituents they contain [16]. These teas can be derived from various parts of plants, including leaves, seeds, bark, and flowers, depending on the solubility of the compounds in water. The growing recognition of the benefits of herbal teas has contributed to the expansion of the herbal tea market, which not only offers additional remedies for ailments but also generates increased employment opportunities.

Nutraceutical is a term that merges "nutrition" and "pharmaceuticals," referring to products derived from food that may offer health advantages. Vegetables qualify as functional foods or nutraceuticals due to their provision of essential minerals and nutrients that promote health [17]. The beneficial properties of numerous traditional vegetables are being increasingly recognized, while innovative food products are being created that incorporate additional nutraceutical elements. Nutraceuticals have recently garnered interest as viable sustainable options for the management and prevention of a wide array of diseases. Their appeal lies in their safety, effectiveness, and potential to provide both nutritional benefits and therapeutic effects. Among the various natural dietary supplements, vegetables stand out due to their low caloric content while being rich in vitamins, minerals, antioxidants, and phytochemicals [17].

The integration of traditional medicine into the conventional healthcare system, particularly in developing nations like Nigeria, has facilitated consistent resort to herbal remedies. This shift has prompted an increased interest in herbal alternatives, leading to the reformulation and development of herbal treatments for managing diseases such as iron deficiency anemia. Presently, the rising costs of conventional medications, along with their associated long-term adverse effects, have driven many individuals in the rural part of Nigeria and Africa at large to consider phytomedicine as viable alternatives. Often times, these low income people depends on herbal remedies for their treatment. These herbs are sometimes localized and may need to be transported to distance places and before they could get to the destination may lose its properties. Also, there is poor dosage standardization of herbal remedies by the trado-medical practioners, thus there is need to ascertain the right dosage and also find a way to preserve the activities of these herbs so that they can be safely delivered in places where they are not localized. The aim of this study is to formulate a nutraceutical in the form of teabag

using the leaves of *Justicia carnea, Ficus capensis* and *Mucuna pruriens* for the management of iron deficiency anaemia.

2. METHODOLOGY

2.1 Sample Collection and Preparation

The leaves was rinsed with deionized water and drained in a filter basket and then spread to air dry in the drying room of Applied Biochemistry Department, Nnamdi Azikiwe University. The dried leaves were ground separately using electric blender and the resulting powder was stored in an airtight container till further use.

2.2 Preparation of Teabags

The ground samples was combined in different ratios based on the dose reported by Oguaka *et al.* [18]. This combination was packaged in form of tea bags which was decocted in hot water and the decoction was used for the study.

2.3 Decoction

One teabag containing a total of 3g of the combined ground leaves was placed in 100ml of hot water in a 250ml beaker. This was simmered for 3mins and the bag was removed. The resultant brick-red solution was allowed to cool and was used for the treatment of iron deficiency anemia.

2.4 Animal Study

A total of 42 Wistar rats were acquired from Mr Onyewuchi's Animal farm, Nnamdi Azikiwe University, Awka and allowed to acclimatize for 7 days through which they were fed with grower's marsh and water *ad libitum.*

2.5 Acute Toxicity

The bioassay was conducted according to the World Health Organizations guideline for the evaluation of the safety and efficiency of herbal medicines [19]. For the study, 12 Wistar rat was divided into four groups of 3 animals each. Animals was deprived of food but not water (16hrs) prior to administration of the decoction. Three groups was given single oral doses of 2, 4 and 8 g/kg of the decoction. The first group used as control received distilled water. Observations was made and recorded systematically at 1, 2, 3, 4 and 24hrs after substance administration. The visual observations included motility, respirations, sensitivity to sound and pinch, smelling of food and feces consistence. The numbers of survivors were recorded after 24 hrs and the animals were observed daily for the next 7 days. The LD50 was determined based on [20], and [21] methods.

2.6 Induction of Iron Deficiency Anemia

Twenty four (24) rats were used to produce iron deficiency anemia model by daily oral administration of 10mg/kg body weight Asunra® for 14days, after which blood was collected from 3 random rats to assay for iron deficiency anemia.

2.7 Grouping of the Animals and Administration of sample

The animals were divided into 5 groups of 6 rats each. Group 1 and 2 were treated with 2ml/kg and 4ml/kg body weight respectively of the hot water decoction of the teabag formulation by oral gavage, Group 3 was treated with 6mg/kg of ferrous sulfate by oral gavage, Group 4 was the negative control (anemic but not treated) and group 5 was the normal control.

2.8 Collection of Blood and Preparation of Serum

At the end of the 28 days, the rats were mildly anaesthetized using ether and blood was collected by ocular puncture. Whole blood was used for hematology and it was collected in EDTA container while the blood for biochemical assays was collected in plain container and allowed to clot. The clotted blood was centrifuged at 4000rpm for 30mins and the serum collected was used for biochemical assay.

2.9 Haematology

The hematological parameters which included the red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), pack cell volume (PCV), and mean corpuscular volume (MCV), were analyzed using Autohematology Analyzer (Icubio iCell-820; Shenzhen iCubio Biomedical Technology C., Ltd.).

2.10 Ferritin

The serum iron ferritin was analyzed using the method of [22]. The serum (0.1ml) was diluted with 0.9ml of normal saline followed by the addition of 1 ml of protein precipitants containing 10% TCA, 3% HCl and 40% thioglycolic acid. This was thoroughly mixed and incubated at 56^{o}C for 15minutes. This was rapidly cooled at

4^{o}C. Two milliliter (2ml) of 1.5M sodium acetate containing bathophenanthrolinedisulfonic acid. After 5minn, the mixed solution was centrifuged at 4000rpm for 5mins.The absorption of the supernatant was measured at 535nm against a blank of distilled water, and the iron concentration was calculated from standard curve prepared with horse spleen apoferritin.

2.11 Total Iron Binding Capacity (TIBC) Assay

The total iron binding capacity was measured according to the method of [23] using Centronic GmbH (Germany) iron TIBC kit.

The total iron binding capacity is the maximum concentration of iron, which can be bound by serum proteins of the organism. In this Assay, 500µl of the serum was mixed 250µl of 89.5mmol $FeCl_3$. This was mixed and incubated for 10mins at 25^{o}C. This was followed by the addition of 50mg of $MgCO_3$ powder and allowed to stand for 45mins with intermittent shaking at 5mins interval. This was centrifuged at 4000rpm for 10mins and 100µl of the clear supernatant was used for iron assay at 578nm using UV-VIS spectrophotometer using Ferene-S method.

2.12 Statistics

The results obtained was subjected to One-way ANOVA using Statistical product and service solutions (SPSS) version 22 and significant values was subjected to Tukey's Post-hoc. P-values less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

None of the doses used led to the death of the animals. Thus the decoction was considered to be non-toxic within the experimental doses.

The result obtained from the acute toxicity (LD50) study of the decoctions indicated that decoction of the teabag possesses no toxic effects as no death or adverse reactions were observed in the mice that administered low to high doses of the extracts after 24hrs of administration. This suggests high tolerance and safety level of the extracts that could be attributed to the extract lacking toxic constituents that could trigger adverse reactions at tested doses. It also implies that the leaves are relatively safe for human and animal consumptions but excessive consumption of the aqueous extract could be chronically toxic as nothing is completely safe.

The result of the hematology shows that all the extracts was able to reverse the effect of Asunra®-Induced iron deficiency in the rats as shown in the red blood cell, hemoglobin, packed cell volume, white blood cell, ferritin, serum iron and total iron binding capacity shown in Table 1. The extract may be rich in bioactive constituents promote activities of haematopoietic cells leading to production of more blood cells in addition to stabilization of blood in circulation [23]. The decoction (formulated product) at a dose of 200mg/kg showed count of 5.76x1012/L red blood cell which is comparable to that of the standard drug. This could be attributed to the abundance macro and micro nutrient compositions of these plant and their rich antioxidant molecules [12]. This result agrees with that of Orjiakor et al. [24] who reported an increase in the circulating red blood cells and hemoglobin in anemic rat after treatment with aqueous extract of *Justicia carnea* leaf. The highest hemoglobin concentration was recorded in the group treated with ferrous sulfate, a standard drug (10.64g/dl). Ferrous sulfate has been shown to replenish iron stores and correct anemia effectively [25]. However, there are also many limitations to their use, with the most common being the frequency and severity of side effects. A systematic review demonstrated that GI side effects were the most problematic with constipation being the most frequent complaint, followed by nausea and diarrhea [25]. The hemoglobin concentration in the group treated with the decoction of the combine herbs was 9.31g/dl which was not significant when compared with that of the standard drug and the normal control ($p > 0.05$).

The administration of 10mg/kg body weight dose of Asunra caused iron deficiency anemia as seen from Table 1. The ferritin content decreased from 48.00±12.18 to 29.75±4.04ng/L. Ferritin is an indirect marker for total body iron stores, low ferritin is highly specific for iron deficiency. It is a blood protein that stores iron. Ferritin is the best indicator of iron deficiency and a low ferritin alone is diagnostic of iron deficiency anemia (IDA) [26]. Iron is stored intracellular as ferritin and in the presence of infection, malignancy or chronic inflammation, the ferritin rises as it is an acute phase protein. Administration of decoction of the teabag (2ml/kg) reversed the iron deficiency anaemia as seen in Table 1. The ferritin level rose from 29.75±4.04ng/L to 42.00ng/L; a value which was not significant from that of the normal control and the standard drug ($p > 0.05$).

Table 1. Effect of decoction of the formulated teabag on iron deficiency anaemia

Parameters	NC	NeC	Fe.S	2ml/kg	4ml/kg
RBC ($x10^{12}$/L)	5.88±0.45	3.58±0.61*	5.85±0.61	5.77±0.65	5.64±0.73
Hb (g/dl)	9.30±1.88	6.51±1.61*	10.64±1.21	9.31±1.61	7.97±1.38
PCV (%)	48.67±3.36	34.25±7.44*	51.25±7.23	52.50±5.15	45.50±5.94
WBC($x10^{8}$/L)	7.49±1.26	7.39±1.10	9.96±2.85	10.14±2.99	7.73±2.96
Ferritin (ng/L)	48.00±12.18	29.75±4.04*	45.25±5.03	42.00±13.11	2.25±12.92
TIBC (µg/L)	208.33±21.15	293.75±21.88*	202.75±27.30	253.25±50.59	22.25±22.86
MCV (fl)	82.87±5.72	72.38±20.63	88.58±13.52	91.07±11.72	90.68±8.20

NC: Normal control
NeC: Negative control
Fe.S: Ferrous sulfate
2mg/kg: Group that received 2ml/kg of the decoction
4ml/kg: Group that received 4ml/kg of the decoction
**: Significantly difference from the treatment group.*

Total iron-binding capacity (TIBC) test measures the blood's ability to attach itself to iron and transport it around the body. In the case of iron deficiency anemia, the iron level will be low while the TIBC will be high. In the TIBC results presented in Table 1, the negative control was significantly higher than that of those treated with the plant extracts and their derivatives ($p < 0.05$). It was also higher than that of the standard drug, ferrous sulfate. Though low level of TIBC may also be an indication of liver damage [27]. Research by Zwain [28] on biological effect of some herbs in improvement of anemia in rats showed that administration of the extracts of cumin, ginger and saffon was able to lower the TIBC of iron-deficient anemic rat and also increased their ferritin level. This is in agreement with the result of this research.

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 the type of anemia in which red blood cells are smaller than usual. A higher MCV shows red blood cells larger than usual which results in macrocytic anemia. Megaloblastic anemia is a type of macrocytic anemia [29]. Deficiencies in cobalamin and folic acid are the most common causes of megaloblastic anemia. The result of the MCV shows that induction of iron deficiency anemia caused reduction in the mcv; treatment with 2ml of the teabag decoction was able to significantly ($p < 0.05$) reverse this decrease.

4. CONCLUSION

The results obtained from this research has shown the potency of the decoction of teabag formulated from the leaf extracts of *Justicia carnea, Ficus capensis* and *Mucuna pruriens* to reverse iron deficiency anemia. The formulation showed a balanced activity against iron deficiency anemia with no recorded deleterious impact. Thus the formulation of teabag from the leaves was successful. The findings highlight the potential of this cost-effective herbal formulation as an alternative treatment for IDA, warranting further research for clinical application.

5. RECOMMENDATION

One of the critical periods that lead to iron deficiency anemia is pregnancy; thus, it is recommended that this decoction be tested on iron deficient pregnant rats to ascertain its effect on gestation.

ETHICAL APPROVAL

Ethical approval was obtained from the Animal ethics committee, Nnamdi Azikiwe University, Awka.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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