

EFFECT OF UCHAKIRI” (*Vitex doniana*), “UTURUKPA” (*Pterocarpus santalinoides*) and “EWA” (*Solanum americanum*) ETHANOLIC EXTRACT ON IRON STATUS OF RATS

Abstract.

Background: Iron deficiency anaemia is a hidden hunger which can affect at every stage of human development especially pregnant women and children. **Aim:** The study was carried out to ascertain the effect of “ewa”, “uturukpa” and “uchakiri” on iron status of rat. **Method:** All the analysis were carried out using a standard method. **Results:** The results obtained were 2.67-3.52mg iron, 0.52-0.89mg copper, 1.56-7.43mg zinc, 2.42-120.70mg calcium, 10.16-39.10mg magnesium, and 68.80-90.40mg potassium. The mean serum ferritin, haemoglobin, packed cell volume and RBC all increased gradually after consumption of leaves extract. **Conclusion:** The result showed that ethanol leaf extract of *Vitex doniana*, “ewa” and “uturukpa” possesses anti-anemic potentials that are capable of reducing the risk of iron deficiency anemia in the society.

Introduction

Background: Iron deficiency anemia is a public health problem that particularly affects young children and pregnant women. According to WHO, 40% of pregnant women and 42% of children under 5 are anemic globally [1]. Nutritional deficiencies, particularly those of iron, folate, vitamins B12 and A, as well as folate, are the main causes of anemia. Infectious disorders like malaria, TB, HIV, and parasitic infections are examples of additional causes. Enrichment with plant ferritin is one approach being studied for enhancing the iron status of people.

In particular, non-hem iron is the subject of experiments to increase its bio

availability, remove obstructions, understand its methods of absorption, and promote its use [2].

Iron supplementation in the human diet has become a need [2]. There are several indigenous vegetables, such as bitter leaf, ewa, bubble bush leaf, uchakiri, uturukpa, and others.

In humans, a high vegetable diet has been linked to a decreased risk of cardiovascular disease [3]. Around 31% of ischemic heart disease and 11% of stroke are thought to be caused by low vegetable intake worldwide. Diets deficient in vegetables, complex carbohydrates, and dietary fiber are thought to contribute to 2.7 million annual deaths, ranking among the top 10 risk factors for mortality in the 2007 World Health Report [4].

"Ewa" (*Solanum americanum*) is a member of the Solanaceae family and is sometimes referred to as American black nightshade and glossy nightshade. Locally, it is known as Gautan kad or Gautan kaaj in Hausa, Oju ologbo in Yoruba, and Anya nwoná in Igbo [5]. In some areas of northeastern Nigeria, the plant has been used to cure diarrhea and dysentery.

The tree species known as "Uturukpa" (*Pterocarpus santalinoides*) belongs to the Fabacea family of legumes. The family Leguminosae includes the genus *Pterocarpus*, which is widespread across the tropics and subtropics. In the South Eastern region of Nigeria, "Uturukpa" (Ibo) leaves from the *Pterocarpus santalinoides* tree are used to make soup. Some tribes in Eastern and Southern Nigeria employ the leaf extracts as antibacterial agents and as a remedy for respiratory issues, convulsions, fever, and headaches, as has also been described for *Sansevieria trifasciata* [6].

Southern Nigeria is home to "Uchakiri" (*Vitex doniana*), a member of the Verbenaceae family popularly known as the black plum [7]. It is the most common species of the genus and is helpful in the treatment of ailments since it is frequently found in savannah regions [8]. In Nigeria, *V. doniana* is known by the Hausa, Fulani, Yoruba, and Igbo names "dinyar," "ori nla," "uchakiri," and "galbihi" [9]. The young leaves are made as vegetable soup and cooked alongside other ingredients, including sauces. Tanning agents, anthraquinones, flavonoids, resins, cardiac glycosides, saponins, and alkaloids were discovered through phytochemical investigations [10].(in the three vegetables or only Uchakiri?)

The size and scope of the issue need the swift implementation of proven solutions. Therefore, it is crucial to promote locally produced food items in order to diversify diets.

Materials and Methods

Procurement of the raw materials

The “*ewa*”, “*uturukpa*” and “*uchakiri*” leaves were purchased from Oriemba market Akpugo in Nkanu West Local Government Area of Enugu State.

Preparation of materials

The vegetable samples were separately plucked and sorted by removing extraneous materials and cleaned by washing with deionized water. The vegetables were milled using electric blender until the desired particle size was obtained (150-180 microns).

Chemical analysis

The Minerals composition of the vegetables were determined in triplicate using AOAC method [11].

Study Design

The anemia studies were carried out using the Completely Randomized Design (CRD). Rats were randomly assigned to the treatments based on their weights. There were five treatments each replicated five times. The rats were the replicates while the different diets were the treatments.

Animal experiment

Animal housing

At the University of Nigeria Nsukka's Department of Veterinary Pathology, 25 adult rats weighing between 40 and 60g were acquired. On the basis of body weight, the animals were separated into 5 groups of 5 rats each. To separate the urine and feces on a base tray, the rats were kept separately in cages. The Industrial Animal Care Committee, University of Nigeria, Nsukka, approved all operations involving animal use in this inquiry in compliance with the moral standards of the European Union's directives for animal testing (Dir 86/609/EEC).

Bioassay (specify the ordinary rat food please)

Throughout the trial, the rats were fed on **ordinary rat food**. The *ewa*, *uchakiri*, and *uturukpa* extract were prepared to give the rats 0.1mg/day of iron. The 28-day trial was conducted. , a 14-day feeding trial, a 7-day anemia-inducing period, (remove the comma) and a 7-day acclimatization period(rearrange as: a 7-day acclimatization period and a 7-day anemia-inducing

period) As a positive control, commercial hematinic-ferrous sulphate was employed. The AIN-93G (American Institution of Nutrition) approach was used to create the diets [12]. Prior to being given access to their various meals, the rats were weighed. **Rat chow was fed to Group 1 alone** (Table 1 shows you fed rat chow to all the groups of animals. **It contradicts this statement. Rewrite as group 1 was fed with only rat chow.**), followed by Group 2 with ferrous sulphate, Group 3 with ewa extract, Group 4 with uchakiri extract, and Group 5 with uturukpa extract. Each day, the animals' weights were noted. For the purpose of calculating nutritional intake, daily food intake and extract were tracked.

Diet composition

The iron content of extract from 100g sample of each vegetable with 100ml of water was used for the study. During the acclimatization period the least quantity of water that was taken by a rat per day was used. The iron need for rat per day is 0.11mg/dl.

Using the dilution of standard solution equation $C_1V_1 = C_2V_2$

Where C_1 = Initial concentration

C_2 = Final concentration

V_1 = Initial volume

V_2 = Final volume

Table 1: Composition of experimental diets

GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Rat chow	Rat chow + ferrous Sulphate	Rat chow + ewa leave extract	Rat chow + uchakiri leave extract	Rat chow + uturukpa leave extract

Blood sample collection (did you give anemia to the rats or you induced anemia? Please rewrite the statement in red colour. Explain how you induced anemia.)

The rats were given anemia for seven days of the trial by having 2 milliliters of blood drawn between 8 and 10 in the morning. Using a heparinized capillary tube, blood was drawn from the rat's orbital sinus's ophthalmic venous plexus. Blood was drawn for hematological analyses on days 0, 7, 12, 17, and 22.

Heamoglobin level

Haemoglobin level was determined using the cyanomethamoglobin technique recommended by The International Committee for Standardization in Hematology [13].

(Chemical analysis should be here)

Determination of Serum ferritin

This was measured by a two-site immune radiometric assay and radioimmuno assay as given by [14].

Determination of the Red Blood Cell in the Rat

The red blood cells count was determined by haemocytometry [15].

Determination of Pack cell volume of the Rat

The packed volume was determined using micro-haematocrit reader according to the method of [16].

Statistical Analysis

The data generated was subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS, version 20) software. Means was separated using the Turkey's Least Significance Difference (LSD) Test at $p < 0.05$.

Results

Table 2: Mineral Composition of the Vegetables (mg/100g)

Sample	Iron	Copper	Zinc	Calcium	Magnesium	Potassium
A	3.01 ^b ±0.21	0.52 ^c ±0.68	7.43 ^a ±0.94	120.70 ^a ±0.35	39.10 ^a ±0.05	68.80±0.14
B	2.67 ^c ±0.10	0.89 ^a ±0.18	2.31 ^b ±0.53	7.30 ^b ±0.94	10.16 ^c ±0.38	75.02±0.55
C	3.52 ^a ±0.31	0.68 ^{ab} ±0.60	1.56 ^c ±0.64	2.42 ^c ±0.02	15.17 ^b ±0.14	90.40±0.47

Values are mean ± standard deviation of 3 replication

Keys: A = Uturukpa
B = *Vitex doniana*
C = Ewa

Table 3: Nutrient Composition of Rat Chow (mg/100g) (please write how you carried out this analysis in your materials and methods)

Nutrients	Rat Chow
Zinc	-
Iron	0.10±0.01

B-Carotene 0.20±0.03

Values are mean ± standard deviation of 3 replication

Table 4: Mean Serum Ferritin (ng/ml) Level of Rat

Days	Group 1 Rat chow	Group 2 Rat chow + Ferrous sulphate	Group 3 Raw chow + <i>V.doniana</i> extract	Group 4 Raw chow + Ewa extract	Group 5 Raw chow + Uturukpa extract
0	23.41 ^a ±0.30	23.78 ^a ±0.13	23.21 ^a ±0.26	23.60 ^a ±0.38	23.54 ^a ±0.40
7	12.33 ^b ±0.10	12.40 ^c ±0.84	12.32 ^d ±0.45	12.28 ^d ±0.36	12.37 ^d ±0.53
12	12.01 ^{bc} ±0.18	20.20 ^b ±0.89	14.01 ^c ±0.51	17.29 ^c ±0.22	15.86 ^c ±0.32
22	11.20 ^c ±0.77	24.04 ^a ±0.39	19.30 ^b ±0.15	22.24 ^b ±0.19	20.10 ^b ±0.04

Values are mean ± standard deviation of 3 replication

Table 5: Mean Haemoglobin (g/dL) Level of Rat

Days	Group 1 Rat chow	Group 2 Rat chow + Ferrous sulphate	Group 3 Raw chow + <i>V.doniana</i> extract	Group 4 Raw chow + Ewa extract	Group 5 Raw chow + Uturukpa extract
0	13.81 ^a ±0.24	13.76 ^a ±0.46	13.98 ^a ±0.21	13.84 ^a ±0.15	13.90 ^a ±0.03
7	7.51 ^a ±0.16	7.38 ^c ±0.36	7.26 ^{cd} ±0.62	7.40 ^c ±0.13	7.33 ^d ±0.10
12	7.50 ^d ±0.32	12.80 ^b ±0.28	8.45 ^c ±0.80	10.20 ^b ±0.28	9.03 ^c ±0.19
22	7.50 ^c ±0.11	14.22 ^a ±0.68	11.74 ^b ±0.47	13.44 ^a ±0.83	12.30 ^b ±0.11

Values are mean ± standard deviation of 3 replication

Table 6: Mean Erythrocytes Level of Rat (10⁶ cell/μl)

Days	Group 1 Rat chow	Group 2 Rat chow + Ferrous sulphate	Group 3 Raw chow + <i>V.doniana</i> extract	Group 4 Raw chow + Ewa extract	Group 5 Raw chow + Uturukpa extract
0	7.12 ^a ±0.10	7.13 ^a ±0.15	7.07 ^a ±0.56	7.15 ^a ±0.83	7.12 ^a ±0.21
7	5.21 ^b ±0.20	5.21 ^b ±0.18	5.18 ^{bc} ±0.16	5.19 ^b ±0.23	5.19 ^b ±0.34
12	5.11 ^b ±0.14	7.09 ^a ±0.56	5.53 ^b ±0.13	5.90 ^b ±0.68	5.75 ^b ±0.60
22	5.10 ^b ±0.35	7.11 ^a ±0.02	6.18 ^b ±0.25	6.53 ^a ±0.32	6.13 ^b ±0.70

Values are mean ± standard deviation of 3 replication

Table 7: Mean Packed Cell Volume (%) Level of Rat

Days	Group 1 Rat chow	Group 2 Rat chow + Ferrous sulphate	Group 3 Raw chow + <i>V.doniana</i> extract	Group 4 Raw chow + Ewa extract	Group 5 Raw chow + Uturukpa extract
0	45.10 ^a ±0.31	45.20 ^a ±0.07	45.30 ^a ±0.10	45.15 ^a ±0.02	45.17 ^a ±1.04
7	35.20 ^b ±0.14	35.13 ^c ±0.16	35.18 ^c ±0.30	35.25 ^d ±0.58	35.00 ^d ±0.54
12	30.00 ^c ±0.54	44.85 ^b ±0.38	36.28 ^{bc} ±0.43	40.25 ^c ±0.22	38.10 ^c ±0.33
22	30.00 ^c ±0.10	44.36 ^b ±0.76	38.05 ^b ±0.32	42.50 ^b ±0.14	41.30 ^b ±0.43

Values are mean ± standard deviation of 3 replication

Discussion

Mineral Composition of the Vegetables

The mineral content of the three leafy vegetables is shown in Table 2. The "uturukpa" leaf included 3.01 mg of iron, 0.52 mg of copper, 7.43 mg of zinc, 120.70 mg of calcium, 39.10 mg of magnesium, and 68.80 mg of potassium. The iron content of vitex doniana leaf was 2.67 mg, copper 0.89 mg, zinc 2.31 mg, calcium 7.30 mg, magnesium 10.16 mg, and potassium 75.02 mg. In contrast, "ewa" leaf had 3.52 mg of iron, 0.68 mg of copper, 1.56 mg of zinc, 2.42 mg of calcium, 15.17 mg of magnesium, and 90.40 mg of potassium.

Iron: The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had relative iron contents of 3.01 mg, 2.67 mg, and 3.52 mg. Comparing the iron level of all the samples studied to the RDA (mg/day), they were all lower. For men of all ages and postmenopausal women, the Recommended Dietary Allowance (RDA) is 8 mg per day; for premenopausal women, it is 18 mg per day. For men, the average daily consumption of iron ranges from 16 to 18 mg, and for women, it is 12 mg [17]. In the human body, iron is a significant trace element with key functions in hemopoiesis, infection management, and cell-mediated immunity. According to estimates, more than a billion individuals worldwide suffer from iron deficiency anemia, which has been called the most common dietary deficiency [18]. Reduced productivity, behavioral and cognitive deficits, as well as lowered infection resistance, are all effects of iron deficiency.

Copper: The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had copper contents of 0.52 mg, 0.89 mg, and 0.68 mg, respectively. For both humans and other animals, copper (Cu) is a crucial trace element. Although the majority of the body's copper is in the cupric (Cu²⁺) form, copper can also exist in the cuprous (Cu¹⁺) form [19]. The importance of copper

in oxidation-reduction (redox) reactions and the scavenging of free radicals is explained by its ease in accepting and donating electrons [20].

Zinc: The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had zinc contents of 7.43 mg, 2.31 mg, and 1.56 mg, respectively. Zn levels were moderately high in all of the leafy vegetable samples, with values ranging from 1.56 to 7.43 mg. This figure is comparable to that which [21;22] reported (2010). For healthy immune system and human growth, zinc is a crucial mineral [23]. According to reports, 20% of the world's population may not get enough zinc [24]. Zinc deficiency affects 20% of children under the age of five, 28.1% of mothers, and 43.9% of pregnant women in Nigeria, according to studies [19]. According to research by [25], zinc is essential for the formation of hemoglobin. Anemia and fragile erythrocytes have been linked to zinc deficiency. Additionally, zinc functions as a cofactor for RBC-SOD, defending the cell's integrity from oxidative stress [26].

Magnesium: The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had relative magnesium contents of 39.10 mg, 10.16 mg, and 15.17 mg. According to earlier research on fluted pumpkin and "uturukpa" by [27], "uturukpa" has the greatest magnesium concentration. The levels found in this study, however, were insufficient to reach the RDA, which is 310 mg for women and 400 mg for males between the ages of 19 and 30. [28]. The structure and operation of the human organism depend heavily on magnesium. The average adult's body has 25 grams of magnesium. More than 60% of the body's magnesium is present in the skeleton, followed by 27% in muscle, 6–7% in other cells, and less than 1% outside of cells [29]. The production of blood and the extracellular and intracellular fluids that make up bodily cells need the minerals calcium and magnesium. They contribute to the formation of bones and teeth as well as the control of nerve and muscle activity [30;31].

Potassium: The three leafy vegetables, "uturukpa," "Vitex doniana," and "Ewa," had potassium contents of 68.80 mg, 75.02 mg, and 90.40 mg, respectively. Potassium is a systematic electrolyte that works with sodium to co-regulate ATP. One important intracellular cation that helps to sustain intracellular osmotic pressure is potassium [32]. Potassium is essential for treating sickle cell anemia. It significantly affects heartbeat and aids in the passage of nerve impulses. Cellular potassium loss and dehydration observed in sickle cell anemia were revealed to be caused by abnormal activation of the potassium chloride co transport system [33].

Nutrient Composition of Rat Chow

Table 3 presents the nutrient composition of the raw chow. From the result, it contained a negligible amount of zinc, 0.10mg of iron and 0.20µg beta-carotene. The nutrient composition of the rat chow were trace amount of zinc, 0.10mg of iron and 0.20µg of beta-carotene respectively. "Chow" means formula feed of pet or laboratory animal. The result of the nutrient content of the rat chow showed that it will not have much effect on the bioassay.

Mean Serum Ferritin Level of Rat

The rat's mean serum ferritin level is shown in Table 4. 28 days were spent doing the study. Day 0 came after acclimatization, Day 7 saw (remove 'saw' and rewrite the statement as anemia was induced on day 7) the induction of anemia, Day 12 saw (remove 'saw' and rewrite the statement as day 12 was the start of recovery testing or recovery testing started on day 12) the start of recovery testing, and Day 22 saw (remove 'saw' and rewrite the statement as recovery testing came to an end or ended on day 22) the end of recovery testing. Serum ferritin levels in Group 1 varied from 11.20 to 23.41ng/ml. On Day 22, Group 1 had the lowest serum ferritin levels (11.20ng/ml), whereas Day 0 had the highest levels (23.41ng/ml). The serum ferritin level was significantly increased by the V.doniana, "ewa," and "uturukpa" extract (19.30, 22.24, and 20.10 ng/ml). The body's type of iron storage is ferritin. The findings indicated that the iron in these vegetables was retained by the body, increasing the ferritin levels of the rats in Groups 3-5 on Day 22. Serum ferritin is utilized as a diagnostic test for iron-deficiency anemia because plasma ferritin is also an indirect indicator of the total amount of iron stored in the body [34]. The presence of a concentration of iron in all plants may be the cause of the increase in serum ferritin of the rat after exposure to plant extract [35].

Mean Haemoglobin level of Rat

The rat's mean hemoglobin level is shown in Table 5. According to the study's findings, giving rats extracts from the vegetables V.doniana, "ewa," and "uturukpa" increased their levels of hemoglobin. From day 7 to day 22, all of the rats fed the vegetable extract had significantly higher hemoglobin levels. This might be explained by the hematopoietic component present in the vegetables. The iron and ascorbate content of the veggies may have contributed to the rise in hemoglobin levels. Your blood's hemoglobin helps carry oxygen from your lungs to your

tissues. In muscle cells, myoglobin receives, transports, stores, and releases oxygen. This suggests that large dosages of "ewa" and "uturukpa," an extract from *V.doniana*, could increase hemoglobin production. Numerous disorders, including thalassemia and iron deficiency anemia, result in the inability to make hemoglobin [36]. Numerous cellular enzymes require iron as a component, and hemoglobin (which contains an iron-containing porphyrin ring) also contains iron as a member of the heme group [37]. Red blood cells hold the majority of the body's iron reserves since they are where hemoglobin is made, which depends on iron. A lack of iron intake or absorption, excessive blood loss from external bleeding, or interference with iron metabolism could all contribute to an iron shortage [38].

Mean Erythrocytes Level of Rat

The mean level of erythrocytes in rats is shown in Table 6. The amount of red blood cells in the experimental groups increased significantly ($p < 0.05$) after the administration of the plant extracts from *V. doniana*, "ewa," and "uturukpa." Particularly in the rats that ingested "ewa" extract, a considerable rise in erythrocyte levels was seen. A similar result was obtained by Asuquo (2013) when ethanol leaf extract of yellow mombin was administered to rats [39]. Red blood cells (RBC), also referred to as erythrocytes, carry oxygen to the body's tissues [40]. Carbon dioxide is released by the tissues as oxygen is converted to energy. According to Asaolu et al. [41], red blood cells also carry carbon dioxide to the lungs for exhalation. As a result, when the methanol leaf extract of *V. doniana*, "ewa" and "uturukpa" was supplied, a significant rise in RBC was seen. Vamsee et al. [42] also discovered a similar outcome after giving anemic rats 400 mg/kg of curry leaf.

Mean Packed Cell (PCV) Volume Level of Rat

The rat's mean PCV level is shown in Table 7. When rats treated with leaf extracts of *V.doniana*, "ewa," and "uturukpa" were compared to control rats treated with ferrous sulphate, the packed cell volume (PCV) of the control rats increased significantly ($p < 0.05$). At day 22, the rats' levels of PCV were significantly higher thanks to the leaf extract. This may be because the *V.doniana*, "ewa," and "uturukpa" leaves have phytochemical content and antioxidant potential [43]. Similarly, "ewa" and "uturukpa" leaves are abundant in phytochemicals and antioxidants, according to [44;45], respectively. Additionally, according to a recent study by [46], "ewa" leaves have antioxidant activity and can both prevent and treat ethanol-induced oxidative stress

in Wistar rats. In a similar vein, [47] revealed the pharmacotherapeutic effect of "ewa" leaves on hyperglycemia and lipidemic parameters of alloxan-induced diabetic mice. This effect was attributed to the leaves' antioxidant capability. Their effects on PCV levels could potentially be explained by their antioxidant functions. Therefore, it is probable that human ingestion of both plants can aid in the prevention of anemia, particularly in women who are pregnant or menstrual. It is known that red blood cell counts and other parameters that are below normal ranges are indicative of anemia, while values that are above normal ranges are indicative of polycythemia [48]. As a result, it is possible that the 22-day treatment with all the plants does not have the potential to result in polycythemia. A possible indicator that "ewa" leaves are more effective haematopoietic agents than *V.doniana* and "uturukpa" leaves is the considerable elevation in PCV that was shown in animals treated with "ewa" leaves compared to those treated with *V.doniana* and "uturukpa" leaves for 22 days.

Conclusion

In conclusion, results obtained from the present study indicate that the ethanol leaf extract of *V.doniana*, "ewa" and "uturukpa" possesses anti-anemic potentials and this may be attributed to the phytochemicals, antioxidant and the nutrient content of *V.doniana*, "ewa" and "uturukpa" leaf. The present study, therefore, supports the therapeutic use of the *V.doniana*, "ewa" and "uturukpa" leaf in the traditional medicine for the treatment of anemia. It is therefore advisable that the use of this extract in herbal medicine should be with some cautious measures to avoid the risk of anemia in patients treated with the extract. Also, the increase in the haematological parameters observed in rats administered extract from the three host plants suggests that *V.doniana*, "ewa" and "uturukpa" extract contains agents that could stimulate the production of iron, therefore the plant extracts could serve as immune booster.

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