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The haemoglobin regeneration potential of fermented and unfermented *Telfaira occidentalis and Gnetum africanum* leaves in iron deficient albino rats

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ABSTRACT

The effect of 10% supplementation of fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves for 21 days on haemoglobin-iron, haematological parameters and serum ferritin was investigated to determined if the leaves could replenish haemoglobin in iron deficient rats. Iron deficiency significantly ($p<0.05$) decreased the relative weight gain, haemoglobin-iron, serum ferritin, haemoglobin (Hb), and mean corpuscular haemoglobin concentration (MCHC), when compared to the iron sufficient control rats. Rats fed with *T. occidentalis* had a significantly ($p<0.05$) increased serum ferritin compared to those fed with *G. africanum* leaves. The levels of haematological parameters of rats in the iron deficient group were not significantly ($p>0.05$) different from the iron sufficient and leaves supplemented groups. However, haemoglobin concentration of rats on iron sufficient diet, fermented and unfermented *T. occidentalis* leaves and those treated with $FeSO_4$ were significantly ($p<0.05$) higher than that of rats in the iron deficient, fermented and unfermented *G. africanum* groups respectively. Percentage change in Haemoglobin-iron was lower for rats supplemented with the fermented forms of both leaves. This study shows that *T. occidentalis* has a significant ($p<0.05$) haemoglobin regeneration potential compared to *G. africanum*, and fermentation did not significantly ($p>0.05$) enhance the haemoglobin regeneration potential of both leaves.

Keywords: Haematological parameters, nutritional anaemia, serum ferritin, vegetables.

INTRODUCTION

The deficiency and excess of essential micronutrients and trace of toxic metals may cause serious effects on human health (Khan et al., 2008). Iron deficiency ranges from iron depletion, which yields little physiological damage, to iron deficiency anemia, which can affect the function of numerous organ systems (Joshi and Mathur, 2009). It is characterized by the reduction or absence of iron stores, low serum concentration of iron, poor haemoglobin concentration, haematocrit reduction and increased platelet count. This type of anaemia affects people of all ages and is prevalent in developing and developed countries (Nirjala and Korah, 2013). Due to its effects on development and growth, resistance to infections and association with the mortality of infants younger than 2 years, it is considered as a major public health problem and the most common nutritional deficiency in the world (Joshi and Mathur, 2009).

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Telfaira occidentalis and *Gnetum africanum* are commonly consumed vegetables in Nigeria. *Telfairia occidentalis* is a tropical Vine grown in West Africa as a leafy vegetable and for its edible seeds. The plant is dioecious, perennial, and drought-tolerant. It is known as Ugu in eastern parts of Nigeria and Pumpkin leaves in English (Idris, 2011). *Gnetum africanum* is a dioecious forest perennial liana, a member of Gnetaceae family, commonly called eru in English, okazi in Igbo and Afang in Ibibio. The leaves are edible and used in the preparation of different dishes (Ekpo, 2007; Orwa et al., 2009).

Iron deficiency anaemia is the most common nutritional problem worldwide (Grosbois et al., 2005). It is the only nutrient deficiency which is also significantly prevalent in industrialized countries. Hence, this work was aimed at determining the effect of fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves supplemented diet on the haemoglobin-iron, haematological parameters and serum ferritin concentrations with a view of investigating whether this leaves could replenish haemoglobin concentration of iron deficient anaemic young rats.

MATERIALS AND METHODS

Collection and identification of plant materials

The matured vegetables (*Telfaira occidentalis* and *Gnetum africanum*) were purchased from Sabon gari market in Zaria, Nigeria. The vegetables were authenticated at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and a voucher specimen number deposited, 23089 and 1259 for *Telfaira occidentalis* and *Gnetum africanum* leaves respectively.

Experimental animals

Forty (40) young Albino rats of both sexes were obtained and kept in well aerated plastic cages in the animal house, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria Nigeria. The animals were acclimatized to the animal house environment for a period of one week before the commencement of the study. They were fed with growers mash and water *ad libitum* (during the acclimatization).

Preparation of plants

The harvested vegetables were washed, and divided into two parts, one part was air dried at room temperature and then grinded and the other part was fermented. Open fermentation was done, the vegetables were soaked in deionized water for 24 hours for *T. occidentalis* and 48 hours for *G.africanum* at room temperature for the inherent fermenting microorganisms and environmental fermenting microorganisms to act. The fermented vegetables were then filtered out and oven dried, then grinded.

Preparation of diet (iron deficient and iron sufficient diet)

Iron deficient and iron sufficient diets were prepared according to the modified method of Yi Ning et al. (2002). The compositions of the diets are presented in Table 1.

Induction of anaemia

Iron deficiency anaemia was induced by feeding the animals exclusively on the formulated iron deficient diet for 8 weeks. Haemoglobin (Hb) concentration was used as index for anaemia and rats with Hb concentration of < 11g/dl (Velmurugan et al., 2010) were used for the study.

Animal grouping

The experimental rats were divided into 8 groups, with 5 animals per group and fed on supplemented diet for 3 weeks.

Group 1: Normal rats (non anaemic) were fed with deionized water and iron sufficient diet *ad libitum.*

Group 2: Anaemic rats continued on the iron deficient diet and deionized water *ad libitum.*

Group 3: Anaemic rats were given iron sufficient diet and deionized water *ad libitum.*

Group 4: Anaemic rats were given iron deficient diet supplemented with 10% *Telfaira occidentalis* and deionized water *ad libitum.*

Group 5: Anaemic rats were given iron deficient diet supplemented with 10% fermented *Telfairia occidentalis* and deionized water *ad libitum.*

Group 6: Anaemic rats were given iron deficient diet supplemented with 10% *Gnetum africanum* and deionized water *ad libitum.*

Group 7: Anaemic rats were given iron deficient diet supplemented with 10% fermented *Gnetum africanum* and deionized water *ad libitum.*

Group 8: Anaemic rats were given iron deficient diet and treated orally with ferrous sulphate (2.79 mg/kg body weight) and deionized water *ad libitum.*

Determination of haematological parameters

Packed cell volume

This was determined by the microhematocrit method as described by Dacie and Lewis (2001). Blood from EDTA bottle was allowed by capillary action to flow through the capillary tube and one end of the tube was sealed by flaming. It was then centrifuged at a speed of 3000 rpm for 10 minutes. The PCV was estimated using a microheamatocrit reader and expressed as percentage erythrocytes the blood contain.

Haemoglobin concentration

This was determined bi-weekly by the Cyanomethemoglobin method (Jain, 1986). The concentration of haemoglobin was marked with Drabkin's method, with the use of a spectrophotometer, at 540nm wavelength. Once Drabkin reagent is mixed with the blood, the solution was incubated at room temperature for the duration of 5 mins and absorbance was measured. The spectrophotometer was set to zero using distilled water.

Red blood cell (RBC) and White blood cell (WBC) Count

These were determined after the animals were sacrificed using the improved Neubauer counting chamber Dacie and Lewis, (2001). The white blood cell and red cell pipettes were used to draw blood and fill to 0.5 mark of both WBC and RBC pipettes. WBC diluting fluid was drawn to 11 and 101 marks respectively. The fluid and the blood were mixed gently and transferred into the counting chamber. It was allowed to settle for 2 mins and the chamber placed on the stage of the microscope for counting. The white blood count was done using the x10 objective while the red blood cell counts were done using the x 40 objective.

Mean corpuscular Volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC)

These were calculated by the method of Dacie and Lewis, (2001). Mean corpuscular Volume (MCV) in fl was calculated as:

$$MCV = 10 X \frac{\text{Hamatocrit}}{\text{Red Blood Cell Count}}$$

Mean corpuscular haemoglobin (MCH) in pg was calculated as:

$$MCH = 10 X \frac{\text{Haemoglobin}}{\text{Red Blood Cell Count}}$$

Mean corpuscular haemoglobin concentration (MCHC) in g/dl was calculated as:

$$MCHC = \frac{\text{Haemoglobin}}{\text{Hamatocrit}} X 100$$

Determination of *in vivo* iron indices

Determination of serum ferritin

Serum ferritin was determined using the Abcam's Ferritin (FTL) Rat ELISA kit according to the manufacturer's protocol. The ferritin present in samples reacts with the anti-ferritin antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After removal of unbound proteins by washing, anti-ferritin antibodies conjugated with horseradish peroxidase (HRP), are added. These enzymes labeled antibodies form complexes with the previously bound ferritin. Following another washing step, the amount

of enzyme bound in complex is measured by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies proportionately with the concentration of ferritin in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of ferritin in the test sample. The quantity of ferritin in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Haemoglobin-iron (Hb-Fe)

This was calculated by adopting the method of Park, (1983) and Buchowski, (1989). The calculation assumes 6.7% body weight (BW) is blood and the iron content of haemoglobin is 3.35 mg/g

Hb Fe (mg) = BW X 0.067 X Grams Hb per mL X 3.35 mg Fe .

Statistical analysis

Data was analysed using statistical package for the social sciences (SPSS), version 20. The data was analysed by analysis of variance (ANOVA). The difference between the various animal groups was compared using the Duncan Multiple Range Test. The results are expressed as mean ± standard deviation (SD) except where otherwise stated. P values less than 0.05 ($p<0.05$) were taken as significant.

RESULTS

Relative weight change of iron deficient rats supplemented with fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves

The relative weight change of iron deficient rats during supplementation with unfermented and fermented *Telfaira occidentalis* and *Gnetum africanum* leaves is shown in Figure 1. At day 7, there was a significant ($p<0.05$) decrease in the weight change of rats in the iron deficient control group when compared to rats in iron sufficient control group, weight change of anaemic rats on iron sufficient diet, and the vegetable supplemented groups were significantly ($p<0.05$) lower when compared to rats in iron sufficient control group, but not significant ($p>0.05$) when compared to rat in iron deficient control group, this trend also occurred at day 14 only that the weight change of rats on unfermented *G.africanum* and those treated with $FeSO_4$ did not differ significantly ($p>0.05$) from that of the iron sufficient control group.

At day 21, the weight change of rats in the treatment groups did not differ significantly ($p>0.05$) from the iron sufficient control group except for rats in groups supplemented with fermented *T.occidentalis* and fermented *G.africanum* respectively which did not differ significantly ($p>0.05$) from that of the iron deficient control group.

Haemoglobin iron concentration of iron deficient rats supplemented with fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves

The effect of Fermented and Unfermented *Telfaira occidentalis* and *Gnetum africanum* Leaves supplementation on the haemoglobin- iron of iron deficient rats is presented in Table 1. There was a significant ($p<0.05$) decrease in the initial haemoglobin iron concentration of rats in all the treated groups except those supplemented with *Telfaira occidentalis* leaf when compared to rats in the iron sufficient control group. Percentage change in haemoglobin iron was significantly ($p<0.05$) higher in anaemic rats supplemented with iron sufficient diet, unfermented *T.*occidentalis and those treated with $FeSO_4$ when compared to that of rats in the iron deficient control group.

Haemoglobin concentration of iron deficient rats supplemented with fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves

The haemoglobin (Hb) concentration of iron deficient rats during supplementation with unfermented and fermented *Telfaira*

occidentalis and *Gnetum africanum* leaves is shown in Figure 2. At day 0, the haemoglobin concentration of rats in all the treated groups were significantly ($p<0.05$) lower than that of the iron sufficient control group but was not significantly ($p>0.05$) different from that of the iron deficient control group. Hb concentration increased at day 7 and 21, respectively. The increase in all the treatment groups at day7 was significantly ($p<0.05$) higher when compared to the iron deficient control group except for those on unfermented *G.africanum* leaf, same trend occurred at day 21 except for rats on unfermented and fermented *G.africanum* leaves respectively which did not differ significantly ($p>0.05$) from the iron deficient control group.

Mean corposcular haemoglobin concentration of iron deficient rats supplemented with fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves

The mean corpuscular haemoglobin concentration (MCHC) of iron deficient rats during supplementation with unfermented and fermented *Telfaira occidentalis* and *Gnetum africanum* leaves is shown in Figure 2. At day 0 and 7, there was a significant ($p<0.05$) decrease in the MCHC of rats in iron deficient control when compared to rats in the iron sufficient control group while the MCHC of rats in the other groups shows no significant ($p>0.05$) change. The MCHC of rats in the treatment groups shows a significant ($p<0.05$) decrease when compared to rats in the iron sufficient control group. At day 21, there was no significant ($p>0.05$) difference in the MCHC of rats between all the groups.

Serum ferritin concentration of iron deficient rats supplemented with fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves

The serum concentration of ferritin in iron deficient rats supplemented with fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves is presented in Figure 4. Serum ferritin shows significantly ($p<0.05$) higher concentration in rats in groups supplemented with iron sufficient diet and unfermented and fermented *T. occidentalis* leaves respectively when compared to the iron deficient control group. Ferritin concentration of rats in groups supplemented with unfermented and fermented *G. africanum* leaves respectively and those treated with $FeSO_4$ did not differ significantly ($p>0.05$) from that of the iron deficient control group.

Haematological parameters of iron deficient rats supplemented with fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves

The haematological parameters of iron deficient rats supplemented with fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves is presented in Table 2. Red blood cell count was significantly ($p<0.05$) higher in group treated with $FeSO_4$ when compared to iron deficient control group and unfermented *G. africanum* group. White blood cell count did not differ significantly ($p>0.05$) between all the groups. Packed cell volume was significantly ($p<0.05$) lower in fermented *G. africanum* group when compared to group on iron sufficient diet and unfermented *G. africanum* leaf respectively, haemoglobin concentration was significantly ($p<0.05$) higher in groups on iron sufficient diet, unfermented and fermented *Telfaira occidentalis* respectively and those treated with $FeSO_4$ when compared to iron deficient control group and rats on unfermented *G. africanum* leaf. MCV, MCH and MCHC did not differ significantly ($p>0.05$) between all the groups.

Table 1: Composition of the formulated iron sufficient and iron deficient diets.

Ingredients(g/kg)	Iron sufficient	Iron deficient
Cornstarch	625.0	625.0
Casein	200.0	200.0
Fiber	50.0	50.0
Groundnut oil	70.0	70.0
Mineral mix	35.0	35.0
Vitamin mix	10.0	10.0
Ferrous sulphate	10.0	0.0

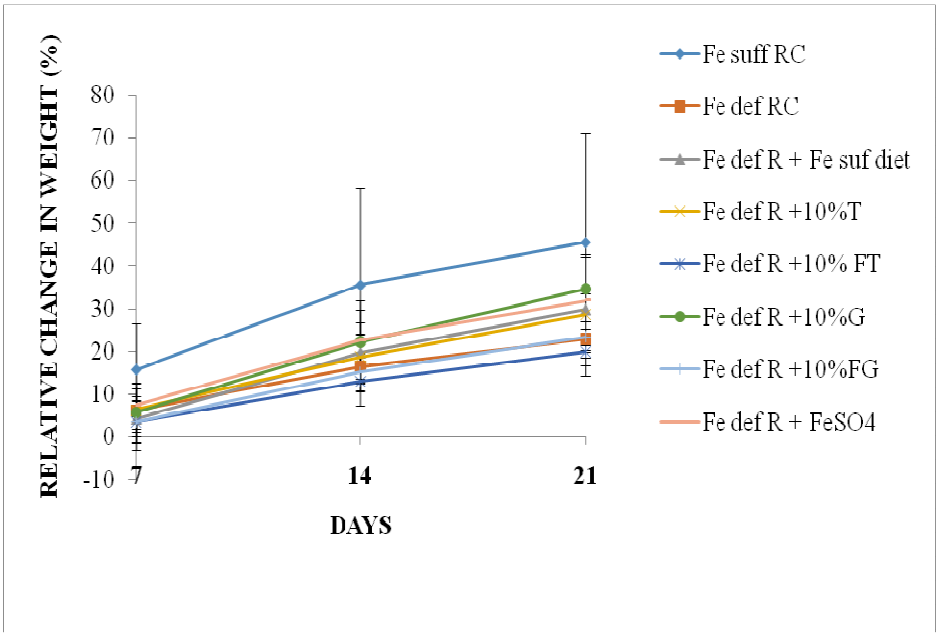

Figure 1: Effect of fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves supplementation on relative change in weight of iron deficient rat. Fe suf RC: Iron sufficient Rat control, Fe def. RC: Iron deficient Rat control, Fe def. R+ Fe suf. Diet: Iron deficient Rat on iron sufficient diet, Fe def. R+10%T: Iron deficient Rat on iron deficient diet supplemented with 10% *Telfaira occidentalis*, Fe def. R+10% FT:Iron deficient Rat on on iron deficient diet supplemented with 10% fermented *Telfaira occidentalis*, Fe def. R+10%G: Iron deficient Rat on iron deficient diet supplemented with 10% *Gnetum africanum*, Fe def. R+10%FG: Iron deficient Rat on iron deficient diet supplemented with 10% fermented *Gnetum africanum* , Fe def. R+$FeSO_4$: Iron deficient Rat on iron deficient diet treated orally with 2.8 mg/kg Ferrous Sulphate.

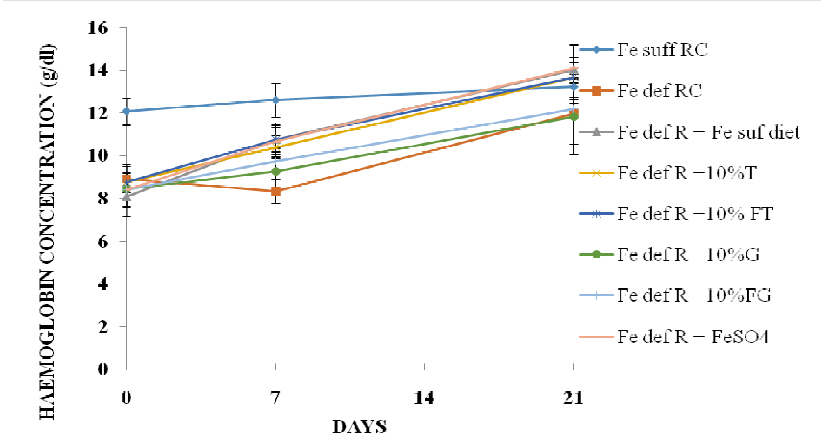

Figure 2: Effect of fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves supplementation on haemoglobin concentration of iron deficient rats. Fe suf RC: Iron sufficient Rat control, Fe def. RC: Iron deficient Rat control, Fe def. R+ Fe suf. Diet: Iron deficient Rat on iron sufficient diet, Fe def. R+10%T: Iron deficient Rat on iron deficient diet supplemented with 10% *Telfaira occidentalis*, Fe def. R+10% FT:Iron deficient Rat on on iron deficient diet supplemented with 10% fermented *Telfaira occidentalis*, Fe def. R+10%G: Iron deficient Rat on iron deficient diet supplemented with 10% *Gnetum africanum*, Fe def. R+10%FG: Iron deficient Rat on iron deficient diet supplemented with 10% fermented *Gnetum africanum* , Fe def. R+$FeSO_4$: Iron deficient Rat on iron deficient diet treated orally with 2.8 mg/kg Ferrous Sulphate.

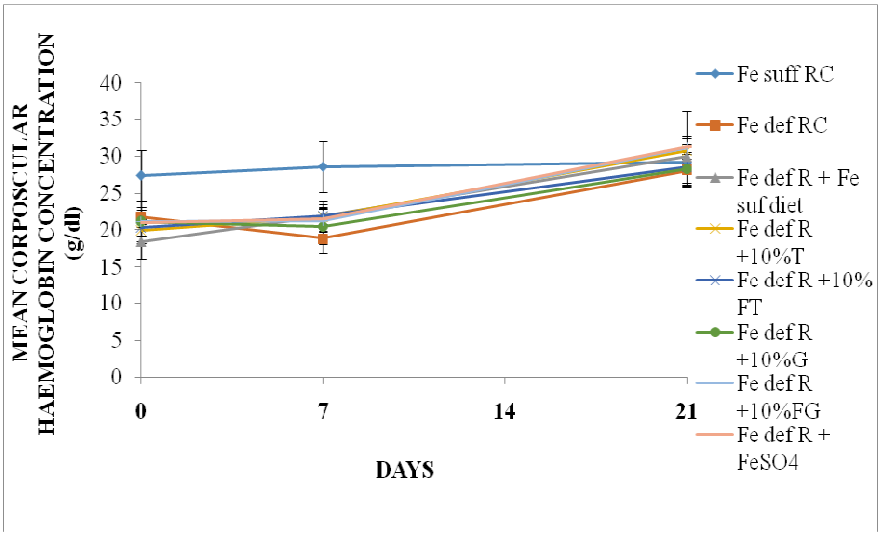

Figure 3: Effect of fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves supplementation on mean corposcular haemoglobin concentration of iron deficient rats. Fe suf RC: Iron sufficient Rat control, Fe def. RC: Iron deficient Rat control, Fe def. R+ Fe suf. Diet: Iron deficient Rat on iron sufficient diet, Fe def. R+10%T: Iron deficient Rat on iron deficient diet supplemented with 10% *Telfaira occidentalis*, Fe def. R+10% FT:Iron deficient Rat on on iron deficient diet supplemented with 10% fermented *Telfaira occidentalis*, Fe def. R+10%G: Iron deficient Rat on iron deficient diet supplemented with 10% *Gnetum africanum*, Fe def. R+10%FG: Iron deficient Rat on iron deficient diet supplemented with 10% fermented *Gnetum africanum* , Fe def. R+$FeSO_4$: Iron deficient Rat on iron deficient diet treated orally with 2.8 mg/kg Ferrous Sulphate

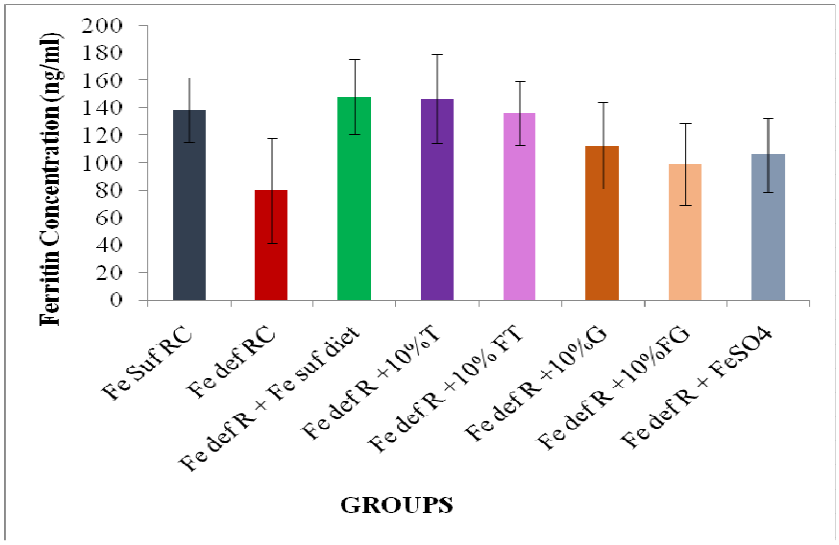

Figure 4: Effect of fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves supplementation on serum ferritin concentration of iron deficient rats. Fe suf RC: Iron sufficient Rat control, Fe def. RC: Iron deficient Rat control, Fe def. R+ Fe suf. Diet: Iron deficient Rat on iron sufficient diet, Fe def. R+10%T: Iron deficient Rat on iron deficient diet supplemented with 10% *Telfaira occidentalis*, Fe def. R+10% FT:Iron deficient Rat on on iron deficient diet supplemented with 10% fermented *Telfaira occidentalis*, Fe def. R+10%G: Iron deficient Rat on iron deficient diet supplemented with 10% *Gnetum africanum*, Fe def. R+10%FG: Iron deficient Rat on iron deficient diet supplemented with 10% fermented *Gnetum africanum* , Fe def. R+$FeSO_4$: Iron deficient Rat on iron deficient diet treated orally with 2.8 mg/kg Ferrous Sulphate.

Table 2: Effect of fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves supplementation on haemoglobin-iron of iron deficient rats.

Groups (n=5)	Initial Hb- fe (day 0)	Final Hb- fe (day 21)	Change in Hb	% Change in Hb-Fe
Fe suff RC	2.51 ± 0.19^{c}	3.97 ± 0.54^{abc}	1.46 ± 0.69^{a}	60.05 ± 33.42^{a}
Fe def RC	2.01 ± 0.41^{ab}	3.36 ± 0.87^{ab}	1.35 ± 0.48^{a}	65.10 ± 16.50^{a}
Fe def R + Fe suf diet	1.69 ± 0.27^{a}	3.81 ± 0.53^{ab}	2.12 ± 0.38^{ab}	126.75 ± 24.81^{d}
Fe def R +10%*T*	2.45 ± 0.33^{c}	4.96 ± 0.83^{c}	2.51 ± 0.51^{b}	102.03 ± 12.54^{bcd}
Fe def R +10% *FT*	2.23 ± 0.25^{bc}	4.16 ± 0.43^{abc}	1.93 ± 0.32^{ab}	87.33 ± 15.17^{abc}
Fe def R +10%G	1.66 ± 0.25^{a}	3.19 ± 0.92^{a}	1.53 ± 0.77^{a}	90.63 ± 38.06^{abc}
Fe def R +10%FG	1.94 ± 0.08^{ab}	3.49 ± 0.34^{ab}	1.55 ± 0.37^{a}	80.01 ± 20.56^{ab}
Fe def R + FeSO4	1.99 ± 0.40^{b}	4.36 ± 0.74^{bc}	2.37 ± 0.39^{b}	121.20 ± 16.13^{cd}

Values with different superscript down the column differ significantly ($p<0.05$). Fe suf RC: Iron sufficient Rat control, Fe def. RC: Iron deficient Rat control, Fe def. R+ Fe suf. Diet: Iron deficient Rat on iron sufficient diet, Fe def. R+10%T: Iron deficient Rat on iron deficient diet supplemented with 10% *Telfaira occidentalis*, Fe def. R+10% FT: Iron deficient Rat on on iron deficient diet supplemented with 10% fermented *Telfaira occidentalis*, Fe def. R+10%G: Iron deficient Rat on iron deficient diet supplemented with 10% *Gnetum africanum*, Fe def. R+10%FG: Iron deficient Rat on iron deficient diet supplemented with 10% fermented *Gnetum africanum* , Fe def. R+$FeSO_4$: Iron deficient Rat on iron deficient diet treated orally with 2.8 mg/kg Ferrous Sulphate.

Table 3: Effect of fermented and unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves supplementation on some haematological parameters in iron deficient rats.

GROUP (n=5)	RBC count ($x10^6$µl)	WBC count ($x10^6$µl)	PCV %	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)
NRC	4.46±0.19^{abc}	5.80±2.01^{a}	45.40±2.30^{ab}	13.22±0.59^{abc}	101.18±9.25^{a}	29.80±2.25^{a}	29.15±1.44^{a}
Fe def. RC							
Fe def. R	4.20±0.37^{ab}	7.26±1.81^{a}	42.71±4.86^{ab}	11.97±1.43^{a}	101.89±11.26^{a}	28.47±1.87^{a}	28.10±2.23^{a}
+ Fe suff. Diet	4.68±0.18^{bc}	8.76±1.50^{a}	47.00±2.45^{b}	14.04±0.34^{c}	100±6.90^{a}	30.02±1.02^{a}	29.94±1.66^{a}
Fe def. R+10%T	4.43±0.39^{abc}	7.23±2.21^{a}	46.00±2.65^{ab}	13.68±1.53^{bc}	96.2±4.95^{a}	29.53±1.68^{a}	30.70±1.79^{a}
Fe def. R+10% FT	4.62±0.29^{abc}	8.04±0.83^{a}	48.00±0.71^{b}	13.68±0.94^{bc}	104.26±7.41^{a}	29.62±0.45^{a}	28.51±2.03^{a}
Fe def. R+10%G	4.08±0.75^{a}	8.38±1.10^{a}	42.00±6.60^{ab}	11.84±1.77^{a}	104.04±13.5^{a}	29.22±1.74^{a}	28.30±2.19^{a}
Fe def. R+10%FG	4.32±0.29^{abc}	6.85±1.33^{a}	40.00±6.16^{a}	12.20±0.25^{ab}	92.54±8.83^{a}	28.28±1.69^{a}	31.09±5.11^{a}
Fe def. R+$FeSO_4$	4.78±0.14^{c}	9.60±6.33^{a}	45.20±2.59^{ab}	14.08±0.65^{c}	94.54±4.14^{a}	29.84±0.48^{a}	31.20±1.49^{a}

Values are mean± SD of three determinations. Means with different superscript down the column differ significantly (P<0.05). NRC:Normal Rat control, Fe def. RC: Fe deficient Rat control, Fe def. R+ Fe suff. Diet:Fe deficient Rat + Fe sufficient diet, Fe def. R+10%T: Fe deficient Rat + 10% *Telfaira occidentalis*, Fe def. R+10% FT: Fe deficient Rat + 10% *Fermented Telfaira occidentalis*, Fe def. R+10%G: Fe deficient Rat + 10% *Gnetum africanum*, Fe def. R+10%FG: Fe deficient Rat + 10% *Fermented Gnetum africanum* , Fe def. R+$FeSO_4$: Fe deficient Rat + 2.8mg/kg Ferrous Sulphate.
PCV: Packed Cell Volume, RBC: Red Blood Cell, WBC: White Blood Cell, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration.

DISCUSSION

The primary cause of anemia in this study was feeding iron deficient diet for a long period (8 weeks). Anemia is considered as one of the most common index of nutritional deficiency worldwide and is caused by iron deficiency store or iron deficiency erythropoiesis (Lin et al., 2003; Soliman et al., 2010). Several authors have reported that iron deficiency anaemia is mainly caused by some food constituents that may contribute to inhibition of iron absorption, hence contribute to the high prevalence of iron deficiency, and iron deficiency anaemia (Lin et al., 2003; Soliman et al., 2010).

There was a significant decrease in the weekly relative weight gain in all the iron deficient rats when compared to the iron sufficient control this might be due to an elevation in metabolic rate in iron-deficient anemic animals, Beard et al. (1995) concluded that iron-deficient rats are hypermetabolic and attributed this to a decreased ability to thermoregulate compared to control rats. This agrees with the reports of Nora (2008) who investigated the protective effect of soybean and thyme on iron deficiency anemia in rats and found that iron deficient rats had reduced weight gain and stated that it may be due to lower plasma thyroid hormone levels (Beard et al., 1998).

The measurement of Hemoglobin is essential for the diagnosis of nutritional anaemia and is one of the most common, easiest and least expensive methods (Kotze et al., 2009). Hemoglobin is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues to the lungs. It is made up of four protein molecules (globulin chains) that are connected together (Kotze et al., 2009).

The haemoglobin concentration of the animals on iron deficient diet met the cut-off value (<11g/dl) after eight weeks of feeding, this is in contrast with the work of Solimon et al., (2010) and Nora (2008), who reported that iron deficiency anaemia was induced by the third week of feeding. This might be because the diet used in this study was not completely deficient in iron also, the amount of iron stores in the rats could also delay the induction. The haemoglobin concentrations for all the rats except those in the Iron sufficient control group were significantly low, this agrees with Nora (2008), Joshi and Mathur (2009) and Solimon et al. (2010). Decreased haemoglobin might be due to the effect of a shortage of iron available to the erythroid precursors in the bone marrow for hemoglobin synthesis (Cook, 2005).

Haemoglobin iron is an index of the iron content of haemoglobin. The percentage change in haemoglobin iron was higher in rats treated with iron sufficient diet, unfermented *T. occidentalis* and $FeSO_4$ respectively; this is probably because absorbed iron was made more available to erythroid precursors for haemoglobin synthesis. This is further shown by their significantly higher Hb concentration.

Haematological paramaters are routinely checked to diagnose anaemia which is characterized by decreased haemoglobin concentration in circulating blood, less than 12 g/dl and less than 13 g/dl in females and males respectively (Okochi et al., 2004). Iron deficiency anaemia may be effectively diagnosed in most cases by full blood examination and serum ferritin level (Pasricha et al., 2010). The Mean corpuscular volume (MCV), represents the average volume of a red cell mean corpuscular haemoglobin (MCH), the mass of haemoglobin per average red cell irrespective of the cell size and mean corpuscular haemoglobin concentration (MCHC), describes the average concentration of haemoglobin per red cell, it takes into account red cell size. These indices are used to classify anemia based on the blood cell morphology. Depending on the MCV, anaemia is classified as microcytic normocytic or macrocytic. These may be further subdivided according to the average amount of RBC haemoglobin (MCHC) into hypochromic or normochromic (Hoffman et al., 2000; Abdelgader et al., 2014). MCHC is a better index for anaemia classification than

MCH because it shows the haemoglobin content in each red blood cell. Therefore, three groups of anaemia are distinguished, Microcytic hypochromic, normocytic normochromic and macrocytic anaemia. The MCHC of all rats decreased significantly at the point of induction, this shows that hypochromic anaemia was induced. This contrasts the reports of Joshi and Mathur (2009), Solimon et al. (2010) and Salawu and Salimon (2014), who reported a non significant change in MCHC.

By day 21 of supplementation, there was a non significant change in RBC, WBC, Hb, MCV, MCH, MCHC and PCV of rats in the treatment groups when compared to the Iron sufficient control group, this is propably due to the significant increase in haemoglobin-Iron. Haemoglobin iron occupies a dominant position in all animals, and high proportion present as haemoglobin indicates that any condition influencing the level of this compound in the blood greatly affect the iron status of the body (Joshi and Mathur, 2009). However, rats in the iron deficient control group which continued on the iron deficient diet showed a non significant increase in Hb concentration, this might be because iron deficiency usually increases the rate of Iron absorption hence there was an increase in the rate of absorbing the little Iron contained in the diet. This also combined with increase depletion of available iron stores (Bothwell et al., 1979; Joshi and Mathur, 2009). This agrees with the findings of Joshi and Mathur (2009) who reported an increase in the blood parameters after 10 days of depletion though the rats were kept on iron deficient diet.

Ferritin is currently considered the most important indicator of iron status as even in the first stage of iron deficiency, its concentration decreases (Knovich et al., 2009). A ferritin molecule consists of 24 subunits of L-ferritin or H-ferritin. H-ferritin has ferroxidase activity, which is necessary for oxidising iron before it can be loaded onto ferritin for storage (Koulanouzidis et al., 2009).

The serum ferritin concentrations of rats in the iron deficient control group were significantly low. This might be because the rats in the face of insufficient iron from diet began to deplete their iron stores in order to meet up with some of their iron needs, this probably explains the non-significant increase in Hb. At the end of the 21 days supplementation, ferritin concentration remained significantly low for rats in the iron deficient control group. However, serum ferritin concentrations of rats in unfermented and fermented *Gnetum africanum* group had a non significant increase when compared to the iron deficient control group. This may be because the iron content and absorption from *Gnetum africanum* leaves were low, this level was lower for the fermented leaves probably because of the fermentation process which might have led to a reduction in the iron content.

Ferritin concentration was significantly higher in rats supplemented with unfermented *Telfaira occidentalis* than fermented *Telfaira occidentalis* this might be due to the effect of fermentation that introduced microorganisms which could have interfered with the iron content of the leaves, thereby reducing it. Ferritin concentrations was significantly higher in rats found in the iron sufficient diet control and *T. occidentalis* groups probably because the iron in these diets were better absorbed hence rats in this group had higher iron stores. The non significant change in serum ferritin concentration in iron deficient rats on ferrous sulphate drug when compared to rats in the iron deficient control group and the rats in the iron sufficient diet control and *T. occidentalis* groups probably indicates that the iron from the drug was not efficiently absorbed, this might be because the action of the first pass effect on drugs taken orally reduced the amount of the iron that finally got into circulation. Also, the drug is known to produce intestinal side effects such as constipation, nausea, and bloating in many users (Hansen 1994; Soliman et al., 2010).

Some forms of ferrous sulfate are enteric-coated to delay tablet dissolving and prevent some side effects, but enteric-coated iron may not absorb as well as iron from standard supplements (Rickettes 1993; Soliman et al., 2010).

Conclusion

Telfaira occidentalis leaf at 10% supplementation significantly increased the haemoglobin-iron, haematological parameters and serum ferritin concentration of nutritionally anaemic rats on iron deficient diet. It also, regenerated the haemoglobin concentrations of the anaemic rats while 10% *G. africanum* leaf supplementation had no significant haemoglobin regeneration potential. Fermentation of both leaves did not significantly enhance their haemoglobin regeneration potentials.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

IO and CAM performed the study and did the final manuscript corrections; OAO and BDJ designed the study and generated the initial manuscript and quality control measures; ABS and BTA were involved with the animal studies and statistical analysis. All authors read and approved the final manuscripts.

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