

Comparative effects of *Telfairia occidentalis* Hook. F. leaf extract and Ranferon-12[®] as dietary supplements on partially starved male Sprague-Dawley rats

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Abstract

This work examined the supplemental effects of *Telfairia occidentalis* Hook.F. leaf extract compared to a standard hematinic drug, Ranferon -12[®], on the performance of thirty-six male Sprague-Dawley rats. The rats were randomly assigned into six groups of six rats each, and were fed standard rat ration at 80 % of *ad lib* within 24 hours. Access to clean water was allowed *ad libitum*. The leaf extract was administered orally at the dose of 200mg/kg, 400mg/kg, 600mg/kg, and 800mg/kg (for groups 1-4 respectively), and group 5 was given Ranferon 12[®] (at 0.3ml/kg), while group 6 (Control) received no extract but water. The extract was administered once daily for 21 days, while feed intake, weight gain, water intake, haematology, and some liver enzymes as well as cholesterol levels were the parameters measured. Results also revealed that weight gain, water intake and total WBC were significantly higher in group 3(600mg/kg) compared to other groups, while group 5 (Ranferon-12[®]) had significantly higher Packed cell volume and total red blood cell counts. There were no significant differences (P>0.05) in the liver enzymes and cholesterol levels of rats in all groups. The results of this study have shown that supplementation with *Telfairia occidentalis* leaf extract was quite beneficial as it produced a significantly higher weight gain at 600mg/kg, and that neither increased dose of extract nor supplementation with Ranferon-12[®] yielded any special advantage.

Keywords: *Telfairia occidentalis*, food intake, rats, leaf concentrate, weight gain

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INTRODUCTION

Telfairia occidentalis is a well-known food crop in West Africa, which is also popular for provision of oil, fibre, and medicines (Fern, 2020). Protein from plant leaves has been shown to be an extremely nutritious food and can be distinguished from ordinary leaf extract as they are made by pressing out the juice containing the proteins, vitamins, and minerals through mechanical means, thus separating them from the indigestible fiber.

According to Feedipedia (2020), the protein extracted from the leaf juice can be used fresh, or coagulated by heating, dried thereafter and stored for future use. *T. occidentalis* also has been reported to be a supplement in micronutrient deficiencies (hidden hunger) for upgrading starchy crops and poor-quality food sources consumed by poor rural populations in sub-Saharan Africa (Lawal *et al.*, 2021).

Many programmes involving the use of plant leaves for the production of alternative protein sources have been approved and funded by many organizations such as United Nations, the European Economic Community, the British Overseas Development Agency, the Rotary Club International, and others (King *et al.* (1991). Leaf extracts have been shown to be very effective in combating malnutrition, anaemia and vitamin A deficiency, which are common in the vulnerable groups (especially children and pregnant women) in some developing countries such as Mexico, India, Bolivia, Sri Lanka, Ghana, Nicaragua and Bangladesh, etc. (Cameron, and Yngve, 1983).

Leaf extracts have also been shown to have huge economic advantage in human nutrition, and after the protein production, it has been suggested that the remnant fibre residue can then be used to feed cows, goats, sheep, horses, rabbits, or guinea pigs (Lowe, 2002; Fellows and Hampton, 1992). Ranferon-12[®] is a standard and very effective hematinic commonly used for replenishment, and for the treatment of iron and folic acid deficiency anaemia in humans (MIMS.com, 2022), although it is contra-indicated in patients suffering from hemochromatosis and hemosiderosis, including those who show hypersensitivity to iron, folic acid, vitamin B₁₂, vitamin C, Zinc, and Cobalt (Pillintrip.com, 2022).

Fluted pumpkin (*Telfairia occidentalis*) is a vegetable food crop of importance that belongs to the family *Cucurbitaceae* and is commonly

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found in the low land humid tropics of West Africa with Nigeria, Ghana and Sierra Leone being the major producers (Nkang *et al* 2003). It has different traditional names; among the Igbos (Ugu) (Akoroda, 1990); in Yoruba (Aporoko), and called "Ubong" in Efik (Badifu and Ogunsina, 1991; Amanke, 2003). According to Kayode *et al.* (2010), *T. occidentalis* Hoof is an important plant used in ethno-medical practice, whereby its supplemental effects have been reported to improve haematological indices in man, thus preventing anaemia which occurs as a result of malnutrition. Its use has also been associated with significant increase in the weight gain of animals (Aregheore, 2007), as well as having both nutritional and medicinal values (Ntinya *et al.*, 2019). Consequently, Emeka and Obidoa, (2009) also reported that the leaf extracts are rich in essential minerals, vitamins, proteins, in addition to antioxidant and antimicrobial properties.

This study investigated the effects of graded doses of *Telfairia occidentalis* leaf extract compared to a standard hematinic drug Ranferon -12[®] on the performance of partially starved male Sprague-Dawley rats.

MATERIALS AND METHODS

Animal Management

Thirty-six male Sprague-Dawley rats weighing between 120 - 140g were used for the study. They were obtained from the Department of Animal Health and Production, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, fed standard rat diet at 80 % ad lib, with clean drinking water provided *ad libitum* throughout the experiment. The animals were housed in metal cages, and were acclimatized for two weeks before the commencement of the experiment.

Preparation of *T. occidentalis* leaf extract

The leaves and stem of *Telfairia occidentalis* were bought from a local market at Orba, near the University, and was identified in the Department of Plant Science and Biotechnology of the University. After botanical identification, two kilogram (2 kg) of freshly cut *T. occidentalis* leaves with the stalk was separated from the stem, washed with clean water to remove dirt and soil, drained, and wilted for thirty minutes.

The leaves were chopped, packed into a muslin bag and then squeezed with a mechanical

screw press to obtain a homogenous extract of the fluted pumpkin leaves. The concentration of the leaf extract was determined by drying 2mls of the leaf extract in a hot air oven (using a watch glass) to constant weight, and thereafter, the actual weight of the extract was determined by difference. The extract was stored at 4°C in the refrigerator until further use. The calculated doses were administered per os (by mouth) in individual rats using gastric lavage.

Experimental design

The experimental animals were divided into six groups of six rats each and were acclimatized for two weeks before the commencement of the experiment. During the experiment, the rats were partially starved, as they were fed standard rat diet at 80% of *ad lib*, with constant availability of fresh water throughout the experiment.

The various groups were as follows; Group 1 (200mg/kg), Group 2 (400mg/kg), Group 3 (600mg/kg), Group 4 (800mg/kg), Group 5 (Ranferon-12[®]) and Group 6 (Control). Groups 1-4 were given *T. occidentalis* leaf extract once a day, using gastric gavage, for twenty-one days. The animals were treated in accordance with the guidelines of Institutional Animal Care and Use Committee (IACUC) Protocol of Faculty of Veterinary Medicine, University of Nigeria. Nsukka, with approval reference number FVM-UNN-IACUC-2022-0688.

Dosage and constituents of Ranferon-12[®]

Ranferon-12[®], a standard hematinic used for this study, was procured from a registered Patent medicine dealer, and was administered at 0.3ml/kg body weight which was based on the recommended dose of 15ml to 30ml daily for an average adult human weighing approximately 60 kg body weight. Components of Ranferon -12[®] include Iron fumarate 305 mg (equiv elemental Fe 100 mg), Folic acid 0.75 mg, Cyanocobalamin 5 mg, Ascorbic acid 75 mg, Zinc sulfate 5 mg, (MIMS.com, 2022). Ranferon-12[®] is made by Ranbaxy Nigeria Limited-a subsidiary company of Sun Pharmaceutical Industries Ltd, Lagos

Determination of Proximate fraction (AOAC, 2012)

The proximate fractions were determined according to the method of AOAC (2012). Crude protein content was obtained by multiplying the value obtained from Kjeldahl's

nitrogen by a protein factor of 5.3, a factor recommended for vegetable analysis.

Ether extract was quantified using the Soxhlet apparatus and petroleum ether (B.P. 60°C-80°C) as a solvent.

Crude fiber was determined by acid-base digestion with 1.25% H₂S₄ (W/V) and 1.25% NaOH (W/V) solutions.

The moisture content of the leaves was determined by drying 5 g of the leaves (in triplicate) in an oven at 105°C until constant weight was attained.

Ash content was determined by burning sample in muffle furnace at 550°C until white ash was obtained.

At the end of the experiment, the parameters measured were weight gain, food and water intakes. The animals were bled for haematological determinations using the orbital technique, into sample bottles containing ethylene-diamine-tetra-acetic acid (EDTA). The sample bottles were shaken gently to mix up the blood with EDTA to prevent clotting. Packed cell volume (PCV) was measured using microhematocrit tubes according to the method of Coles (1986), while the total red blood cells (TRBC), as well as the total white blood cells (TWBC) were determined using the method of Schalm *et. al.* (1975). Serum biochemical parameters such as Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), were measured as described by Cartwright (1979), while cholesterol levels were determined according to the method of Schalm *et. al.* (1975).

Statistical analysis

Statistical significance between groups was analyzed using One-way analysis of variance (ANOVA with computer software (SPSS) version 20.0 (IBM). Values were expressed as mean ± Standard error of mean and separated using Duncan's multiple range test. The means were considered significant at P<0.05 (Steel and Torrie, 2000).

RESULTS

Table 1 shows the components of the rat ration in addition to the constituents of the premix as well as the calculated values of Lysine and Methionine.

Table 1. Composition of feed nutrients.

Ingredients	Values
Dry matter%	65.3
Ether extract%	5.40
Crude fibre%	5.60
Crude protein %	14.78
Ash%	6.30
Energy (MJME/kg)	12.05
Calcium%	1.09
Phosphorous%	0.80
*Lysine%	1.02
*Methionine%	0.45

*Calculated values

Constituents of Premix:

Vitamin A 15,000 I.U, vitamin D₃ 13000 I.U, thiamin 2mg, riboflavin 6mg, pyridoxine 4mg, niacin 40mg, cobalamine 0.05g, Biotin 0.08mg, cholinechloride 0.05g, manganese 0.096g, zinc 0.06g, iron 0.024g, copper 0.006g, iodine 0.014g, selenium 0.24mg, cobalt 0.024mg and antioxidant 0.125g. The premix was added to the feed at the rate of 2.5 kg/tonne of feed.

Table 2. Nutrient composition of *T. occidentalis*

Parameter	Value
Dry matter (as fed basis)	13.6%
Ash	6.5%
Crude fibre	15.6%
Crude protein	16.2%
Ether extract	6.2%
Gross energy MJ/kgDM	18.4

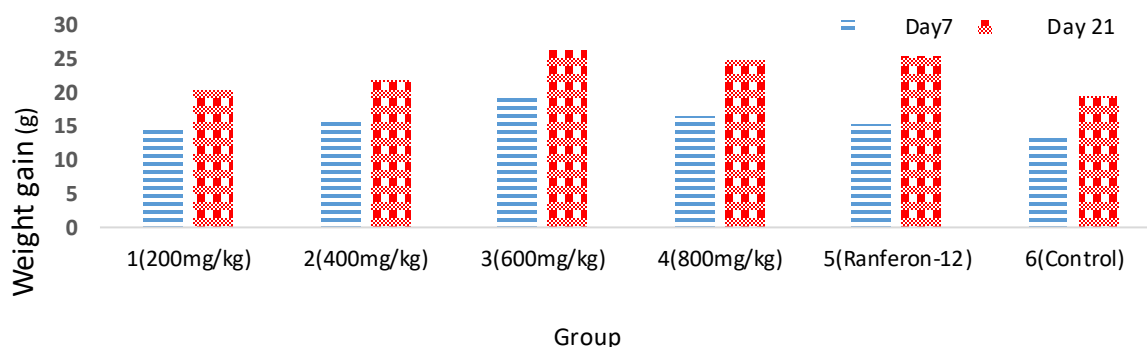


Figure 1. Weight gain of groups.

The weight gain of the various groups is presented in Figure 1 below. The result shows that Group 3 (600mg/kg) had a significantly higher weight compared to the Control(P<0.05).

Figure 2 shows water consumption of different groups. The result shows that water consumption was significantly higher (P<0.05) in group 3(600mg/kg), which was closely followed by the water consumption in groups 4 (800mg/kg) and group 5 (Ranferon-12).

The feed intake in Figure 3 shows that rats in group 3(600mg/kg) consumed a mean ration quantity of 116g by day 21, at the dose of 600mg/kg. This value was significantly higher (P<0.05) when compared to the Control and other groups.

The Packed cell volume and the Total red blood cell counts of the various groups are presented in Fig.4. Consequently, there was no significant difference in the Packed cell volume of the groups. However, Group 5 (Ramferon-12®) had non-significantly higher values (P>0.05) in both Packed cell volume and the Total red blood cell counts compared to the rest of groups. The results in Table 3 shows significantly higher values (P<0.05) only in the Total white blood cell counts for group 3 (600 mg/kg) compared to other groups except for the Ranferon -12® group. There was no significant difference(P>0.05) in the values of other parameters across different groups

The result in Fig.5 shows that there was no significant difference (P>0.05) in the liver enzymes and the cholesterol levels across the groups.

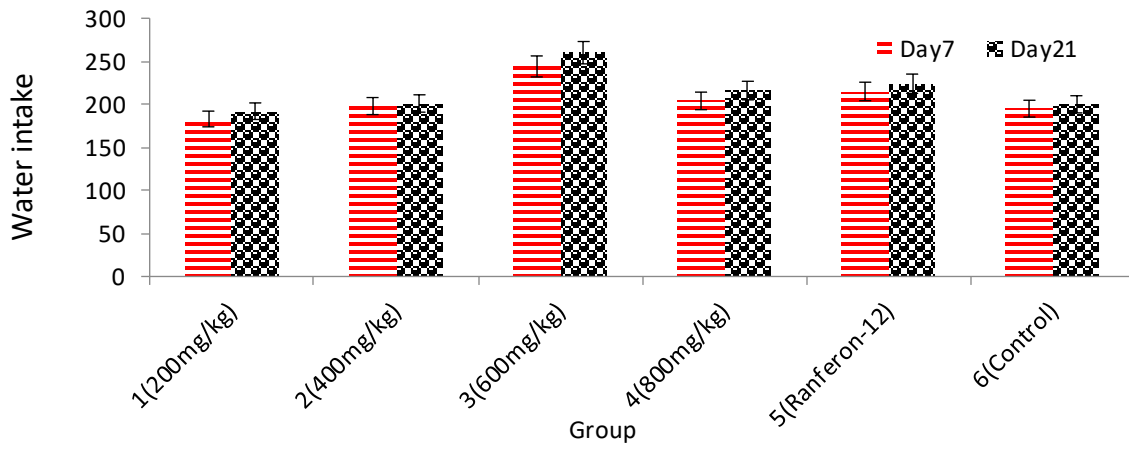


Fig.2. Water intake of groups.

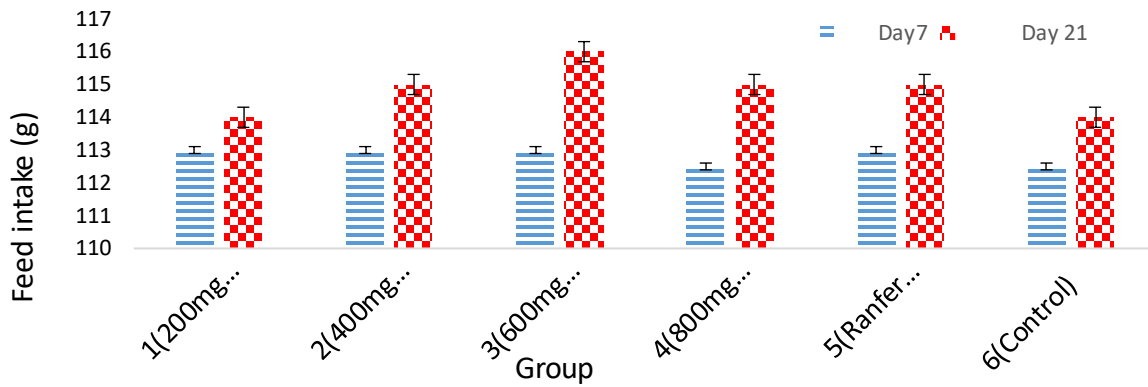
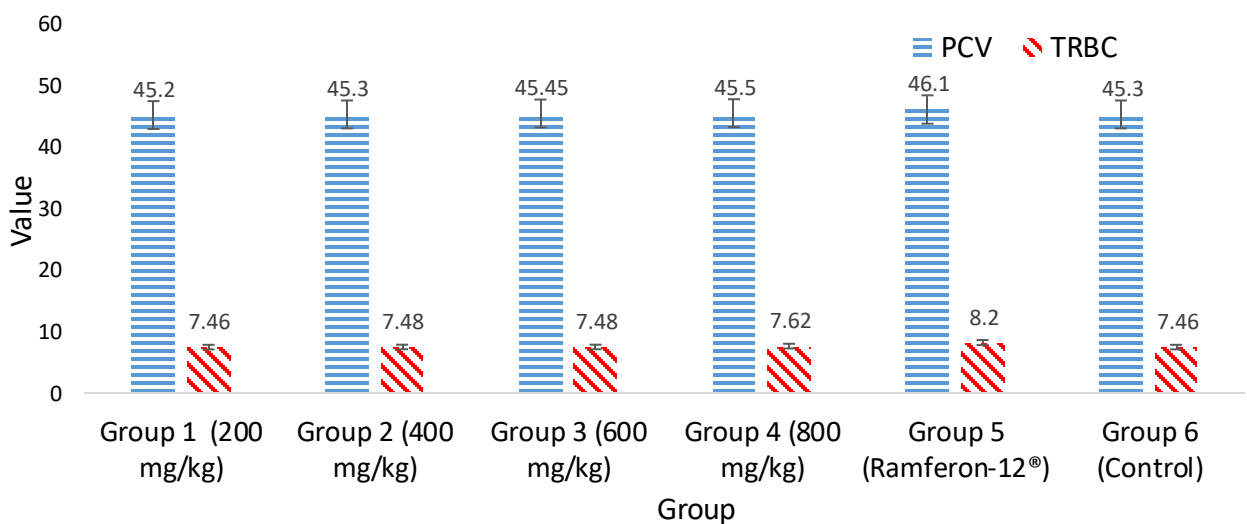


Fig. 3. The feed intake of different groups.



Packed cell volume (PCV %), Total red blood cell (TRBC) (10³)

Fig 4. Mean Packed cell volume and the Total red blood cell counts of groups

Table 3. The white blood cell profile of the groups.

Groups	TWBC ($10^6/\mu\text{l}$)	Neutrophil ($10^6/\mu\text{l}$)	Lymphocytes ($10^6/\mu\text{l}$)	Monocytes ($10^6/\mu\text{l}$)	Eosinophils ($10^6/\mu\text{l}$)	Basophils ($10^6/\mu\text{l}$)
200 mg/kg	12.10 \pm 0.56 ^a	2.58 \pm 0.27 ^a	10.07 \pm 0.48 ^a	0.16 \pm 0.16 ^a	0.12 \pm 0.06 ^a	0.03 \pm 0.03 ^a
400 mg/kg	12.22 \pm 0.42 ^a	2.66 \pm 0.16 ^a	9.41 \pm 0.94 ^a	0.16 \pm 0.24 ^b	0.09 \pm 0.04 ^a	0.00 \pm 0.00 ^a
600 mg/kg	13.05 \pm 1.21 ^b	2.82 \pm 0.24 ^a	16.93 \pm 0.40 ^b	0.15 \pm 0.06 ^a	0.20 \pm 0.10 ^a	0.05 \pm 0.05 ^a
800 mg/Kg	12.30 \pm 0.44 ^a	2.63 \pm 0.32 ^a	10.01 \pm 1.10 ^a	0.15 \pm 0.07 ^a	0.16 \pm 0.11 ^a	0.00 \pm 0.00 ^a
Ranferon - 12 [®]	12.94 \pm 1.57 ^{ab}	2.74 \pm 0.32 ^a	12.63 \pm 0.32 ^a	0.15 \pm 0.07 ^a	0.11 \pm 0.32 ^a	0.0 \pm 0.00 ^a
Control	12.08 \pm 1.7 ^a	2.54 \pm 0.12 ^a	10,54 \pm 1.12 ^a	0.15 \pm 0.32 ^a	0.12 \pm 0.10 ^a	0.05 \pm 0.00 ^a

a, b = Values with different superscripts vertically are statistically different ($P \leq 0.05$).

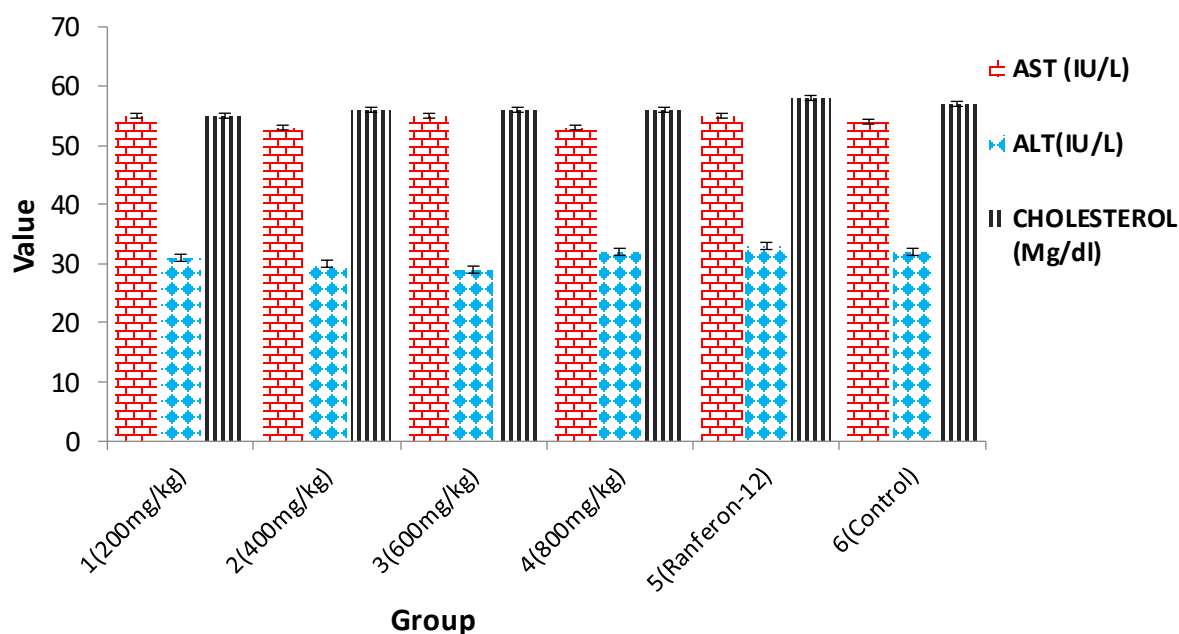


Figure 5. Serum analysis of groups on day 21.

DISCUSSION

The leaf extract of *Telfairia occidentalis* Hook. F. which was used in this study as dietary supplement has shown unique nutritional attributes capable of maintaining physiological activities in partially starved murine animal model. The proximate fractions of *Telfairia occidentalis* leaf extract from this study (crude protein 16.2% , crude fibre 15.6%, ether extract 6.2% and ash 6.5%) were within the ranges reported by Taiga *et al* (2008) which were, 17.5% for protein, ash (7.9%) ether (6.8%) and crude fibre (16.2%). However, Aregheore (2007) reported higher crude protein of 21.9% , while fat (5.9%), crude fibre (15.9%) and

ash (12.9%) were within our range. This is one important reason why samples must be analyzed in order to determine differences in value. More so, the results on weight gain showed that all the supplemented groups had significantly higher ($P < 0.05$) weight gain compared to the Control. However, the rats in group 3 (600mg/kg) had significantly highest weight gain, compared to those in group 4 (800mg/kg) and other groups, except group 5 which ranked closer. The growth increase observed in rats in this study could be attributed to a balance in the rich nutrient content of *T. occidentalis* in terms of its amino acids, fatty acids, minerals, and vitamins as reported by other reseachers such as Emeka and Obidoa (2009), Omimakinde *et al.* (2018), in addition to the reports of Fagbemi

(2007), Oliviero and Fogliano (2016). Aregheore (2007) also observed significant increase in weight due to dietary supplementation with *T. occidentalis* leaf extracts, although no reference was made in the work concerning any dose. Consequently, Lowe (2002) also reported that the leaf extracts of *Telfairia occidentalis* are readily digestible, and well tolerated in the gastrointestinal tract, while Lawal *et al.* (2021) reported on the palatability and acceptability of the leaf extracts when used to fortify low-nutrient diets for human consumers. Water intake was also significantly higher ($P < 0.05$) in the treated groups (though highest in Group 3 (600mg/kg) compared to the Control. This increase in water intake could be attributed to higher protein content of the leaf extract. This finding agrees with the observations of McDonald *et al.* (2011) who reported that consumption of protein diets provoke increased water intake, adding that water is necessary in protein digestion, as well as in the elimination of toxic products from protein metabolism. Although the animals were fed 80% *ad lib* of their rations, but Group 3 (600mg/kg) had significantly higher ($P < 0.05$) intake at day 21, compared to control and the other treated groups. Although there was no significant difference ($P > 0.05$) between Groups 4 (800mg/kg) and 5 (Ranferon-12). Consequently, this suggested that at 600mg/kg, the rats had better efficiency of nutrient utilization, hence better gain in weight. It was also an indication that *T. occidentalis* leaf extract was most beneficial at this level compared to the other doses as recorded in this study. The above observation supports the findings of Ogunka-Nnoka *et al.* (2017) who reported best performance of oral administration of *T. occidentalis* leaf extract at 500mg/kg in male adult Wistar rats. It is also possible that the leaf extract of *T. occidentalis* at 800mg/kg may likely contain more quantity of anti-nutrients such as tannins, saponins, etc. (Dube *et al.*, 2001; Sofowora, 1996; Harborne, 1973), which may likely interfere with intake, digestion and absorption of nutrients, and this may have affected their feed intake weight gain compared to Group 3. However, increased intake and weight gain should be evidence of balanced nutrients, leading to improved food digestibility as well as enhanced food absorption. The beneficial effects of leaf extracts were also documented by Lowe (2002) who reported better growth rate and overall performance in malnourished Bolivian school children whose diets were supplemented with leaf extracts. Similarly, other evidence concerning the effects of *T. occidentalis* on digestibility and conversion rate in animals have

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also been provided in the reports of Adedapo *et al.* (2008), while the use of the extract for biofortification in human diets have also been documented according to Bouis. and Saltzman (2017). Haematological results indicated that the group supplemented with standard haematinic drug (Ranferon- 12[®]) had significantly higher ($P < 0.05$) Packed cell volume as well as Total red blood (TRBC) counts compared to the other groups, whereas a higher Total white blood cell (TWBC) count was observed in Group 3 (600mg/kg) in comparison to the other groups. The reason for the differences in haematology could be explained based on the reports of Alada (2000) and Agbede *et al.* (2008), which emphasized that the haematological result of *T. occidentalis* leaf extracts may not necessarily follow any regular pattern, even though it has been repeatedly shown to be protective against anaemia. The cholesterol levels in the treated groups were not significantly higher ($P > 0.05$) compared to the Control, while no significant changes ($P > 0.05$) were also observed in the cardinal liver enzymes, Alanine amino transaminase (ALT), aspartate transaminase (AST). These non-significant changes may suggest some level of safety for the animals, particularly the liver, as well as other vital organs of their body. This observation also corroborates the findings of Oboth (2005) and Emeka and Obidoa (2009), in addition to the observations of Aderibigbe *et al.* (2008) concerning the safety of *Telfairia occidentalis* leaf extract.

Conclusion

The results of this study suggested that supplementation with *Telfairia occidentalis* leaf extract was quite beneficial as it produced a significantly higher weight gain at 600mg/kg, and no signs of toxicity in the liver and blood. Consequently, this study has shown that *Telfairia occidentalis* leaf extract was most beneficial at 600mg/kg, such that neither increased dose of nor supplementation with Ramferon-12[®] yielded special advantage in the partially starved rats.

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Author contribution

CON designed the study, analyzed the work and wrote the first draft of the manuscript. CUN was involved in the data collection, gathering of materials and review of the manuscript. LS provided reagents and performed laboratory analysis. CON and LS interpreted the data. All authors read and approved the final version of the manuscript.

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