International Journal of Biochemistry Research & Review

24(2): 1-6, 2018; Article no.IJBCRR.43088
ISSN: 2231-086X, NLM ID: 101654445

Immune Modulatory Properties of Powdered *Telfairia occidentalis* Supplemented Vital Feed Diet on Male Wistar Rats

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Authors' contributions

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

Article Information

DOI: 10.9734/IJBCRR/2018/43088
Editor(s):
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Complete Peer review History: http://www.sciencedomain.org/review-history/27696

Original Research Article

Received 01 July 2018
Accepted 29 November 2018
Published 08 December 2018

ABSTRACT

This study was carried out to investigate the immune modulatory properties of different graded stored powdered of *Telfairia occidentalis* supplemented diet on male wistar rats. Twelve male wistar rats weighing 120g±20g were purchased, acclimatised and randomly allotted into four (T_1, T_2, T_3, T_4) *Telfairia occidentalis* supplemented groups (0% 5%, 10% and 15%). The rate of their feeding was monitored for three weeks after which the animals were decapitated and the blood and organs were

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removed for haematological and endogenous antioxidant analysis. The result showed that the highest feed intake was observed in animal fed with the highest level of *Telfairia occidentalis* supplementation. Also from the haematological analysis, the white blood cell of the highest supplementation was boosted when compared to other graded level likewise the endogenous Antioxidant level was enhanced when compared with the control group.

Keywords: Endogenous antioxidants; exogenous antioxidant; haematological parameters; immune modulatory properties; Telfairia occidentalis.

1. INTRODUCTION

Plants have been known to have medicinal properties, hence their use as herbs or as components of medicines cannot be over emphasised examples *Ocimum sanctum* and *Axonopus compressus*, and the root extracts of *Ophiopogon japonicus* has long been traditionally used and have been found to possess antidiabetic activity [1]. Bitter leaf (*Vernonia amygdalina*) is utilised in Africa to treat dysentery and other intestinal ailments. Celery (*Apium graveolens*) is employed as a diuretic; the leaf extract of Eucalyptus (*Eucalyptus globulus*) is employed as an antipyretic (febrifuge) and its oil, employed in cold and cough analgesics. The leaves of *Corchorus olitorius*, which is widely consumed in Nigeria is rich in antioxidants andused in the treatment of pain, fever, chronic cystitis and tumours [2]. An important medicinal benefit of some plants is the improvement or the boosting of human immunity, achieved via different mechanisms such as the stimulation of the production of the protective white blood cells and antioxidants [3,4]. Indigenous to Southern Nigeria, the leaves of fluted pumpkin (*Telfairia occidentalis*), a widely consumed vegetable in Nigeria has been shown to possess several medicinal benefits and is extensively used in herbal medicines, *Telfairia occidentalis* has been shown to possess better erythropoietin potential [5,6].

Considering the versatile medicinal benefits of fluted pumpkin and its common use in herbal medicines, it is important to ascertain the immune modulatory potentials via examining the haematological parameters, endogenous antioxidant of the rat and exogenous antioxidant of this widely utilised vegetable when supplemented with vital feed.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Fresh leaf samples of *Telfairia occidentalis* were collected from the Nigerian Stored Products Research Institute (NSPRI) farm, identified and authenticated at the Herbarium Section of the University of Ibadan, Oyo state, Nigeria.

2.2 Processing of Plant Samples

The fresh leaves of *Telfairia occidentalis*, were rinsed, destalked, and air-dried at room temperature (23.5^{o}C). The dried samples were homogenised using an automated electric blender and stored in an airtight container prior to the supplemental formulation.

2.3 Formulation of Experimental Diet

Four experimental diets were prepared: The *Telfairia occidentalis* supplemented diets varied at 5%, 10%, and 15% *Telfairia occidentalis* inclusions, and the control diet containing 0% inclusion of *Telfairia occidentalis*.

2.4 Procurement of Experimental Animals and Grouping

Twelve (12) male wistar rats weighing between 120g±20g were purchased NSPRI farm house and randomly allotted into three treated groups (T_2, T_3, T_4), and a control group (T_1). The rats were fed different levels of supplemented *Telfairia occidentalis ad-libitum* for three (3) weeks. The rats in each group were sacrificed and their blood and liver were collected for haematological and endogenous antioxidant analysis.

2.5 Feed Intake Determination

The average feed intake (AFI) for the various supplemented and the control group for 3 weeks was calculated via the method described by Ogungbemi [7].

Feed Intake (g/week) = Average feed intake for three weeks (g)/ 2a3

2.6 Haematological Parameter

Haematological parameters were determined using an automated haematological analyser

(SYSMEX KX-21N), according to the manufacturer's instructions.

2.7 Catalase Activity

Catalase (CAT) activity was measured using the method of Abie [8]. 10µL each of kidney or liver homogenate was added to test tubes containing 2.80 mml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of freshly prepared 30Mm H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm at 5 min interval in a spectrophotometer (Manufacturer and model). A molar extinction coefficient of 0.041 mM^{-1} cm^{-1} was used to calculate the catalase activity using the formula:

1 unit of catalase activity = Moles of H_2O_2 degraded / min / mg Protein.

2.8 Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was evaluated according to the method described by Martin et al*.,* (1987) based on the principle that anti-oxidation of haematoxylin is inhibited by SOD at the assay of pH of 7.8 and the percentage of the inhibition is linearly proportional to the amount of SOD present within a specific concentration range. The amount of SOD in the sample was determined in the 'standard cytochrome C' SOD unit, by measuring the ratio of auto-oxidation rates in the presence and absence of the samples. The method can be summarised thus: 920µL of phosphate buffer (0.05M, pH 7.80) was dispensed into clean test-tubes and 40µL of sample homogenates were added. A reagent test was also prepared by replacing the sample with 40µL of sample dilution buffer. The mixture was incubated for 2 minutes at 25ºC before the addition of 40µL of haematoxylin. After the addition of 40µL haematoxylin, absorbance of the sample test and reagent test was read in duplicate at 560nm immediately against the sample blank, distilled water.

2.9 Extraction and Estimation of Total Polyphenolic Content

10ml of 70% methanol was introduced into a glass test tube containing 0.1g of the grinded sample. This was followed by heating in a water bath for 2 hours at 37ºC with agitation every 15 minutes. The mixture was then allowed to cool to room temperature before being centrifuged at 3000rpm for 10 minutes. The supernatant was collected for total flavonoids determination. Absolute methanol was used for extraction for this procedure. The Folin-Ciocalteu method was used to determine the total amount of phenolic compounds [9].

2.10 Determination of Total Flavonoid Content

The total flavonoids content was determined using the aluminium chloride colorimetric method described by Chang et al. [10]. The principle is based on the formation of stable complex between aluminium chloride and keto and hydroxyl groups of flavones and flavonoids. Briefly, 100µl of each extract were mixed with 1.5ml of 95% ethanol, 100µl of 1M potassium acetate and 2.8ml of deionised water and the absorbance of the reaction mixture were determined at 415nm after incubation at 80^{o}C degree Celsius for 35 minutes. The total flavonoid content was expressed as milligrams quercetin equivalent per gram extract (mgQE/g extract) following extrapolation from a quercetin standard curve.

3. RESULTS

Feed Intake: The results obtained from feed intake in Table 1 showed a significant increase in the average feed intake with increasing percentage *Telfairia occidentalis* supplementation with 15% supplementation having the highest and control group the lowest feed intake.

3.1 Effect of *T. occidentalis* Feed Supplement on Haematological Parameters

The white blood cell (WBC) count increases with increase in percentage supplementation of *Telfairia occidentalis* with highest supplementation showing the highest WBC counts (12.32±0.91). There was no significant increase observed between 5% (11.23±1.01) and 10% (11.52±0.51) and 15% (12.32±0.91) supplementation. Likewise, in the red blood cell count (RBC) an increase RBC counts with increasing supplementation was observed with the highest being 15% (9.42±0.063) *Telfairia occidentalis* supplementation when compared to the control feed (8.01±0.20), respectively. An increase in percentage lymph was observed as the percentage feed supplementation increases. A slight increase is observed between 5% and

10% supplementation. Haemoglobin (HGB) levels show an increase with increasing percentage feed supplementation.

3.2 Endogenous Antioxidant of the Liver

Superoxide dismutase and Catalase activity increases with increase in the level of supplementation of *Telfairia occidentalis*. The increase in activity was significantly high for 10% and 15% supplementation when compared to the control feed.

3.3 Exogenous Antioxidant Determination

Polyphenols concentration increases with increasing feed supplementation; 15% *Telfairia occidentalis* contained a highest concentration (171.45±6.5) of polyphenols when compared with the control feed. Flavonoids concentration also increases with increasing percentage of feed supplementation, with 15% (101.32± 5.62) feed supplementation containing a high flavonoid content compared to the control feed.

4. DISCUSSION

The consumption of the leaves of *Telfairia occidentalis* supplemented with the animal standard diet to boost the immune system has provided a substantial result as seen in this study. From the result obtained, there was an appreciable feed intake observed in all level of the supplemented diets when compared to the control group with 15% of the supplemented diet

Table 1. Cumulative feed intake of *Telfairia occidentalis*

Supplementation	Average Feed Intake (3 weeks) (g)
Control feed	568.8±32.7[a]
5% *Telfairia occidentalis*	812.2±34.3[b]
10% *Telfairia occidentalis*	878.6±40.6[b]
15% *Telfairia occidentalis*	916.6±132[b]

Values are expressed as means± SD and are mean of three replicates (n=3);
values mean with different superscript down the column are significantly different (P<0.05)
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Values mean with superscript a shows no significant different with other mean with superscript a, ab
Values mean with superscript b shows no significant different with other mean with superscript b, ab

Table 2. Effect of *T. occidentalis* food supplement on haematological parameterS

Supplementation	WBC (x10^9/L)	RBC (10^{12}/L)	Lymph (%)	HGB (×g/L)
0% Supplementation	9.88±1.35[a]	8.01±0.20[a]	78.78±12.11[a]	115.32±8.32[a]
5% Supplementation	11.23±1.01[b]	8.10±0.30[ab]	78.78±12.11[a]	133.32±6.21[a]
10% Supplementation	11.52±0.51[b]	8.80±0.80[ab]	81.42±2.30[a]	135.00±17.32[a]
15% Supplementation	12.32±0.91[b]	9.42±0.10[b]	82.31±3.60[a]	141.34±24.50[a]

Values are expressed as means± SD and are mean of three replicates (n=3); values mean with different superscript down the column are significantly different (P<0.05)
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Table 3. Effect of *T. occidentalis* feed supplement on endogenous antioxidant

Treatment	SOD (µmol/mg protein)	Catalase (µmol/min/mg protein)
Control feed	58.45±2.32[a]	86.00±3.45[a]
5% *T. occidentalis* diet Suppl.)	62.32±6.15[ab]	87.10±9.21[ab]
10% *T. occidentalis* diet Suppl.)	64.86±8.11[ab]	109.54±3[b]
15% *T. occidentalis* diet Suppl	75.96±5.11[b]	110.43±4[b]

Values are means of three determination ± SD
Values Mean with different superscript down the column are significantly different (P<0.05).
Values mean with superscript a shows no significant different with other mean with superscript a, ab
Values mean with superscript b shows no significant different with other mean with superscript b, ab

Table 4. Exogenous antioxidant determination

Supplementation	Polyphenols	Flavonoids
Control feed	62.54±0.00	54.63±0.41
5% *T. occidentalis* diet Suppl	82.23±0.02	65.15±2.52
10% *T. occidentalis* diet Suppl	100.32±6.5	82.62±6.28
15% *T. occidentalis* diet Suppl	171.45±6.5	101.32±5.62

Values are means of three determination ± SD
Values Mean with different superscript down the column are significantly different (P<0.05).

having the highest feed intake which can be attributed to the palatability of *Telfairia occidentalis* [11] and the high level of minerals and vitamins which has an appetite stimulating property [12]. *Telfairia occidentalis* has shown the ability to combat certain disease due to its exogenous antioxidant and antimicrobial properties and its minerals (especially iron), vitamins and high protein content [5]. This was in agreement with the result obtained in Table 3 which showed an appreciable increase SOD and catalase activity with increasing supplementation, the increase in activities can be attributed to the high level of exogenous antioxidant found in the plant and the level of the feed intake. The body has several mechanisms to counteract oxidative stress generated by free radicals by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants) [13]. The roles of antioxidants are to neutralise the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention and by boosting immunity. Exogenous antioxidant such as flavonoids on human health mainly reside in their potent antioxidant activity that have been reported to prevent or delay a number of chronic and degenerative ailments such as cancer, cardiovascular diseases, arthritis, aging, cataract, memory loss, stroke, Alzheimer's disease, inflammation, infection [14]. Similarly, there was an increase in the WBC counts as seen in Table 2 with increasing level of supplementation which can be attributed to the boosting effect of the *Telfairia occidentalis* via the possession of some immune boost phytochemicals and minerals [15], also the RBC counts increases with increasing leaf supplementation due to it erythropoietin potentials [16,17] and minerals associated with RBC such as Fe, Cu, Co etc. the polyphenols and flavonoid evaluation as seen in Table 4 agrees with the endogenous antioxidant which sees the ability of the exogenous antioxidant boosting the endogenous antioxidant in-turn modulating the immune system by working alongside the WBC in the defense of the system. Plants are being used for protective and therapeutic properties.

5. CONCLUSION

It can be concluded that *Telfairia occidentalis* is a very palatable vegetable that has a very high immune modulatory properties enhanced by its rich exogenous antioxidant and thus be recommended to patient in order to boost or enhance their immunity.

DISCLAIMER

This paper is based on preliminary dataset. Readers are requested to consider this paper as preliminary research article, as authors wanted to publish the initial data as early as possible. Authors are aware that detailed statistical analysis is required to get a scientifically established conclusion. Readers are requested to use the conclusion of this paper judiciously as statistical analysis is limited. Authors also recommend detailed statistical analysis for similar future studies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27696