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Original Research Article

Telfairia Occidentalis as a Phytotherapeutic Agent for Chemical (Phenylhydrazine) Induced Anaemia

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Abstract: ***Introduction***: Anaemia is a medical condition characterized by a decrease in the number of red blood cells and the amount of haemoglobin in the blood leading to inadequate oxygen delivery to tissues and organs. *Telfairia occidentalis* is a dietary leafy vegetable with phytochemicals that are beneficial to human health. ***Methodology*:** Anaemia was induced in rats by oral administration of phenylhydrazine and subsequently followed by administration of aqueous extract of T. occidentalis at 100mg and 200mg/kg respectively. At the expiration of the study, blood samples were taken from the animals for evaluation of red blood cell parameters. ***Results***: The red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration of the Non extract anaemic group were significantly depressed. The corresponding values for the extract groups were similar to those of the control. ***Conclusion***: The extract of Telfairia occidentalis was able to reverse the anaemia induced by oral administration of phenylhydrazine in animal model.

Keywords: Phenylhydrazine, anaemia, Telfairia occidentalis.

INTRODUCTION

Anaemia is a medical condition characterized by a decrease in the number of red blood cells and the amount of haemoglobin in the blood, leading to inadequate oxygen delivery to tissues and organs. Anaemia has a very high prevalence world wide and more worrisome amongst women in reproductive age especially those from low and medium income countries [1]. Anaemia has several causes that form the basis for classification. One of the classes is haemolytic anaemia and this is characterized by excessive destruction of red blood cells. This type may be due to genetic error as exemplified by sickle cell hemoglobinopathy or ingestion of chemical substances such as drugs and alcohol. Phenylhdrazine has been used to induce haemolytic anaemia in animal model [2].

Telfairia occidentalis also known as fluted pumpkin, is a tropical vine plant primarily cultivated for human nutrition but has some medicinal use in herbal medicine in West Africa. Common name for the plant include fluted gourd and fluted pumpkin. In South East Nigeria (Igbo), it is known as ugu; while among the Efik and Ibibio of South-South Nigeria, it is called ikong-ubong. *T. occidentalis* is a member of the family of Cucurbitaceae and its indigenous to Southern Nigeria [3]. Although the fruit is inedible, the seeds produced by the gourd are high in protein and fat, and can therefore contribute to a well-balanced diet. The plant is a drought-tolerant, dioecious perennial that is usually grown trellised. Its leaf extract has been reported to possess antioxidant, anti-inflammatory and hematopoietic properties [4].

Anaemia is a major cause of morbidity and mortality in children, pregnant women, nursing mothers especially in Sub-Saharan Africa that is plagued with malnutrition and undernutrition sequel to food and nutritional insecurity that are potentiated by poverty, armed conflicts and insurgencies.

Management of anaemia in human include iron supplements (oral and injectable), vitamins, transfusion of blood or its components, bone marrow transplant. All these therapeutic modalities are not that affordable especially in Low and Middle Income Countries. Thus the need to find affordable and readily accessible alternatives becomes pertinent. Most of the edible plants be the leaves, stems, fruits, seeds or roots contain phytochemicals that are of medicinal benefits.

Phytochemicals such as flavonoids and phenols are known to stimulate and promote erythropoiesis [5]. The phytoconstituents of *Telfairia occidentalis* include terpenoids, flavanoids, saponins, tanins, sterols and alkaloids. These phytochemicals make consumption of *T. occidentalis* safe as a dietary supplement [6, 7].

In an effort to find an alternative naturally occurring blood boosting alternative, we decided to elucidate the hematinic propensity of the leaves of *T. occidentalis* in wistar rats with chemical (phenylhrdazine) induced anaemia hence the justification of this study.

MATERIALS AND METHODOLOGY

Area of study

The research study was carried out in the animal holding facility located in the Department of Human Anatomy, Faculty of Basic Medical Science, Chukwuemeka Odumegwu Ojukwu University, Uli campus, Anambra State.

Plant Materials

Plant collection: Farm fresh leaves of Telfairia occidentalis were sourced from Nkwo market being a major grocery outlet situated in Nnewi, Anambra State.

Preparation of aqueous extract: The leaves were washed and subsequently left to dry till nil moisture at room temperature. The dried leaves were grinded to obtain a fine textured powder and about 250 g of it was refrigerated till use.

Animals

This study utilized thirty adult Wistar rats weighing 150-200g sourced from the Animal holding facility of the Physiology Department, Nnamdi Azikiwe University, Okohia Otolo, Nnewi in Anambra state, Nigeria. They had an initial two-week acclimatization in an ambient and conducive laboratory environment having unrestricted access to normal rodent ration and water.

Design of the Experiment

The induction of anaemia and the dosage of the *T. occidentalis* extract administered form the bases for allocation into experimental groups. Thus the animals were randomly allotted into five equal groups as detailed below-;

1. Control (CN) –had normal rat feed and water
2. Non-extract anaemic (NEA)- anaemia was induced but extract was not administered
3. Low dose extract only (LE)- had low dose of the extract. Anaemia was not induced.
4. Low dose extract anaemic (LEA)-had low dose of the extract following induction of anaemia
5. High dose extract anaemic (HEA)-had high dose of the extract following induction of anaemia

Induction of Anaemia

Based on empirical evidence in the literature of phenylhydrazine being the chemical of choice for induction of anaemia in laboratory animals; crystalline solution of phenylhydrazine was administered orally at a dose of 60 mg/kg on alternate days for three weeks to three of the of the groups (NEA, LEA & HEA).

Conduct of the Experiment

The control group (CN) had only rat diet with water while the non-extract anaemic group (NEA) had phenylhydrazine solution as stated earlier. The low dose extract group (LE) had oral daily administration of the aqueous extract at 100 mg/kg. For the low dose and high dose extract anaemic groups, the extract was administered after the completion of phenylhydrazine administration at respective dose of 100mg and 200 mg /kg body weight. The extract was administered for twenty-one days. Thereafter, blood samples were collected for haematological analyses which included packed cell volume (PCV), red blood cell (erythrocyte) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Data Analysis and Processing:

The values obtained were analysed with Statistical Package for the Social Sciences (SPSS) version 24 and expressed as percentages, means plus standard deviation of means (SD). The Student t-test was used for intergroup comparison and the level of significance set at $p<0.05$.

RESULT

There were marked differences between the initial and final mean weights of the animals across the groups. The mean weight change (in percentage) was most pronounced in the Non-extract anaemic group (NEA) and least pronounced in the extract groups ie Low dose extract (LE), Low dose extract anaemic (LEA) and High dose extract anaemic (HEA) groups. The percentage mean weight change of groups LE, LEA and HEA were significantly lower to those of the control and NEA groups (Table 1).

The red blood cell (RBC) count of the NEA was significantly lower than that of the control and low dose extract groups. The NEA group had the least packed cell volume (PCV) while the HEA group had the highest

PCV with the difference between the two groups being significant.

Also the haemoglobin concentration of the NEA was the least and in fact significantly lower than those of the Control, LEA and HEA groups. The mean corpuscular volume (MCV) of the NEA group was significantly lower than those of the other groups and the HEA group had the highest MCV. The mean corpuscular haemoglobin (MCH) of the LEA and HEA were significantly higher than that of the control. The mean corpuscular haemoglobin concentration (MCHC) was similar across the groups (Table 1).

Table 1: Mean Values of Red Blood Cell (Erythrocyte) parameters

Parameters	Control (CN)	Non-Extract Anaemic (NEA)	Low Dose Extract (LE)	Low Dose Extract Anaemic (LEA)	High Dose Extract Anaemic (HEA)
Weight Change (%)	25.12	28.41	$11.47^{\alpha\beta}$	$11.35^{\alpha\beta}$	$9.87^{\alpha\beta}$
Red Blood Cell count ($x10^{12}$)/L	7.02±0.39	$4.87\pm0.06^{\alpha}$	$6.78\pm0.60^{\beta}$	6.31±0.28	6.71±0.02
Packed Cell Volume (PVC)- %	40.40±1.28	34.78±0.23	39.93±1.10	41.36±0.84	$45.40\pm1.98^{\beta}$
Haemoglobin Concentration(g/dl)	$14.43\pm0.74^{\beta}$	10.01±0.21	13.43±0.78	$15.00\pm0.21^{\beta}$	$15.47\pm0.52^{\beta}$
Mean Corpuscular Volume (MCV) (fl)	$62.47\pm1.45^{\beta}$	49.01±2.01	$62.33\pm3.84^{\beta}$	$68.03\pm1.48^{\beta}$	$77.53\pm2.60^{\beta}$
Mean Corpuscular Haemoglobin (MCH) (pg)	19.33±0.54	21.25±0.47	23.26±0.76	$25.70\pm0.67^{\alpha}$	$27.10\pm0.34^{\alpha}$
Mean Corpuscular Haemoglobin Concentration (MCHC) (g/dl)	$314.00\pm1.15^{\beta}$	339.33±3.48	$319.67\pm2.91^{\beta}$	$322.67\pm0.88^{\beta}$	$314.33\pm1.86^{\beta}$

α-indicates significant difference from that of the Control group
β-indicates significant difference from that of the Non Extract Anaemic group
CN- Control; NEA- Non-Extract Anaemic; LE- Low dose Extract; LEA- Low dose Anaemic; HEA- High dose Anaemic.

DISCUSSION

Anaemia can cause loss of body weight through reduced tissue metabolism. Also most causes of weight loss can have anaemia as a comorbidity this may be tthrough reduced appetite and food intake, altered digestion and or absorption of nutrients. Thus there is a symbiotic relationship between anaemia and body weight dynamics. Phenylhydrazine being a chemical substance stimulates the release of some proinflammatory cytokines that ultimately resulted in weight loss as exemplified by the non-extract anaemic group that had the highest weight change. The administration of *Telfairia occidentalis* extract resulted in marginal weight change in a dose dependent fashion. This could suggest the ability of the plant extract to reverse the weight loss caused by phenylhydrazine administration.

One of the indices of anaemia is reduction in the number of the red blood cell-RBC (erythrocyte) count. The Non extract anaemic (NEA) group had the least RBC count while the counts for the extract groups were not appreciably different from that of the control group. Thus it could be reasonably deduced that extract of T. *occidentalis* ameliorated the reduction in RBC count occasioned by the administration of phenylhrdazine.

Packed cell volume (PCV) is a measure of the proportion of RBC in a given volume of blood expressed in percentage and is an acceptable laboratory benchmark for the diagnosis of anaemia. The NEA group had the least PCV while both the low and high extract groups (LEA & HEA) had high PCV with that of the HEA even higher than that of the control. This further strengthens the assertion that extract of *T.occidentalis* has erythrogenic propensity.

Haemoglobin is an iron pigment of the erythrocyte. Its haem moiety is responsible for oxygen transport from the lungs to the organs and tissues. Thus the quantity of heamoglobin in the blood is proportionate to the amount of red blood cells in circulation. Thus in anaemic conditions either due to under production or increased destruction of RBC, there will be a concomitant reduction in the amount of haemoglobin. The haemoglobin concentration of the NEA group was significantly lower than those of the control and the low and high extract groups ie LEA and HEA. Thus the aqueous extract of *T.occidentalis* is capable of reversing chemical induced hemolytic anaemic in experimental animal.

The average volume of a red blood cell is known as mean cell volume (MCV) thus shrinkage of red cell as occurs in hemolysed cell will result in reduced MCV. In this study, the MCV of the NEA was significantly lower to those of the control, low extract

only, low extract anaemic and high extract anaemic groups.

The average content of the hemoglobin in a red cell is measured by the Mean corpuscular haemoglobin (MCH). Thus a value that is lower than the control is indicative of anaemia while a value higher than the control is suggestive of the interventional agent being erythrogenic. In this study, the MCH of all the extract groups though higher than that of the control; but only those of groups LEA and HEA were significantly higher.

The concentration of haemoglobin in a given volume of packed red cells is measured by the Mean corpuscular haemoglobin concentration (MCHC). All the extract and control groups had significantly lower MCHC values than that of the control. This might have resulted from the destruction of erythrocyte by the anaemia inducing agent ie phenylhydrazine.

Phenylhydrazine is among the drugs known to induce nonindigenous redox processes in the erythrocytes reacting with haemoglobin and ultimately leading to hemolysis of the red cell. This is the mechanism through which phenylhydrazine causes anaemia [8-11]. Results from this study clearly showed that phenylhydrazine caused hemolytic anaemia as evidenced by the red cell parameters of the NEA (Non extract anaemic) group.

A cocktail extract derived from the roots of perennial plants indigenous to Republic of Korea, China and Japan had been used as therapeutic in animals with phenylhydrazine induced anaemia and pattern of the haematological profile was similar to ours [12].

The vinegar prepared from apple contains an array of bioactive compounds with remarkably high antioxidant potential [13]. This made it to be used as an interventional agent in a related study in which anaemia was induced in rats with phenylhydrazine (PHZ) and subsequently followed by oral administration of apple vinegar [14]. The results of the haematological parameters of that study were similar to ours.

Punarnavadi mandura, an Indian Ayurvedic formulation prepared from different parts of about twenty plants was administered to rats that had PHZ induced anaemia and the results showed the restoration of the haematological parameters hitherto depressed by PHZ [15].

Also, in another similar PHZ induced anaemia study, *Sesamum indicum*, an oil seed was administered as an interventional agent and the restoration on the red cell parameters was similar to what we obtained [16].

In a previous study some years back, in which the aqueous and ethanolic extracts of *T. occidentalis* leaf were administered following induction of benign prostatic hyperplasia in male rat; we documented reduction in the associated prostatitis, shrinkage of the prostate gland and marked reduction in the size of the prostate [17].

Arising from the findings of this study, it is obvious that *T. occidentalis* has an important role in the management of diseases, although caution has to be exercised in extrapolating results of animal studies to human. The phytotherapeutic benefits of T. *occidentalis* may be due to its rich content of antioxidants such as vitamins A and C [3, 18-20].

CONCLUSION

In summary, the aqueous extract of *T. occidentalis* restored the following parameters of the red blood cell-; Packed cell volume, Haemoglobin concentration, Red blood cell count, Mean corpuscular volume, Mean corpuscular haemoglobin and Mean corpuscular haemoglobin concentration that were all depressed following administration of phenylhydrazine in rats. This restoration was independent of the dose of the extract. Thus the extract of *T. occidentalis* is capable of reversing phenylhydrazine induced anaemia. This may be of immense benefit in the non drug management of anaemia in humans since *T.occidentalis* is normally consumed as vegetable thus, it may be formulated to haematinics.

Ethical Approval

The study was carried out with approval of the Institutional Animal Care and Use Committee of Chukwuemeka Odumegwu Ojukwu University. Also the 'Principles of laboratory animal care' as contained in the NIH publication No. 85-23, revised 1985 were duly observed.

REFERENCES

1. Kinyoki, D., Osgood-Zimmerman, A. E., Bhattacharjee, N. V., Local Burden of Disease Anaemia Collaborators, Kassebaum, N. J., & Hay, S. I. (2021). Anemia prevalence in women of reproductive age in low- and middle-income countries between 2000 and 2018. *Nature medicine*, *27*(10), 1761–1782. https://doi.org/10.1038/s41591-021-01498-0
2. Roque, M., D´Anna, C., Gatti, C., & Veuthey, T. (2008). Hematological and Morphological Analysis of the Erythropoietic Regenerative Response in Phenylhydrazine-induced Hemolytic Anemia in Mice. *Scandinavian Journal of Laboratory Animal Science*, *35*(3), 181–190. https://doi.org/10.23675/sjlas.v35i3.149.
3. Akoroda, M. O. (1990). Ethnobotany of Telfairia occidentalis (cucurbitaceae) among Igbos of Nigeria. *Society for Economic Botany*, 44, 29-39.
4. Agte, V. V., Tarwadi, K. V., Mengale, S., & Chiplonkar, S. A. (2000) Potential of indigenous green vegetables as natural sources of fortification of eight micronutrients. *J Food Comp Anal*, 13, 885-891.

5. Fejes, S., Blázovics, A., Lugasi, A., Lemberkovics, E., Petri, G., & Kéry, A. (2000). In vitro antioxidant activity of Anthriscus cerefolium L. (Hoffm.) extracts. *Journal of ethnopharmacology*, *69*(3), 259–265. https://doi.org/10.1016/s0378-8741(99)00171-3
6. Am Doughari, J. H., Human, S. I., Bennade, S., & Ndakidemi, P. A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *Journal of Medicinal Plants Research*, *3*(11), 839- 848.
7. Arowicz, R., Naczk, M., & Shahidi, F. (2000). Antioxidant activity of crude tannins of Canola and Rapeseed hulls. *JAOCS*, 77, 957–961.
8. Shetlar, M. D., & Hill, H. A. (1985). Reactions of hemoglobin with phenylhydrazine: a review of selected aspects. *Environ Health Perspect*, 64, 265-281. doi:10.1289/ehp.8564265
9. Berger, J. (2007). Phenylhydrazine haematotoxicity, *J Appl Biomed*, 5, 125-130.
10. Hashmi, A. N., & Saleemuddin, M. (1996). Phenylhydrazine causes sulfhydryl oxidation and protein aggregation in hemoglobin-free human erythrocyte membranes. *Biochem Mol Biol Int*, 40, 543-550.
11. Itano, H. A., Hirota, K., & Hosokawa, K. (1975). Mechanism of induction of haemolytic anaemia by phenylhydrazine. *Nature*, *256*(5519), 665–667. https://doi.org/10.1038/256665a0
12. Lee, H. W., Kim, H., Ryuk, J. A., Kil, K. J., & Ko, B. S. (2014). Hemopoietic effect of extracts from constituent herbal medicines of Samul-tang on phenylhydrazine-induced hemolytic anemia in rats. *International journal of clinical and experimental pathology*, *7*(9), 6179–6185.
13. Bakir, S., Toydemir, G., Boyacioglu, D., Beekwilder, J., & Capanoglu, E. (2016). Fruit Antioxidants during Vinegar Processing: Changes in Content and in Vitro Bio-Accessibility. *International journal of molecular sciences*, *17*(10), 1658. https://doi.org/10.3390/ijms17101658
14. Ousaaid, D., Ghouizi, A. E., Laaroussi, H., Bakour, M., Mechchate, H., Es-Safi, I., Kamaly, O. A., Saleh, A., Conte, R., Lyoussi, B., & El Arabi, I. (2022). Anti-Anemic Effect of Antioxidant-Rich Apple Vinegar against Phenylhydrazine-Induced Hemolytic Anemia in Rats. *Life (Basel, Switzerland)*, *12*(2), 239. https://doi.org/10.3390/life12020239
15. Rajendran, K., Chellappan, D. R., Ramakrishnan, V., & Krishnan, U. M. (2024). Therapeutic efficacy of Punarnavadi mandura against phenylhydrazine-induced hemolytic anemia in rats. *Journal of Traditional and Complementary Medicine*, https://doi.org/10.1016/j.jtcme.2024.03.017.
16. Prasad, Y.P.S., Hari, P., Shajina, M., Mirshad, P. V., & Rahiman, O. M. F. (2018). Hematinic and antioxidant potential of aqueous extract of Sesamum indicum seeds against phenylhydrazine-induced hemolytic anemia in albino rats. *Natl J Physiol Pharm Pharmacol*, *8*(8), 1092-1096.
17. Ajani, R. S., & Akinsola, R. A. (2016). Telfairia occidentalis Leaf and Seed Extracts as Possible Preventive and Therapeutic Agents for Induced Benign Prostatic Hyperplasia. *European Journal of Medicinal Plants*, *12*(1), 1-11.
18. Oboh, G., & Akindahunsi, A. A. (2014). Change in the ascorbic acid, total phenol and antioxidant activity of some sun-dried green leafy vegetables in *Nigeria*. *Journal of Nutrition Health and Aging*, 18, 29-36.
19. Oboh, G. (2005a). Hepatoprotective property of ethanolic and aqueous extracts of *Telfairia occidentalis* (Fluted Pumpkin) leaves against garlic-induced oxidative stress. *Journal of Medicinal Food*, 8, 560-563.
20. Oboh, G., & Rocha, J. B. (2006) Istribution and antioxidant activity of polyphenols in ripe and unripe tree pepper (*Capsicum pubescens*). *Journal of food Biochemistry*, 31, 456-473.