Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2022.144.151

Research Article
Biochemical and Haematological Effects of *Telfairia occidentalis* and *Amaranthus viridis* Extracts on Anaemic Rats

[1]B.N. Enemchukwu, [2]J.T.C. Onwuatuegwu, [3]A.C. Ofomatah, [4]O.C. Ezeigwe, [5]O.F. Orinya, [4]E.L. Iloanya, [4]C.M. Okeke and [6]E.I. Onyedinma

[1]Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, P.M.B. 1007, Afikpo, Ebonyi, Nigeria
[2]Department of Microbiology, Tansian University, Umunya, Anambra, Nigeria
[3]National Centre for Energy Research and Development, University of Nigeria, Nsukka, Enugu, Nigeria
[4]Department of Applied Biochemistry, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra, Nigeria
[5]Department of Medical Biochemistry, Ebonyi State University, P.M.B. 053, Abakaliki, Ebonyi, Nigeria
[6]Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia, Nigeria

Abstract

Background and Objective: The beneficial use of medicinal plants for many diseases treatment has prompted an investigation of locally acclaimed anti-anaemic vegetables. The biochemical and haematological effects of ethanol leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis* on phenylhydrazine-induced anaemic rats were analyzed using standard methods. **Materials and Methods:** Ethanol leaf extraction employed the use of the Soxhlet extraction technique and through ocular puncture, 2 mL of blood were collected and used for biochemical and haematological analysis. **Results:** Phytochemicals include, tannins (7.65, 4.75), flavonoids (29.38, 33.08), alkaloids (58.33, 51.39), phenolics (78.92, 44.09) and saponins (0.47, 0.52) for *T. occidentalis* and *A. viridis*. The results showed a significant decrease ($p<0.05$) in total cholesterol, low-density lipoprotein and triglyceride, white blood cells at 200 mg kg^{-1} b.wt., of extracts, no significant difference ($p<0.05$) in alanine transaminase, alkaline phosphatase, total and direct bilirubin, urea, creatinine, Na^+, Cl^-, K^+ and BCO_3^- and a significant increase ($p<0.05$) in packed cell volume and haemoglobin count. **Conclusion:** The leaf extracts showed no significant difference ($p>0.05$) in biochemical parameters and the haematological studies significantly boost blood production and could be useful in the treatment of anaemia.

Key words: Anaemic, biochemical, haematological, parameters, phenylhydrazine, leaves, extracts

Citation: Enemchukwu, B.N., J.T.C. Onwuatuegwu, A.C. Ofomatah, O.C. Ezeigwe and O.F. Orinya *et al*., 2022. Biochemical and haematological effects of *Telfairia occidentalis* and *Amaranthus viridis* extracts on anaemic rats. J. Appl. Sci., 22: 144-151.

Corresponding Author: B.N. Enemchukwu, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, P.M.B. 1007, Afikpo, Ebonyi, Nigeria Tel : +2348034009418

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have been beneficial for the management of many diseases due to bioactive compounds called phytochemicals present in them[1].

Vegetables generally are leafly outgrown plants or plants parts that are useful in soup-making which serve as integral parts of the main sources of the human diet. Its consumption provides the quick and cheapest means of enough dietary vitamins, minerals and fibre supplies. Nutrients contained in vegetables when absorbed by the body could be useful for bodybuilding, energy sources, regulatory and provision of protective materials[2].

Traditionally, a great number of medicinal plants and vegetables have been employed for the alleviation of anaemia among which are, *Telfairia occidentalis*, *Amaranthus viridis*, *Cocos nuciferia*[3] and *Tectona grandis*[4].

Telfairia occidentalis also known as fluted pumpkin is a dark green leafy vegetable employed mainly for culinary and folk medicine for the management of many diseases in Nigeria. The aqueous extract has been documented to possess haematinic potential, contain vitamin C and E which are antioxidants that scavenge oxygen free radicals and prevent oxidative stress[5].

The nutritional and mineral composition of this vegetable that forms a crucial part of the human diet has been reported to include, carbohydrates, lipids, proteins, ash, fibres and moisture[5,6], potassium, sodium, calcium, iron, phosphorus, zinc, magnesium and copper[6]. Ethno-pharmacologically, *Telfairia occidentalis* in the form of herbal decoctions/ concoctions serves as a blood tonic for the treatment of sudden attacks of convulsions, pain, malaria and anaemia[7]. The phytochemical contents of the leaves, seed and stem of *Telfairia occidentalis* have been found to contain tannins, flavonoids, alkaloids, saponins, terpenoids, phenolic compounds, phytosterols, anthraquinones, reducing sugars, chlorophyll and glycosides which have been known to exhibit chemo-suppressive activity[7,8]. Eseyin *et al.*[9] using 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical reported a high free radical scavenging activity, two pure compounds and four oily isolates from the seed of *Telfairia occidentalis*. Research has shown that *Telfairia occidentalis* leaves and seeds extracts are used to treat anaemia, convulsion, atherosclerotic cardiovascular disorders, high blood pressure, hyperglycemia, arthritis, liver problems and inflammatory conditions[10].

Amaranthus viridis L. also known as spinach, which is probably native to South America, is eaten as a leafy vegetable among the South Indian populace and the decoction can be used for the treatment of dysentery and inflammation[11]. *Amaranthus viridis* leaves constitute a cheap and rich source of protein, carotenoids, vitamin C and fibre[12], minerals (calcium, zinc and iron)[13].

Phytochemicals are biological active food components mainly found in vegetables, fruits, complete products of grains, nuts and seeds. In as much as there are a large number of phytochemicals, only a very small number of them are of known identity and have been isolated from plants[14,15]. Most common foods' phytochemicals include, flavonoids, alkaloids, saponins, tannins, polyphenols, carotenoids etc[16,17]. Varieties of nutrients of plant origin can give the distinctive and extended source of wealth in finding out a bioactive and a new useful food[18,19].

Phenylhydrazine (PHZ) was the first hydrazine derivative to be characterized[20] and lipid peroxidation of red blood cells, decreased glutathione (GSH) and increased reactive oxygen species (ROS) have been reported to be caused by phenylhydrazine[21]. PHZ activity has also been associated with oxidative damage in haemoglobin and membrane phospholipid and induction of acute obstruction in the circulation of blood[22].

Anaemia is a clinical condition that develops when the rate of bone marrow production of red blood cells is inadequate to meet up with loss or destruction resulting in an insufficient number of red blood cells or their oxygen-carrying capacity to meet physiologic needs[23].

Synergism is the working together of two things to produce an effect greater than the sum of their individual effects. Traditionally, vegetables could be effective in alleviating anaemia among which are, *Telfairia occidentalis* and *Amaranthus viridis* without any scientific investigation of their synergistic effects. The study aims to investigate the biochemical and haematological effects of a combination of ethanol leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis* on phenylhydrazine induced anaemic rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria from 18th November, 2020 to 30th September, 2021.

Samples procurement and identification: The leaves of *Telfairia occidentalis* and *Amaranthus viridis* were purchased from Eke Market Afikpo, Afikpo North Local Government Area Ebonyi State, Nigeria and were identified and authenticated by the herbarium curator Botanist in the Department of Science Laboratory Technology, Akanu Ibiam

Federal Polytechnic, Unwana, Afikpo, Ebonyi State, Nigeria. Voucher numbers were assigned to the samples and specimens were deposited in the herbarium of the Department for reference purposes.

Pre-extraction preparation of leaf extracts of *T. occidentalis* and *A. viridis*: We thoroughly washed the leaves, rinsed and air-dried at room temperature and blended them to a fine powder using a household electric blender and then filtered with a white 10 mm sieve and then kept for future use.

Phytochemical screening of *Telfairia occidentalis* and *Amaranthus viridis* leaves: A portion of each of the ground crude samples was used for phytochemical analysis following the scientific guide as recommended by Trease and Evans[24].

Extraction of plant materials: Plants materials extraction employed the use of the Soxhlet procedure as recommended by Vijayan *et al.*[25] to obtain the ethanol extracts of the leaves. In this procedure, a 100 g part of the ground crude samples was tied in thimbles and then placed in an extraction chamber that was fitted with a 50 mL capacity round bottom flask containing anti pumping chips to prevent pumping of the solvent during heating. On top of this flask was mounted the condenser and about 250 mL of the solvent (ethanol) was poured into the extractor to soak the sample. Heating the flask with a heating mantle, the solvent evaporated into the condenser mounted on top of the round bottom flask where it condensed and converted into liquid and flowed slowly drops by drops into the extraction chamber containing the sample, promoting extraction. This procedure continued until complete extraction ensures that a clear solvent in the extraction chamber is indicative of complete extraction. The extract was then concentrated in a water bath at 65°C and then reconstituted 1 g in 5 mL of phosphate-buffered saline (PBS) and the combining ratio of 1:1 of the extracts was used for the administration.

Test animals: Thirty adult Wistar Albino rats purchased were used for the experiment. The animals were randomly grouped into six groups of five rats per group and were maintained and housed in cages in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka and were allowed to acclimatize with conditions of the animals housing facility with ambient temperature 26-28°C and adequate ventilation for 1 week before use. Vita growers' mash pellets purchased from Vita Feed Distributor at Awka, Anambra State and clean water fed *ad libitum* were the fed ration for the animals at this period.

Study design: Haematological parameters of the organisms were determined and recorded before the administration of phenylhydrazine at a dose of 50 mg kg^{-1} b.wt., via the intra-peritoneal route (I.P) according to the method outlined in Et Al[26] for two consecutive days.

The Albino rats in their respective cages received normal feeding for 48 hrs after which the Packed Cell Volumes (PCV) of the organisms were determined and the rats with lower than 50% were anaemic and therefore suitable for the study. The organisms were then separated, weighed and grouped into six (n = 6) according to their weights and marked for easy identification. The groups were labelled, A, B, C, D, E. and F. Group A was the normal control (no induction and no treatment), group B was the negative control (anaemic untreated). Group C was the positive control (received 50 mg kg^{-1} b.wt.) of the standard drug (emzeron tonique), groups D, E and F received 100, 200 and 400 mg kg^{-1} b.wt., of ethanol leaf extracts, respectively. Administration with the extracts lasted for 2 weeks and then followed the collection of blood samples through the ocular puncture and then used for the various analysis.

Blood sample collection and determination of biochemical parameters: About 2 mL of blood were collected through the ocular puncture into plain tubes and allowed to clot and were then centrifuged at 3000 g for 10 min, the serum was separated into plain test tubes for biochemical analysis.

The samples were analyzed for lipid profile, Total Cholesterol (TCHOL), High-Density Lipoprotein (HDL-C), Low-Density Lipoprotein (LDL-C) and Triglycerides (TRIG), for liver function parameters the following, Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), also Total Bilirubin (T. Bil.) and Direct Bilirubin (D. Bil.) and then for kidney function, urea, creatinine and electrolytes (Na^+, Cl^-, K^+ and BCO_3^-). All the biochemical analyses were carried out using Vitros-360 auto analyzer (model SE-410, Germany) according to the manufacturer's instruction for use. This system makes use of a thin film analyzer that uses "dry reagent" spread in an extremely thin layer on a plastic slide to which the sample is added. When light is incident on the slide, light passes through different layers of the slide, namely the spreading layer, reagent layer, indicator layer and support layer. The amount of light that enters the slide is different from the amount that leaves the slide due to the absorption of light

at the reagent layer. The difference in light intensity is directly proportional to the quantity of analyte present in the sample that was useful in computing the value of the analyte. Each biochemical parameter uses a different slide with the apparatus.

Blood sample collection and determination of haematological parameters and indices: About 2 mL of blood samples were collected by "ocular puncture", placed in tubes containing dipotassium Ethylene Diamine Tetraacetic Acid salt (EDTA) and mixed thoroughly. The samples were analyzed for Packed Cell Volume (PCV), haemoglobin concentration (HB), White Blood Cell (WBC), Mean Cell Haemoglobin Concentration (MCHC), Neutrophils (Neut), lymphocytes (Lymp) and monocytes (Mono) using an auto-analyzer (sysmex XE-2100 haematology automated analyzer Germany). This machine performs haematological analysis according to the radio frequency/direct-current detection method. Direct current resistance (DC) and the density of the blood cell interior detected the size of blood cells by changes in Radio Frequency (RF).

Statistical analysis: The data obtained from the experiment were statistically analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 25 (SPSS Inc., Chicago, Illinois, USA) and the results were presented as Mean±Standard mean of Error (SEM). Students t-test was used to analyze the results to determine the existence of a significant difference between the mean of the treated group and the control and statistical significance was taken at $p<0.05$ confidence level.

RESULTS

The phytochemical screening results shown in Table 1 indicated the following phytochemicals of *Telfairia occidentalis* and *Amaranthus viridis* leaves determined on a dry weight basis (g/100 g sample), Tannins (7.65, 4.75), flavonoids (295.38, 33.08), alkaloids (58.33, 51.39), phenolics (78.92, 44.09±1.20) and Saponins (0.479, 0.526) for *Telfairia occidentalis* and *Amaranthus viridis*, respectively.

The results of the effects of ethanol leaf extracts on the body-weight of phenylhydrazine-induced anaemic rats shown in Table 2 revealed a decrease in weight in all the groups after the phenylhydrazine induction of anaemia. In weeks one and two of the experiment, no significant difference ($p<0.05$) in weight was noticed in groups C (standard drug) and D (100 mg kg^{-1} b.wt., of extracts) treated groups, respectively compared to the anaemic untreated group. However, within this period, a significant increase ($p<0.05$) in weight was recorded in groups D (200 mg kg^{-1} b.wt., of extract) and F (400 mg kg^{-1} b.wt., of extract) treated groups, respectively compared with the anaemic untreated rats.

Lipid profile analysis: The results of the lipid profile analysis shown in Table 3 revealed a significant decrease ($p<0.05$) in all the groups treated with the extracts and the standard drug in total cholesterol, low-density lipoprotein cholesterol and triglyceride concentrations compared with the anaemic untreated group (B). High-density lipoprotein cholesterol concentration significantly increased ($p<0.05$) in the extract-treated groups compared with the anaemic untreated group.

Liver function parameters: The results of the liver function analyses presented in Table 4 showed no significant difference ($p<0.05$) in Alanine transaminase, Alkaline phosphatase enzyme concentrations, total bilirubin and direct bilirubin levels in the treated groups compared with the anaemic untreated group. Aspartate transaminase enzyme concentration recorded a significant decrease ($p<0.05$) compared with the anaemic untreated group (Table 4).

Kidney function parameters: Treatment with ethanol leaf extracts of *T. occidentalis* and *A. viridis* and the standard drug showed no significant difference ($p<0.05$) in urea, creatinine, Na^+, Cl^-, K^+ and BCO_3^- levels compared with the anaemic untreated group (B). However, a significant increase ($p<0.05$) in urea, creatinine and BCO_3^-, was observed in the group treated with 400 mg kg^{-1} b.wt., of the extracts compared with the anaemic untreated group (Table 5).

Table 1: Phytochemical screening of ethanol leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis* determined on a dry weight basis

Parameters	*Telfairia occidentalis* (g/100 g samples dry wt.)	*Amaranthus viridis* (g/100 g samples dry wt.)
Tannins	7.65±1.20	4.75±2.10
Flavonoids	29.38±0.50	33.08±2.05
Alkaloids	58.33±1.50	51.39±1.00
Phenolics	78.92±2.10	44.09±1.20
Saponins	0.48±1.20	0.53±0.20

Values are the Mean±SD of duplicate determinations

Table 2: Effects of ethanol leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis* on the body-weight of phenylhydrazine-induced anaemic rats

Groups	Initial weight (g)	Day 0 b.wt. (g)	Week 1 b.wt. (g)	Week 2 b.wt. (g)
A	86.80±3.03	87.8±3.77	93.80±3.27	99.60±3.05
B	87.20±5.54	82.67±2.08	83.00±1.00	85.50±3.54
C	87.40±5.98	82.30±3.21	84.00±4.36$^{\#}$	86.30±6.11$^{\#}$
D	87.20±10.13	83.10±4.92	84.00±3.65$^{\#}$	87.30±6.11$^{\#}$
E	87.00±5.98	82.30±3.21	89.00±4.36*	96.30±6.11*
F	87.40±4.34	83.00±5.51	93.00±4.36*	115.30±3.79*

Values are the Mean±SD of duplicate determinations, *Significant increase at p<0.05 compared to the anaemic untreated group and $^{\#}$No significant difference at p<0.05 compared to the anaemic untreated group

Table 3: Lipid profile of phenylhydrazine induced anaemic rats treated with ethanol leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis*

Groups	TCHOL (mmol L^{-1})	HDL-C (mmol L^{-1})	LDL-C (mmol L^{-1})	TRIG (mmol L^{-1})
A	4.20±0.20	2.27±0.17	1.73±0.20	2.50±0.44
B	4.30±0.26	2.13±0.20	1.87±0.15	2.67±0.12
C	2.27±0.10$^{\#}$	3.40±0.30*	0.77±0.06$^{\#}$	1.53±0.12$^{\#}$
D	2.40±0.10$^{\#}$	3.33±0.15*	0.60±0.10$^{\#}$	1.33±0.15$^{\#}$
E	2.07±0.15$^{\#}$	3.57±0.11*	0.70±0.10$^{\#}$	1.37±0.56$^{\#}$
F	2.20±0.17$^{\#}$	3.99±0.30*	0.57±0.12$^{\#}$	1.40±0.10$^{\#}$

Values are the Mean±SD of duplicate determinations, *Significant increase at p<0.05 compared to the anaemic untreated group, $^{\#}$Significant decrease at p<0.05 compared to the anaemic untreated group, TCHOL: Total cholesterol, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol and TRIG: Triglycerides

Table 4: Liver function parameters of phenylhydrazine induced anaemic rats treated with ethanol leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis*

Groups	ALT (IU L^{-1})	ALP (IU L^{-1})	AST (IU L^{-1})	T. Bil (mg dL^{-1})	D. Bil (mg dL^{-1})
A	27.67±0.57	41.50±9.19	22.33±1.53	11.33±1.15	3.33±0.58
B	28.33±1.52	39.00±1.00	32.67±3.21	12.67±0.58	3.67±1.15
C	28.333±1.52^{a}	40.33±3.22^{a}	22.00±1.00$^{\#}$	10.67±0.58^{a}	3.00±0.00^{a}
D	26.33±0.57^{a}	38.00±1.00^{a}	20.33±1.53$^{\#}$	11.00±1.00^{a}	3.33±0.58^{a}
E	27.33±0.57^{a}	38.00±2.00^{a}	22.67±1.59$^{\#}$	11.00±1.00^{a}	3.33±0.58^{a}
F	28.66±1.52^{a}	41.33±3.51^{a}	24.00±2.64$^{\#}$	10.67±0.57^{a}	3.00±0.00^{a}

Values are the Mean±SD of duplicate determinations, $^{\#}$Significant decrease at p<0.05 compared to the anaemic untreated group, ^{a}No significant decrease at p<0.05 compared to the anaemic untreated group, ALT: Alanine transaminase, ALP: Alkaline phosphatase AST: Aspartate transaminase, T. Bil: Total bilirubin and D. Bil: Direct bilirubin

Table 5: Kidney function parameters of anaemic rats treated with ethanol leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis*

Groups	Urea (mmol L^{-1})	Creatinine (mmol L^{-1})	(Na^{+}) (mmol L^{-1})	(Cl^{-}) (mmol L^{-1})	(K^{+}) (mmol L^{-1})	(BCO_3^{-}) (mmol L^{-1})
A	4.90±0.76	0.83±0.67	135.00±0.58	96.00±1.15	3.90±0.31	21.33±0.33
B	4.40±0.57	0.77±0.03	136.33±0.88	96.67±1.20	3.60±0.12	22.67±0.33
C	4.67±0.41^{a}	0.73±0.03^{a}	137.67±1.45^{a}	97.33±1.45^{a}	3.97±0.15^{a}	23.00±1.15^{a}
D	4.40±0.81^{a}	0.90±0.58^{a}	137.33±2.19^{a}	96.67±2.60^{a}	4.10±0.26^{a}	23.00±1.15^{a}
E	4.27±0.18^{a}	0.63±0.03^{a}	135.67±0.67^{a}	96.67±1.76^{a}	3.50±0.06^{a}	23.00±1.52^{a}
F	6.70±0.25*	1.97±0.03*	137.67±2.22^{a}	96.00±1.00^{a}	3.77±0.33^{a}	26.33±1.20*

Values are the Mean±SD of duplicate determinations, * Significant increase at p<0.05 compared to the anaemic untreated group and ^{a}No significant decrease at p<0.05 compared to the anaemic untreated group

Table 6: Haematological parameters of anaemic rats treated with ethanol leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis*

Groups	PCV (%)	HB.C (g dL^{-1})	MCHC (% dL g^{-1})	WBC (×109 L^{-1})	NEUT (%)	LYMP (%)	MONO (%)
A	51.67±0.88	16.33±0.67	32.60±0.82	23.40±5.96	31.67±5.67	76.00±5.51	2.33±0.33
B	41.67±0.88	14.20±0.23	33.80±0.44	12.77±1.24	20.67±5.60	77.00±5.36	3.00±0.58
C	50.00±1.73*	16.50±0.51*	33.00±0.35	15.37±3.10*	28.67±1.20*	78.00±2.08	2.33±0.88
D	53.33±4.26*	16.20±0.99*	32.40±0.24	17.97±3.50*	23.67±5.78*	77.33±3.18	3.00±0.58
E	52.00±2.08*	17.07±0.58*	32.83±0.24	7.97±1.37$^{\#}$	26.00±2.65*	78.07±3.18	5.33±0.67*
F	50.67±2.33*	16.80±0.72*	33.17±0.23	14.97±2.94*	22.67±2.33*	76.33±1.76	3.00±0.58

Values are the Mean±SD of duplicate determinations,*Significant increase at p<0.05 compared to the anaemic untreated group, $^{\#}$Significant decrease at p<0.05 compared to the anaemic untreated group, PCV: Packed cell volume, HB.c: Haemoglobin count, MCHC: Mean corpuscular haemoglobin concentration, WBC: White blood cells, NEUT: Neutrophils, LYMP: Lymphocytes and MONO: Monocytes

Haematological analysis: The haematological analyses results presented in Table 6 revealed a significant increase (p<0.05) in the packed cell volume, haemoglobin concentration, white blood cells and neutrophils in the treated groups compared with the anaemic untreated group. However, a significant decrease (p<0.05) in white blood cells was observed in

200 mg kg^{-1} b.wt., of the extracts treated group. The results also recorded no significant difference ($p<0.05$) in mean cell haemoglobin concentration and the lymphocytes at all extracts concentrations compared with the anaemic untreated group. Monocytes showed a significant increase ($p<0.05$) in 200 mg kg^{-1} b.wt., of the extracts treated group compared with the anaemic untreated group (Table 6).

DISCUSSION

The phytochemical analysis is a veritable tool in the bioactive component evaluation of fruits and other plants parts. Phenylhydrazine activity has been associated with lysis of red blood cells and free radicals' production resulting in oxidative damage in haemoglobin, lipid peroxidation, membrane phospholipid degradation and induction of acute obstruction in the circulation of blood[22,27].

The present study undertakes to investigate the biochemical and haematological effects of a combination of ethanol leaf extracts of *T. occidentalis* and *A. viridis* on phenylhydrazine-induced anaemic rats. The phytochemical screening results indicated that *T. occidentalis* and *A. viridis* leaves contain an appreciable amount of bioactive pharmacological ingredients such as phenolics, alkaloids and flavonoids.

Flavonoids possess anti-inflammatory, anti-hypertensive and anti-microbial activities as reported by Cushnie and Lamb[28]. The presence of flavonoids in *Amaranthus hybridus* has been reported by Akubugwo *et al.*[29] which justifies the use of the plant in the treatment and management of hypertension[30].

The results showed that the induction of anaemia by phenylhydrazine had a decrease in body weight effect on the experimental organisms. However, in weeks one and two of the experiment, no significant difference ($p<0.05$) in body weight in groups C (standard drug) and D (100 mg kg^{-1} b.wt., of extracts) were observed. Groups E (200 mg kg^{-1} b.wt., of extract) and F (400 mg kg^{-1} b.wt., of extract) recorded a significant increase ($p<0.05$) in body weight compared to the anaemic untreated rats. This significant increase ($p<0.05$) in body weight as indicated in the extracts treated groups were observed to be dose-dependent (Table 2).

Lipid profile analysis: The results of lipid profile analysis shown in Table 3 revealed a significant decrease ($p<0.05$) in all the groups treated with the extracts and the standard drug in total cholesterol, low-density lipoprotein cholesterol and triglyceride concentrations compared with the anaemic untreated group (group B). This result agrees with the work of Igbodaro and Omole[31], who reported the anti-cholesterolemic potential of plant extracts that are useful in lipid peroxidation management. High-density lipoprotein cholesterol concentration significantly increased ($p<0.05$) in the extract-treated groups compared with the anaemic untreated group. The capability of causing a significant increase ($p<0.05$) in HDL and a significant decrease ($p<0.05$) in LDL levels by the combination of the extracts of *T. occidentalis* and *A. viridis* portrays them as a potential candidate for HDL/LDL level regulation in living organisms.

The results of the liver function analyses shown in Table 4 showed no significant difference ($p<0.05$) in alanine transaminase, alkaline phosphatase, total and direct bilirubin levels and a significant decrease ($p<0.05$) in aspartate transaminase levels in all the treated groups compared to the anaemic-untreated group. The ethanol leaf extracts did not cause any disruptive effect on the liver as seen from the levels of the alanine transaminase, aspartate transaminase and alkaline phosphatase of the rats treated with these extracts for 14 days (Table 4). These results suggest that the combination of leaf extract of *T. occidentalis* and *A. viridis* modulated the liver function favourably without causing any known damage to the liver.

Treatment with ethanol leaf extracts of *T. occidentalis* and *A. viridis* showed no significant difference ($p<0.05$) in urea, creatinine, Na^+, Cl^-, K^+ and BCO_3^- levels in the treated groups compared to the anaemic untreated group. However, there was a significant increase ($p<0.05$) in urea, creatinine and BCO_3^-, in the group treated with 400 mg kg^{-1} b.wt., of the extract compared to the anaemic untreated group (Table 5). Increased levels of urea, creatinine and BCO_3^- in the group treated with 400 mg kg^{-1} b.wt., of the extract can be an indication of toxicity to the kidney. Creatinine is a metabolic waste released into the blood from the breakdown of a high-energy molecule, phosphocreatine. High levels of serum creatinine can mean spontaneous metabolism of phosphocreatine which is an indication of malfunction of the kidney[32]. Packed cell volume and haemoglobin concentrations bare the major indices for circulatory red blood cells evaluation and are significant in the diagnosis of anaemia, which could serve as a veritable tool for assessment of bone marrow capacity to produce red blood cells[33]. Packed Cell Volume and haemoglobin are major indices for evaluating circulatory red blood cells are significant in the diagnosis of anaemia and serve as useful indices of the bone marrow capacity to produce red blood cell[33]. It is therefore adequate to say that *Telfairia occidentalis* and *Amaranthus viridis* leaves contain bioactive constituents, which stimulated the process of haemopoiesis that have, a direct influence on the

production of red blood cells in the bone marrow. These findings agreed with an earlier work of Taiwo *et al.*[30], who reported increased levels of packed cell volume and haemoglobin concentration by aqueous extract of *Nauclea latifolia*.

Induction of anaemia by phenylhydrazine had a decrease in body weight as well as the packed cell volume levels effects on the experimental organisms indicating that a decrease in body weight is one of the indices of anaemia. The haemopoietic effect exhibited by the extracts in this study is important and therefore holds promise in the formulation of a pharmaceutical regimen that can compete favourably in the treatment of anaemia that is in agreement with the background information concerning its acclaimed anaemia alleviating potential.

CONCLUSION

Telfairia occidentalis and *Amaranthus viridis* leaves contain important phytochemicals such as alkaloids, flavonoids and phenolics which justifies their already locally established function in the treatment and management of hypertension. Phenylhydrazine induced had a decrease in body weight effect on the experimental organisms. The study revealed that the leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis* showed no significant difference ($p>0.05$) in most of the biochemical parameters, however, haematological parameters significantly boost blood production and hence the leaves could be useful in the treatment of anaemia and the related ailments.

SIGNIFICANCE STATEMENT

The study reveals the devastating phenylhydrazine induction effect and the potential synergistic effect of the combination of ethanol leaf extracts of *Telfairia occidentalis* and *Amaranthus viridis* that confer haemopoietic effect on the test organisms. The study will guide the scientific researcher towards curbing the devastating effect of phenylhydrazine and discovering what the prospects of this combination are especially in the formulation of a pharmaceutical regimen that can compete favourably in the treatment of anaemia.

ACKNOWLEDGMENTS

The authors wish to acknowledge the entire staff of Joe-Medics Diagnostic Laboratory, Nnewi, Anambra State, Nigeria and Mrs. Ify Enemchukwu for assistance and encouragement throughout the research.

REFERENCES

1. Fasuyi, A.O., 2007. Bio-nutritional evaluations of three tropical leaf vegetables (*Telfairia occidentalis*, *Amaranthus cruentus* and *Talinum triangulare*) as sole dietary protein sources in rat assay. Food Chem., 103: 757-765.
2. Saidu, A.N. and N.G. Jideobi, 2009. The proximate and elemental analysis of some leafy vegetables grown in minna and environs. J. Appl. Sci. Environ. Manage., 13: 21-22.
3. Tchogou, A.P., M. Senou, T.V. Dougnon, A. Agossadou and F. Assogba *et al.*, 2016. The aqueous extract of *Cocos nucifera* L. (arecaceae) effectively treat induced anemia. experimental study on wistar rats. Int. J. Biol., Vol. 8. 10.5539/ijb.v8n3p1.
4. Diallo, A., M. Gbeassor, A. Vovor, K. Eklu-Gadegbeku and K. Aklikokou *et al.*, 2008. Effect of *Tectona grandis* on phenylhydrazine-induced anaemia in rats. Fitoterapia, 79: 332-336.
5. Idris, S., 2011. Compositional studies of *Telfairia occidentalis* leaves. Am. J. Chem., 1: 56-59.
6. Enujiugha, V.N., T.F. Oluwole, J.Y. Talab and A.I. Okunlola, 2014. Selected bioactive components in fluted pumpkin (*Telfairia occidentalis*) and amaranth (*Amaranthus caudatus*) leaves. J. Exp. Agric. Int., 4: 996-1006.
7. Osukoya, O.A., D. Adegbenro, S.A. Onikanni, O.A. Ojo and A. Onasanya, 2016. Antinociceptive and antioxidant activities of the methanolic extract of *Telfairia occidentalis* seeds. Anc. Sci. Life, 36: 98-103.
8. Eseyin, O.A., M.A. Sattar, H.A. Rathore, A. Ahmad and S. Afzal *et al.*, 2014. Hypoglycemic potential of polysaccharides of the leaf extract of *Telfairia occidentalis*. Annu. Res. Rev. Biol., 4: 1813-1826.
9. Eseyin, O.A., A. Daniel, T.S. Paul, E. Attih and E. Emmanuel *et al.*, 2018. Phytochemical analysis and antioxidant activity of the seed of *Telfairia occidentalis* Hook (Cucurbitaceae). Nat. Prod. Res., 32: 444-447.
10. Akindele, A.J., J.A. Oladimeji-Salami and B.A. Usuwah, 2015. Antinociceptive and anti-inflammatory activities of *Telfairia occidentalis* hydroethanolic leaf extract (cucurbitaceae). J. Medic. Food, 18: 1157-1163.
11. Ashok, K.B.S., K. Lakshman, K.N. Jayaveera, R. Nandeesh, B. Manoj and D. Ranganayakulu, 2010. Comparative *in vitro* anthelmintic activity of three plants from the amaranthaceae family. Arch. Biol. Sci. Belgrade, 62: 185-189.
12. Shukla, S., A. Bhargava, A. Chatterjee, J. Srivastava, N. Singh and S.P. Singh, 2006. Mineral profile and variability in vegetable amaranth (*Amaranthus tricolor*). Plant Foods Hum. Nutr., 61: 21-26.
13. Kadoshnikov, S.I., I.G. Kadoshnikova, Y.A. Kulikov and D.M. Martirosyan, 2008. Researches of fractional composition of protein of amaranth. Curr. Nutr. Food Sci., 4: 196-205.

14. Cao, H., T.T. Chai, X. Wang, M.F.B. Morais-Braga and J.H. Yang *et al*., 2017. Phytochemicals from fern species: Potential for medicine applications. Phytochem. Rev., 16: 379-440.
15. Singh, D. and P.K. Chaudhuri, 2018. A review on phytochemical and pharmacological properties of holy basil (*Ocimum sanctum* L.). Ind. Crops Prod., 118: 367-382.
16. Xiao, J., 2017. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? Crit. Rev. Food Sci. Nutr., 57: 1874-1905.
17. Zhao, C., C. Yang, B. Liu, L. Lin and S.D. Sarker *et al*., 2018. Bioactive compounds from marine macroalgae and their hypoglycemic benefits. Trends Food Sci. Technol., 72: 1-12.
18. Bacanlı, M., S. Aydın, A.A. Başaran and N. Başaran, 2017. Are all phytochemicals useful in the preventing of DNA damage? Food Chem. Toxicol., 109: 210-217.
19. Chen, L., H. Teng, Z. Jia, M. Battino and A. Miron *et al*., 2018. Intracellular signaling pathways of inflammation modulated by dietary flavonoids: The most recent evidence. Crit. Rev. Food Sci. Nutr., 58: 2908-2924.
20. Berger, J., 2007. Phenylhydrazine haematotoxicity. J. Appl. Biomed., 5: 125-130.
21. Amer, J., A. Goldfarb and E. Fibach, 2004. Flow cytometric analysis of the oxidative status of normal and thalassemic red blood cells. Cytometry Part A, 60A: 73-80.
22. Sato, H., T. Sakairi, H. Fujimura, J. Sugimoto, E. Kume, K. Kitamura and K. Takahashi, 2013. Hematological and morphological investigation of thrombogenic mechanisms in the lungs of phenylhydrazine-treated rats. Exp. Toxicol. Pathol., 65: 457-462.
23. Levison, D., R. Reid, A.D. Burt, D.J. Harrison and S. Fleming, 2018. Muir's Textbook of Pathology. 14th Edn., CRC Press, United States, London, ISBN-13: 9780429100505, Pages: 584.
24. Trease, G.E. and W.C. Evans, 2000. Phytochemicals. In: Pharmacognosy, Evans, W.C. (Ed.)., Saunders Publishers, London, ISBN: 9780702026171, pp: 42-44.
25. Vijayan, P., C. Raghu, G. Ashok, S.A. Dhanaraj and B. Suresh, 2004. Antiviral activity of medicinal plants of Nilgiris. Indian J. Med. Res., 120: 24-29.
26. Et Al, O., 2000. Medical Laboratory Science: Theory and Practice. Tata McGraw-Hill Education, New York, ISBN: 97800 74632239, Pages: 1338.
27. Zangeneh, M.M., M. Pooyanmehr and A. Zangeneh, 2017. Evaluation of the anti-anemic potential of *Glycyrrhiza glabra* aqueous extract in phenylhydrazine-treated rats. Iran. J. Pharmacol. Ther., Vol. 15.
28. Cushnie, T.P.T. and A.J. Lamb, 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents, 26: 343-356.
29. Akubugwo, I.E., N.A. Obasi, G.C. Chinyere and A.E. Ugbogu, 2007. Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Afikpo, Nigeria. Afr. J. Biotechnol., 6: 2833-2839.
30. Abidemi, T.A., O.J. Adebayo, O. Idowu and M.O. Agbotoba, 2009. Nutrient content and anti-nutritional factors in shea butter (*Butryospermum parkii*) leaves. Afr. J. Biotechnol., 8: 5888-5890.
31. Ighodaro, O.M. and J.O. Omole, 2012. Effects of Nigerian *Piliostigma thonningii* species leaf extract on lipid profile in wistar rats. ISRN Pharmacol., Vol. 2012. 10.5402/2012/387942.
32. Adepoju, G.K.A. and O.O. Odubena, 2009. Effects of *Mucuna pruriens* on some haematological and biochemical parameters. J. Med. Plants Res., 3: 73-76.
33. Chineke, C.A., A.G. Ologun and C.O.N. Ikeobi, 2006. Haematological parameters in rabbit breeds and crosses in humid tropics. Pak. J. Biol. Sci., 9: 2102-2106.