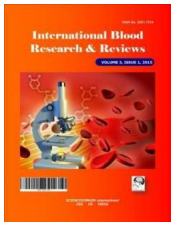

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Evaluation of Hematological and Tissue Weight Changes Associated with Sub Acute Exposure of Rats to *Telfairia occidentalis* Root, Pod and Stem Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Author EAO designed and carried out literature searches, experimental process and compiled manuscript. Author POU supervised research design and experimentation and revised the initial manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The effect of twenty eight (28)-day exposure to aqueous extract of *Telfairia occidentalis* root, pod and stem on hematological parameters of Wistar rats was assessed in this study.
Methodology: Sixty eight (68) wistar rats were separated into 17 groups of 4 animals per group and distilled water, root, pod, and stem extracts of *Telfairia occidentalis* were administered at doses of 250, 750, 1500, 2250, and 3000 mg/kg body weight (bw). Test animals received extracts for 27 days, and sacrificed on the 28th day by jugular laceration under mild chloroform anesthesia. The hematological parameters were determined using standard laboratory protocol.

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Results: The result showed that PCV, Hb and RBC were significantly (P<0.05) reduced when root extract was administered at 1500 mg/kg bw. The extract also caused significant (P<0.05) reduction in WBC at all dose levels, except 750 mg/kg bw. The pod extract caused significant (P<0.05) increase in the PCV, Hb, and RBC when administered at the doses of 250 mg/kg bw and 3000 mg/kg bw. The WBC increased significantly only at 2250 mg/kg bw dose administration of the pod extract, while the PLT count reduced significantly (P<0.05) at 3000 mg/kg bw pod extract. The stem extract, at different doses, significantly (P<0.05) reduced the PCV value without corresponding changes in Hb and RBC. Although insignificant (P>0.05), WBC values increased at all the administered dose levels. *T. occidentalis* root, pod and stem extracts caused insignificant (P>0.05) changes in the kidney, liver and heart weight of experimental animals.
Conclusion: While the root extract of *T. occidentalis* may possess immunomodulatory potentials and may cause anemia at higher concentration, the pod extract may have some antianemic, immune stimulatory and thrombocytopenic effect at higher concentration. Whereas the stem extract may possess antidiuretic potential, none of the extracts showed any sign of organ toxicity at the exposed doses.

Keywords: Hematology; subacute; Telfairia occidentalis; root; pod; stem; immunomodulatory; antianemic; antidiuretic.

1. INTRODUCTION

The use of plants as remedies for ailments has been in existence for centuries [1]. Most plants are known to contain secondary metabolites, which possess biological activities that can be harnessed in drug development. Several disorders are known to affect the blood, which is the medium of transport in most vertebrates. Conditions that affect quality or quantity of blood, and cause the disruption of its biological function include, hemoglobinopathies, thalassemia, anaemia, cytopenia, thrombocytopenia haemophilia, etc. The consequences of these conditions can range from metabolic dysfunction to fatality [2].

Organ toxicity, blood poisoning and other negative drug/herbal interaction may result from consumption of herbs or extracts of plant origin [3]. Some herbs while having efficacy for a particular ailment may impact negatively on the blood composition and function [4]. Thus there is need to screen plants, especially those of potential food and drug, for hematological effects to forestall inadvertent toxicity when in use.

Telfairia occidentalis (Family: Curcurbitaceae), commonly known as Fluted Pumpkin is a common food vegetable plant in Nigeria, West African and sub Saharan African countries [5]. Some workers have reported the nutritional and antioxidant potentials of the leaves [6-8]. The antiplasmodial, antimicrobial, and antidiabetic properties of the leaves have also been reported [9,10,11,12]. The toxicity of extracts of *T. occidentalis* root and leaves has been reported [13,14]. While the leaf and the seed are edible, other parts of the plant are usually discarded for "lack" of nutritional or medicinal importance [15]. There have been recent enquiries into the nutritional and pharmacological potentials of these unused parts of the plant: root, pod and stem [15,16,17]. The effect or otherwise of their extracts on some hematological parameters and organ weight is the subject of investigation in this sub-acute exposure study.

2. MATERIALS AND METHODS

2.1 Plant Sample

Telfairia occidentalis (Voucher # UPH/V/1186) root, pod and stem were sourced in Port Harcourt, Nigeria and identified at Plant Science and Biotechnology Department, University of Port Harcourt, where voucher specimen was deposited. They were washed, chopped into bits and air-dried. Samples were pulverized and 600 g of each, macerated in aqueous solvent for 24 hours. Resulting solutions were filtered and filtrate concentrated at 40℃ to obtain crude extracts which were stored in the refrigerator until used.

2.2 Animals

Sixty eight (68) Wistar rats of both sexes (150-175 g), were obtained from Enugu, and kept in the Biochemistry Department Animal House,

University of Port Harcourt. Animals were placed in wire cages and were acclimatized for two (2) weeks with access to water and normal rat chow ad libitum.

Animals of were divided into 17 groups with 4 animals in each group. Different animal groups received distilled water (2 groups) and different extracts at the doses of 250, 750, 1500, 2250, and 3000 mg/kg body weight. Animals were treated for four weeks after which they were weighed and sacrificed under mild chloroform anesthesia. Blood was collected through vena jugularis into Ethylenediaminetetraacetic acid (EDTA) sample bottle for analysis and the animal organs; heart, liver and kidney, were excised and weighed.

2.3 Hematological Analysis

2.3.1 Packed Cell Volume (PCV) determination

This was determined using micro-haematocrit method according to method described earier [18]. In brief, a plain capillary was filled to about ¾ with well mixed EDTA anticoagulated blood and sealed. The filled capillary was spun in a haematocrit centrifuge for 5 min at a relative centrifugal force (RCF) of 12000. The PCV was read, immediately after centrifuging, in a hand-held haematocrit reader. The reading was expressed in percentage.

2.3.2 Haemoglobin (Hb) concentration determination

This was conducted by light-emitting diode (LED) modified method described earlier [19]. In brief, 20 µl of blood was dispensed into 2 ml of ammonia diluting fluid in a test tube. The tube was covered and the solution mixed. The performance of the meter was checked by inserting the control standard glass in the cuvette aperture. The test tube content was transferred into a clean 1mm light-path cuvette. The cuvette was placed in the cuvette holder and at an audible sound; the haemoglobin value was read from the display.

2.3.3 Red Blood Cell (RBC) count determination

This was determined according to method described before [20]. In brief, 1:200 dilution of blood was made in formal citrate solution. Mixture was allowed to develop by standing for 5 min. Blood was placed in a charged Improved Neubauer counting chamber and incubated for 2 minutes at room temperature. With chamber mounted on microscope, stained cells within the central large square were counted using x10 or x40 magnification.

2.3.4 White Blood Cell (WBC) count determination

WBC count was determined using haemocytometer according to method described before [21]. In brief, to 0.38 ml of diluting fluid was added 20 µl of well mixed EDTA anticoagulated blood and placed in a counting chamber. The chamber was placed in the microscope stage and using the x10 objective, the rulings and the white cells were focused. Then the four large corner squares of the chamber were counted. The number of the white cells per litre of blood was calculated thus:

$$WBC = \frac{\text{Total number of cells counted}}{2 \times 10} \times 10^9$$

2.3.5 Platelet (PLT) count determination

Platelet count was also determined using haemocytometer according to method described earlier [21]. In brief, 0.38 ml of filtered ammonium oxalate diluting fluid was mixed with 20 µl of well mixed anticoagulated venous blood. Mixture was placed in the counting chamber and allowed to stand for 20 min. The chamber was then placed on the microscope stage and using x10 objective to focus the grid rulings and x40 objective to focus the small platelets. The Platelet Count was reported as number of platelets counted multiplied by 10^9.

2.3.6 Differential WBC counts

These were also determined according to method described earlier [21]. In brief, drop of immersion oil was placed in the lower third of the leishman stained blood film. The cells were focused using x10 objective and the staining and distribution of cells were checked. A part of the film where the red cells were just beginning to overlap was located and the x100 objective was brought into place. The blood film was systematically examined and the different white cells seen in each field was counted using a mechanical differential white cell counter.

About a hundred (100) white cells were counted and each cell type expressed as a percentage.

2.4 Statistical Analysis

Analysis of Variance (ANOVA) was used to analyze data obtained and Bonferroni's test was used for Post-hoc comparisons. $P<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

The results of twenty eight (28)-day exposure to *Telfairia occidentalis* root extract on hematological parameters (PCV, Hb, RBC, WBC, PLT, NEU, LYMP and MONO) are shown in Table 1. At all the doses administered, except for 1500 mg/kg bw, the root extract caused insignificant increase or decrease in PCV, Hb and RBC compared with control. Administration 1500 mg/kg bw dose caused a significant ($P<0.05$) reduction in PCV, Hb, and RBC values compared with control. This may indicate toxicity of the extract to the blood. Decreased PCV, Hb concentration and RBC count has been associated with retardation of haemopoesis, destruction and shrinking of RBC and oxidative injury to cell membrane [22,23]. Thus the extract at this dose may lead to anaemia.

The root extract also caused significant reduction in WBC at all dose levels, except 750 mg/kg bw. Reduction in total WBC count has been associated with immunosuppression [24]. Reduction may result from reduced production, redistribution into tissues or destruction of white blood cells [25]. Also bone marrow depression and toxin competition for folic acid utilization has been reported to cause reduced total WBC count [26]. The lowering of WBC count at most of the root extract doses may occur through any of those means.

At all dose levels, the root extract caused insignificant ($P>0.05$) change in the amounts of blood platelet, neutrophil, lymphocyte, and monocyte.

In Table 2, the pod extract caused significant increase in the PCV, Hb concentration, and RBC count when administered at doses of 250 mg/kg bw and 3000 mg/kg bw. Erythrocytes (RBC), PCV and Hb concentration are interrelated [27] and RBC, platelet and WBC originate from the stem cells in the bone marrow [28]. The significant ($P<0.05$) increase in the values of PCV, Hb, and RBC when the pod extract was administered at lowest and highest doses may result from stimulation of growth and differentiation factors in the bone marrow, which causes the formation/ proliferation of blood cells or improved erythropoietin production [29].

The WBC increased significantly only at 2250 mg/kg bw dose of the extract. At all dose levels, the pod extract caused insignificant change in the hematological parameters: neutrophil (NEU), lymphocyte (LYMP), and monocyte (MONO). Thus, the increased WBC count caused by lowest dose administration of pod extract may not be associated with increased production. Hemoconcentration due to dehydration or renal disease has also been reported to cause increased WBC count [29,30,31].

The significant reduction ($P<0.05$) in the value of PLT count at the highest dose administration (3000 mg/kg bw) may indicate antisickling potentials [24] of the pod extract. Increased platelet count has been implicated in vaso-occlusion occurring in sickle cell disease patients [32].

In Table 3, the stem extract, administered at different doses, significantly ($P<0.05$) reduced the PCV value compared to control. The extract had no effect on the Hb concentration and RBC, but although insignificantly ($P>0.05$), increased the WBC values at all the administered dose levels. At all dose levels, the stem extract caused no significant change in the amount of platelet (PLT), neutrophil (NEU), lymphocyte (LYMP), and monocyte (MONO). The significant reduction in PCV without proportional changes in Hb and RBC may be related to hemodilution resulting from antidiuretic effect [29].

Telfairia occidentalis root, pod and stem extracts caused no significant ($P>0.05$) change in the kidney, liver and heart weight of experimental animals (Table 4). Insignificant changes in organ weight have been associated with non toxicity by some workers [33,34]. This is an indication of absence of organ toxicity.

Table 1. Effect of *Telfairia occidentalis* root extract on hematological parameters

Group	Treatment	PCV (%)	Hb (g/dl)	RBC (x10^9)	WBC (X10^9)	PLT (X10^9)	NEU (%)	LYMP (%)	MONO (%)
1	Distilled water	49.5±1.8	16.5±0.7	11.3±0.6	13.0±0 .8	739.3±64.9	19.3±7.0	77.2±7.6	3.5±0.6
2	Root extract (250 mg/kg bw)	47.3±1.2	15.9±0.6	10.0±0.3	8.7±0.4 [a]	524.7±37.6	15.0±2.6	82.0±3.1	3.0±0.6
3	Root extract (750 mg/kg bw	50.0±0.6	16.5±0.3	11.1±0.5	11.9±0.6	584.7±56.6	13.0±3.2	83.7±4.5	3.3±1. 3
4	Root extract (1500 mg/kg bw)	27.0±1.0[a]	7.8±0.1[a]	5.1±0.0[a]	2.7±0.1 [a]	381.0±6.0	17.0±1.0	82.5±2.5	5.0±1.0
5	Root extract (2250 mg/kg bw)	50.7±2.9	16.5±1.0	11.2±1.1	6.8±1.0 [a]	600.5±84.5	15.7±9.2	81.3±10.2	3.0±1.0
6	Root extract (3000 mg/kg bw)	48.5±2.5	15.5±0.9	9.2±0.2	5.7±0.9 [a]	782.7±107.8	17.3±5.3	78.8±6.2	4.0±0.9

n=4; Values are presented as Mean± Standard error mean (SEM). [a]Significant difference (P< 0.05) compared to Group 1

Table 2. Effect of *Telfairia occidentalis* pod extract on hematological parameters

Group	Treatment	PCV (%)	Hb (g/dl)	RBC (x10^9)	WBC (X10^9)	PLT (X10^9)	NEU (%)	LYMP (%)	MONO (%)
1	Distilled water	40.8±0.5	13.5±0.2	8.4±0.2	6.7±1.4	791.0±37.3	6.7±1.3	91.7±1.7	1.7±0.3
2	Pod extract (250 mg/kg bw)	50.0±1.5[a]	16.6±0.3[a]	10.8±0.3[a]	7.2±0.9	695.7±81.1	12.3±1.2	86.8±1.7	2.5±0.3
3	Pod extract (750 mg/kg bw	44.7±1.9	14.8±0.7	9.2±0 .7	8.2±0.7	932.7±15.9	10.05±0.5	87.0±1.0	2.7±0.3
4	Pod extract (1500 mg/kg bw)	44.0±0.7	14.7±0.3	9.2±0.2	9.3±0.3	854.3±44.8	6.7±0.9	91.3±0.9	2.0±0.0
5	Pod extract (2250 mg/kg bw)	39.8±0.6	13.4±0.3	8.2±0.2	14.0±0.4[a]	711.0±28.6	7.25±0.8	90.3±0.9	3.0±0.6
6	Pod extract (3000 mg/kg bw)	46.5±0.5[a]	15.6±0.2[a]	10.0±0.0[a]	6.3±0.6	411.5±67.5[a]	7.0±3.0	90.5±3.5	2.5±0.5

n=4; Values are presented as Mean± Standard error mean (SEM). [a]Significant difference (P< 0.05) compared to Group 1

Table 3. Effect of *Telfairia occidentalis* stem extract on hematological parameters

Group	Treatment	PCV (%)	Hb (g/dl)	RBC (x10^9)	WBC (X10^9)	PLT (X10^9)	NEU (%)	LYMP (%)	MONO (%)
1	Distilled water	40.8±0.5	13.5±0.2	8.4±0.2	6.7±1.4	791.0±37.3	6.7±1.3	91.7±1.7	1.7±0.3
2	Stem extract (250 mg/kg bw)	31.0±0.6[a]	11.5±0.3	7.1±0.2	9.4±0.7	928.7±21.7	8.3±1.5	93.3±1 .2	1.3±0.3
3	Stem extract (750 mg/kg bw	32.7±1.2	13.0±0.7	7.8±0.5	8.3±0.9	755.0±27.6	11.7±1.2	87.0±1.5	1.3±0.3
4	Stem extract (1500 mg/kg bw)	31.8±0.9[a]	12.3±0.3	7.4±0.1	17.2±1.5	869.7±42.2	4.3±0.8	94.8± 0.8	1.0±0.0
5	Stem extract (2250 mg/kg bw)	31.7±0.7[a]	12.3±0.3	7.3±0.2	17.1±1.6	903.7±36.8	14.3±1.7	84. 0±2.0	1.5±0.3
6	Stem extract (3000 mg/kg bw)	36.0±1.0	13.0±0.7	8.1±0.3	20.4±4.9	842.8±40.7	14.7±1.3	84.8±1.8	2.0±0.4

n=4; Values are presented as Mean± Standard error mean (SEM). [a]Significant difference (P< 0.05) compared to Group 1

Table 4. Effect of *Telfairia occidentalis* root, pod and stem extracts on tissue-body weight ratio

Group	Treatment	Tissue weight (g/100 g Body Weight)--								
		Root			Pod			Stem		
		Kidney	Liver	Heart	Kidney	Liver	Heart	Kidney	Liver	Heart
1	Distilled water	0.43±0.03	2.73±0.25	0.38±0.02	0.38±0.04	3.50±0.19	0.42±0.04	0.38±0.04	3.50±0.19	0.42 ±0.04
2	250 mg/kg bw	0.39±0.04	2.98±0.23	0.33±0.01	0.31±0 .04	2.44±0.29	0.32±0.05	0.49±0.05	5.64±0.69	0.47±0. 04
3	750 mg/kg bw	0.37±0.06	2.71±0.41	0.29±0.03	0.38±0 .03	3.25±0.35	0.49±0.06	0.44±0.02	4.14±0.16	0.42±0. 04
4	1500 mg/kg bw	0.43±0.02	2.55±0.05	0.33±0.02	0.36± 0.03	3.09±0.14	0.43±0.02	0.42±0.03	4.75±0.42	0.49±0 .03
5	2250 mg/kg bw	0.47±0.05	3.03±0.34	0.35±0.04	0.32±0.01	2.92±0.17	0.31±0.02	0.50±0.04	4.89±0.32	0.55±0.04
6	3000 mg/kg bw	0.48±0.03	3.04±0.27	0.38±0.03	0.34± 0.02	2.76±0.27	0.37±0.03	0.41±0.04	3.93±0.54	0.37±0 .01

n=4; Values are presented as Mean± Standard error mean (SEM)

4. CONCLUSION

In conclusion, while the root extract of *Telfairia occidentalis* may be immunomodulatory and causing anemia, at higher concentration, the pod extract may be anti-anemic, immune stimulatory and thrombocytopenic at higher concentration. Whereas the stem extract may possess anti-diuretic potential, none of the extracts showed any sign of organ toxicity. The non correlation of dose and effect observed in this study is consistent with an earlier observation [17]. The reported toxicity of *T. occidentalis* root [13,14] could not be ascertained in this study, thus the root, pod and stem may be useful in animal nutrition and medicine. However, further investigations are necessary.

ETHICAL APPROVAL

Author hereby declare that this research, involving animal studies, was carried out after due approval (of research relevance and design, including research ethics in animal handling and patients consent) by the Departmental Board of Postgraduate Studies, Department of Biochemistry, Faculty of Life Sciences, University of Benin, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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