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# Investigation of Haemolytic Properties of Ethanolic Leaf and Seed Extracts of *Telfairia occidentalis* in Wistar Rats

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author AIA conceptualized and designed the study and also wrote the manuscript. Author EOA managed the analyses of the study. Author EOO managed the literature searches. Author UO wrote the protocol while authors OAA and JAE performed the statistical analysis. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** Investigation of haemolytic properties of ethanolic leaf and seed extracts of *Telfairia occidentalis* in Wistar rats is the aim of this study.
**Methods:** Fresh plants of *T. occidentalis* and a seed pod were purchased from Oja-Oba Market in Ibadan, Nigeria and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. The seeds were removed from the pod and were split opened. The white seed and the leaves were air dried at room temperature in an open laboratory space until they were completely dried and were milled into powder. The

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extraction was done using soxhlet apparatus and ethanol as the solvent. Fifteen (15) healthy adult male Wistar rats with body weight between 150 and 163 g were used for this study. They were randomly divided into three groups of five rats each. Animals in group 1 were administered saline solution; those in group 2 were administered *T. occidentalis* leaf extract while those in group 3 were administered *T. occidentalis* seed extract. The administration was done 12 hourly for twenty-eight days at 100 mg/kg body weight via oral route since the plant is consumed orally. At the end of the treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture into heparinized bottles. Haematological parameters were determined using standard methods.

**Results:** A significant increase was observed in the PCV, Hb, RBC, MCV, WBC, lymphocyte of control animals when compared with those treated with leaf and seed extract of *T. occidentalis* respectively at $p<0.05$. However, treatment had no significant difference in the platelet of animals.

**Conclusion:** It is obvious from this study that consumption of the leaf and seed of *T. occidentalis* enhanced various heamatological parameters and would therefore improve the physiological and nutritional status of its consumers. The study has further justified the ethnobotanical use of both leaf and seed of *T. occidentalis* as a blood tonic and antianemic. Instead of focusing on the leaves for its haemolytic properties, the seeds can be used as well.

*Keywords: T. occidentalis; haemolytic properties; ethanolic leaf; seed extracts; anaemia.*

## 1. INTRODUCTION

Haemolysis occurs in many haematologic and non-haematologic diseases and can be defined as the removal of senescent or damaged red blood cells (RBCs) from the circulation [1]. Haemolysis also occurs after transfusion of stored blood. In particular, there is increasing evidence to suggest that increasing the storage period between blood donation and transfusion results in a decrease in RBC recovery and consequently an increase in post-transfusional haemolysis [2,3]. Haemolysis is also spelt as hemolysis. It involves the rupturing (lysis) of red blood cells (erythrocytes) and the release of their contents (cytoplasm) into surrounding fluid (e.g. blood plasma) [4]. Hemolysis may occur in vivo or in vitro (inside or outside the body). One cause of hemolysis is the action of hemolysins, toxins that are produced by certain pathogenic bacteria or fungi. Another cause is intense physical exercise [5]. Hemolysins damage the red blood cell's cytoplasmic membrane, causing lysis and eventually cell death [6].

The affordability of herbs over expensive pharmaceutical drugs to treat diseases among non-industrialized societies is fast becoming revolutionized. In some countries, it has been integrated into the health scheme despite advances in orthodox medicine. It is believed that the natural products if utilized in the correct form and dosage are less harmful than synthetic products, which most often elicit some side effects [7].

*Telfairia occidentalis* (Fluted pumpkin) is one of the popular and widely grown vegetable crops in Nigeria particularly in the eastern part (Anambra, Imo, Enugu, Abia and Ebonyi States) and mid-western areas (Edo and Delta States) and to an appreciable degree in the south western states (Ondo, Ogun, Osun, Ekiti, Oyo and Lagos). It is often referred to as “ugu” [8]. It is a pot-herb cultivated mainly for its succulent young leaves and shoots which are used as vegetables [9]. It is a high-climbing perennial with partial drought tolerance and parenting root system. The crop is grown close to trees, walls, fences and structures on which the shoots are allowed to climb. It could be allowed to creep on the ground or staked [10]. Its scientific classification is as follows: Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Cucurbitales, Family: Cucurbitaceae, Genus: Telfairia, Species: occidentalis [11].

*T. occidentalis* is a vegetable consumed for its medicinal values. Airaodion et al. [13] has reported the phytochemical content and antioxidant potential of its leaves. The presence of pharmacological active compounds was responsible for the analgesic activity in *T. occidentalis* [14]. *T. occidentalis* possesses pro-fertility properties that can be exploited in fish fingerling production by hatchery operators [15] while Kuku et al. [16] equally reported that *T. occidentalis* seeds had a positive effect on nutrient metabolism and the performance of growth in rats. The medicinal values of *T. occidentalis* have been reported on

**Fig. 1. *Telfairia occidentalis* plant with its seed pod [12]**

hematological indices of starter broilers [17], concentration of hemoglobin and packed cell volume [18], lipid peroxidation amelioration and cardiovascular disease reduction [19]. Its hepatoprotective effects on the liver have been reported [20], while Ajani and Akinyemi [21] highlighted its therapeutic efficacy on induced benign prostatic hyperplasia. Furthermore, Airaodion et al. [12] has reported its therapeutic effect against acute ethanol-induced oxidative stress in Wistar rats. Its pharmacotherapeutic effect of on glycemic and lipidemic indexes of alloxan-induced diabetic rats has also been reported [11]. Finally [22], reported that its seeds and leaves supplement had positive effects on biochemical enzymes on lipid profiles in the liver at varying dosages. Several people in Nigeria take the extract of the leaves to boost their blood with little or no regards for the seed. It was on this basis that we decided to compare the effect of the leaves of *T. occidentalis* on haematological parameters with that of its seeds using Wistar rats.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Extraction of Plant Materials

Fresh plants of *T. occidentalis* and a seed pod were purchased from Oja-Oba Market in Ibadan, Nigeria and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. The seeds were removed from the pod and were split opened. The white seed and the leaves were air dried at room temperature in an open laboratory space until they were completely dried. They were milled into powder using an electronic blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the method described by Airaodion et al. [23]. About 25 g of the powder was packed into the thimble of the soxhlet extractor and 250 mL of ethanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle. The solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35ºC with a yield of 1.78 g which represents a percentage yield of 7.12% for the leaves while the seed yielded 2.31 g representing a percentage yield of 9.24%. The extracts were preserved in the refrigerator for further analysis.

### 2.2 Experimental Design

Fifteen adult male Wistar rats with body weight between 150 and 163 g were purchased from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. They were housed in Imrat animal house, Ibadan. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into three groups of five rats each. Animals in group 1 were administered saline solution; those in group 2

were administered *T. occidentalis* ethanolic leaf extract while those in group 3 were administered *T. occidentalis* seed extract. The administration was done 12 hourly for twenty-eight days at 100 mg/kg body weight via oral route since the plant is consumed orally. At the end of the treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture into heparinized bottles. The blood samples were centrifuge for 10 minutes using a bench-top centrifuge (Centromix) and the supernatant plasma was then used for the determinations of the biochemical parameters.

### 2.3 Determination of Haematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to Jain [24], using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis [25]. Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells [26]. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to Jain [24].

### 2.4 Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean ± standard deviation. One way analysis of variance (ANOVA) was used for comparison of the means followed by Turkey's (HSD) multiple comparison test. Differences between means were considered to be significant at $p<0.05$.

## 3. RESULTS

Results of this study are presented in Tables 1 and 2.

## 4. DISCUSSION

Anaemia increases in prevalence and severity as renal function decreases, it becomes much more common at reduced glomerular filtration rate. Depending on the severity, some of the symptoms of anaemia may include: pale skin, fatigue, weakness, loss of appetite, low haematocrit and hemoglobin in a RBC etc. Factors likely to contribute to anaemia in chronic kidney diseases include blood loss, shortened

**Table 1. Effect of ethanolic leaf and seed extracts of *T. occidentalis* on erythrocyte parameters after 28 days of administration in Wistar rats**

| Parameters | Control | Leaf extract | Seed extract |
|---|---|---|---|
| PCV (%) | $37.08\pm3.45^{a}$ | $45.09\pm3.14^{b}$ | $44.70\pm3.77^{b}$ |
| Hb (g/dL) | $9.41\pm0.47^{a}$ | $12.70\pm1.08^{b}$ | $11.29\pm1.36^{b}$ |
| RBC ($X10^{12}$/L) | $6.79\pm0.67^{a}$ | $10.45\pm1.44^{b}$ | $9.42\pm1.06^{b}$ |
| MCV (FL) | $54.65\pm6.80^{a}$ | $43.13\pm3.99^{b}$ | $40.86\pm6.54^{b}$ |
| MCH (pg) | $14.87\pm1.19^{a}$ | $12.15\pm2.18^{ab}$ | $11.79\pm2.27^{b}$ |
| MCHC (g/dL) | $25.38\pm2.74^{a}$ | $30.17\pm3.09^{b}$ | $27.89\pm4.03^{ab}$ |

*Values are presented as Mean±standard deviation, where n = 5. Values with different superscript along the same row are significantly different at p<0.05*

*Legend: PCV = Packed Cell Volume; Hb = Haemoglobin; RBC = Red Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration*

**Table 2. Effects of ethanolic leaf and seed extracts of *T. occidentalis* on white cells parameters and platelets after 28 days of administration in Wistar rats**

| Parameters | Control | Leaf Extract | Seed extract |
|---|---|---|---|
| WBC ($X10^{9}$/L) | $13.02\pm1.96^{a}$ | $20.10\pm0.72^{b}$ | $17.89\pm1.21^{c}$ |
| Lymphocyte (%) | $38.08\pm6.79^{a}$ | $57.10\pm5.42^{b}$ | $46.89\pm4.35^{c}$ |
| Neutrophil (%) | $61.98\pm6.79^{a}$ | $32.90\pm5.42^{b}$ | $53.11\pm4.35^{c}$ |
| Platelet ($X10^{9}$/L) | $418.02\pm1.96^{a}$ | $413.10\pm0.72^{a}$ | $412.89\pm1.21^{a}$ |

*Values are presented as Mean±standard deviation, where n = 5. Values with different superscript along the same row are significantly different at p<0.05. WBC = White Blood Cell*

red cell life span, vitamin deficiencies, the "uremic milieu," erythropoietin (EPO) deficiency, iron deficiency and inflammation [27]. However, the typical "anaemia of chronic renal insufficiency" is a result of a decreased production of red blood cells by the bone marrow. This defect in red blood cell production is largely explained by the inability of the failing kidneys to secrete hormone erythropoietin. This hormone is a necessary stimulus for normal bone marrow to produce red blood cells. Several researchers have reported the beneficial effect of *T. occidentalis* leaves especially its haemolytic effect but very little is known about its seeds. This study is therefore aimed at assessing the effect of both the leaves and seeds on the haematological parameters of Wistar rats.

In this study, a significant increase was observed when the blood levels of erythrocyte parameters (packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), Mean Corpuscular Volume (MCV)) of control animals were compared with animals treated with leaf and seed extract of *T. occidentalis* respectively at $p<0.05$ as presented in Table 1. This is an indication that there may be increased production of red blood cells therefore, suggesting the non-toxic nature of both the leaf and seed extracts to red blood cells at this period of administration. This might be due to the presence of phytochemical content and antioxidant potential of the *T. occidentalis* leaves as reported by Airaodion et al. [13]. Similarly, Daramola et al. [28] has also reported that *T. occidentalis* seed has antioxidant activity which is able to protect against lipid peroxidation and cell damage in ovary tissue. Its action on blood levels of erythrocyte parameters might also be attributed to its antioxidant activity.

The increase in the blood levels of erythrocyte parameters observed in this study might be suggestive that *T. occidentalis* leaf and seed have possible potentials to enhance erythropoietin released from the kidneys, which is the humoral regulator of RBC production and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and haemoglobin (Hb) are very important in transferring respiratory gases [29,30]. This may be due to the high content of iron and proteins in the leaf and seed of the plant. It is therefore possible that consumption of *T. occidentalis* leaves and seeds by humans can help prevent anaemia especially in menstruating and pregnant women. It has also been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia [31], thus, the 28-day treatment with *T. occidentalis* leaves and seed may not have the potential to induce anemia or polycythemia.

*T. occidentalis* leaves have been reported to contain saponins [13]. Oboh [32] has reported that saponins have strong haemolytic properties when ingested orally. Although saponins usually remain in the gastro-intestinal tract, but can sometimes leak into the blood during intestinal mucosa erosion, or damage [33]. Following such a leakage, a number of pathological abnormalties have been identified among which we may include liver damage, haemolysis, respiratory failure, convulsion and coma [34].

In this study, a non-significant difference was observed when the blood levels of erythrocyte parameters of animals treated with leaf extract of *T. occidentalis* were compared with those treated with *T. occidentalis* seed extract. This implies that instead of concentrating on *T. occidentalis* leaves for haemolytic benefit, the seeds can also be used.

The results of this study revealed a significant difference in the white blood cells parameters but non-significant in platelet of control animals when compared with those treated with leaf and seed extract of *T. occidentalis* respectively at $p<0.05$ as presented in Table 2. White blood cells, platelet, neutrophil, and lymphocytes are used to provide useful information for diagnosis in routine clinical practice evaluation of the state of health of a patient. Changes in the haematological system have a higher predicative value for human toxicity [35].

The increase in WBC and lymphocyte counts may be due to the presence of anti-nutritional compounds such as saponins, flavonoids and steroid glucosides in *T. occidentalis* leaves [13]. It has been emphasized that the high percentage of WBC especially lymphocytes are associated with the ability of the animals to perform well under very stressful conditions [36]. This increase in the WBC and percentage lymphocyte counts suggests that the phytochemical compounds present in the extracts elicited stress responses. The effect of both extracts on the total WBC count could be due to the presence of glycosides. This compound has an anti-inflammatory property and so has vital effects on inflammatory processes of some pathological

states such as bacterial infection, malaria and liver diseases [37]. This might also imply that *T. occidentalis* leaves and seeds may strengthen the immune system through many cytokines regulation.

The result on reduced level of neutrophil count caused by the plant extracts may be one of the reasons for the benign ethnic neutropenia experienced by Africans. This probably indicates that the body's ability to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) was compromised [38].

The non-significant difference in platelet count at $p<0.05$ observed in this study may be an indication that leaf and seed extracts of *T. occidentalis* has no effect on the actions of platelet activating factor (PAF) and thus on the blood clotting potentials. It could also be an indication that it does not have the potential to stimulate thrombopoietin production with the hemostatic capability of the blood maintaining the status quo since platelets mediate in the blood–clotting mechanism [39].

## 5. CONCLUSION

It is obvious from this study that consumption of the leaf and seed of *T. occidentalis* enhanced various heamatological parameters and would therefore improve the physiological and nutritional status of its consumers. The study has further justified the ethnobotanical use of both leaf and seed of *T. occidentalis* as a blood tonic and antianemic. Instead of focusing on the leaves for its haematinic properties, the seed can be used as well. Both leaves and seeds of *T. occidentalis* have also shown to have the propensity to boost the immune system due to significant increase observed in the white blood cell parameters in this study.

## 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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