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ORIGINAL RESEARCH

Effects of aqueous leaf extract of *Telfairia occidentalis* on haematological parameters and liver enzymes in male Wistar rats

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Abstract

Background: The leaves of *Telfairia occidentalis* (locally known as Ugu) are widely consumed as part of a staple in the southern region of Nigeria. Its nutritional benefits include its rich mineral contents and antioxidant properties. It has been suggested that the leaf extracts may affect blood parameters.
Objectives: To investigate the effects of aqueous extracts of *T. occidentalis* leaves on haematological indices and liver enzymes in rats.
Methods: Twenty-four male Wistar rats weighing between 150g and 200*g* were used for the study. They were categorized into four groups of six rats each *viz*: high-dose, medium-dose, low-dose, and control groups. The leaf extract was administered in doses of 300mg/kg, 200mg/kg, and 100 mg/kg, respectively, while the control group received distilled water rather than leaf extracts.
Results: There was a dose-dependent decrease in the concentrations of liver enzymes and an increase in blood parameters. There was a significant difference (p = 0.000) in the mean red blood cells countof the control group ($7.5\pm0.2\times10^{12}$/L) compared to the low-dose group ($9.1\pm0.1\times10^{12}$/L), the medium-dose group ($11.7\pm0.2\times10^{12}$/L) and the high-dose group ($13.3\pm0.2\times10^{12}$/L).For the liver enzymes, there was a significant decrease in the mean AST levels in the high-dose group (42.8±3.5 IU/L), **the** medium-dose group (53.7±5.7IU/L) and the low-dose group (68.5±3.5IU/L) were compared to the value for the control group ($88.6\pm2.5\times10^{12}$/L).
Conclusions: Using an animal model, *Telfairia occidentalis* may have hepatoprotective and haemopoietic properties.

Keywords: Haematological indices, Liver enzymes, Red blood cells, Telfairia occidentalis.

Introduction

Plants are important sources of nutrients, minerals, and antioxidants which are of immense benefit to the well-being of humans. They are also considered to have protective and therapeutic properties that are of interest to folks in traditional medicine. [1] Plants contain, in varying compositions, numerous active chemical compounds such as saponins, tannins, alkaloids, flavonoids and other phytochemicals that are responsible for the healing properties of some plants. [2]

Telfairia occidentalis (T. occidentalis) is a leafy plant, with oil-rich seeds; it is grown and consumed in Nigeria as staple vegetables but it is also reputed for its medicinal values. [3] It

is a member of the sub-family Cucurbitaceae and belongs to the Joliffieae. Although it is known by other traditional names, it is popularly called Ugu among the Southeastern people of the country. Besides being consumed as luxuriant vegetables, its seeds can be fermented to prepare a local condiment known as Ogiri. It has been reported that the seeds of this plant may contain 27-31% proteins depending on the species. [4] There are three species of *T. occidentalis*- but these proteins contain little or no sulfur-containing amino acids. [3] Also, the seeds have about 54% fats and oil containing about 61% unsaturated fatty acids. [5]

The leaves of this plant are rich in iron and phosphorus and also contain other vital minerals such as potassium, sodium, calcium, and magnesium, [5] which are essential for normal metabolic functions. The plant is known for its antioxidants properties because they contain significant amounts of vitamins and related compounds such as thiamine, riboflavin, ascorbic acid, and nicotinamide. [6] They are good sources of leucine, an essential amino acid as well as other important amino acids such as aspartate, glycine, and alanine. [6] Interestingly, frequent consumption of *T. occidentalis*, as observed in places where it is a staple, has direct correlations with decreased symptoms of severe Protein Energy Malnutrition, such as kwashiorkor and marasmus. [7]

Not only that the leaves are prepared as soup and salad, but they are also prepared as herbal concoctions and infusions which are used for traditional treatment of malaria, anaemia, convulsion and low sperm count. [7] For instance, the young leaves could be sliced and mixed with coconut water and salt and could be stored in bottles as on-the-shelf herbal medicine. (7) Despite, the benefits of *T. occidentalis* as food and phytomedicine, there is a report that the roots, which contain resin, alkaloids, and saponins are toxic to rats and mice; even the purely extracted alkaloids and saponins from the leaves may be injurious to these animals. [8] Whether such toxicity occurs in humans at a significant level, which may be a cause for concern, remains a question that requires thorough investigations.

The present study addresses the hepatic and haematologic effects of *T. occidentalis* since it is rich in iron. Despite the wide use of *T. occidentalis*, there are contrasting findings in the literature as regards its effects on liver enzymes and haematological parameters. Some researchers reported that *T. occidentalis* caused no significant increase in white blood cells count, [9] while others reported a significant increase in white blood cells count. [10] As such, this study was designed to investigate the possible effects of the aqueous leaf extract of *T. occidentalis* on some liver enzymes and haematological indices in male Wistar rats.

Methods

Preparation of Plant Extract

The fresh leaves of *T. occidentalis* (Ugu) were picked from a local farmland in Ikenne-Remo, Ogun State, Nigeria. The leaves were rinsed to remove sand and other debris. The plant was authenticated by a Botanist in the Department of Botany, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The method of Salman *et al* [11] was adopted for the extraction. The leaves were dried under the shade and were reduced to a powdery form by grinding with an electric blender. Five hundred (500) grams of the powdered sample was soaked in one litre of distilled water for 48 hours; this solution was stirred at intervals, after which it was sieved with a piece of clean white cloth. The filtrate was then evaporated in water-bath at 40^0C to dryness to obtain a solid extract. A stock solution was prepared by dissolving 20g of the extract in 10ml of distilled water to give a concentration of 2000mg/ml.

Handling of Experimental Animals

Twenty-four (24) male Wistar albino rats weighing between 150gand 200gobtained from the Animal House of the Department of Physiology, Olabisi Onabanjo University, Ago-Iwoye, were used for this study. The rats were housed in wire mesh cages under ambient conditions (Temperature 24 - 28^{o}C, 12-hour light, and 12-hour darkness cycles); they were fed with standard rat pellet diet purchased from Animal Care Nigeria Limited and water was administered *ad libitum*. The experimental animals were handled carefully and following the protocols highlighted in the guidelines of the National Institute of Health for Laboratory Animal Care and Use. [12] The ethical approval for the study was provided by the departmental committee on the use of animals for research in the Department of Physiology.

Experimental Design

The experimental design was completely randomized involving the random distribution of the twenty-four rats into four groups of six rats each. The groups were labelled as control, high-dose, medium-dose, and low-dose. The control group received distilled water only while the other three groups were the test groups that received different doses of aqueous extract of *T. occidentalis* by oral means, using an intubator, daily and for 21 days. The low-dose group received 100mg/kg of aqueous extract of *T. occidentalis*, the medium-dose group received 200mg/kg extract and the high-dose group received 300mg/kg of the aqueous extract.

Collection of Blood Samples

Twenty-four (24) hours after administering the last dose of the extract, the animals were anaesthetized using diethyl ether and the blood samples were collected by cardiac puncture after the animals have been sacrificed. The blood samples were kept in heparinized bottles. Subsequently, the samples were centrifuged at 5000rpm for 5 minutes. The plasma was kept in clean specimen bottles placed in ice-bucket and refrigerated at -4^{0}Cuntil they were needed for haematological assays.

Haematological Counts

The tests carried out included: Packed Cell Volume (PCV) and haemoglobin concentration (Hb),red blood cells count (RBC) and white blood cells count (WBC). The results obtained for PCV, Hb, RBC, and WBC were adequate enough to indicate changes observed in the haematological indices of the Wistar rats. The experimental protocols for each of these tests have previously been documented.[13]

Liver Enzyme Assay

Randox™ diagnostic kits (Randox Laboratories Limited, UK.) were used for the quantification of liver enzymes. These included aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in liver homogenate.

Statistical Analysis

Statistical analyses of the data were performed using the SPSS 15 software. [14]The One-way Analysis of Variance (ANOVA) statistical method was used to compare the mean values recorded for the groups. The level of significance was tested at $p< 0.05$ with Games-Howell posthoc test.

Results

The reference ranges of RBC, WBC, and PCV are 8.20- 9.50×10^{12}/L, 3.40- 9.50×10^{9}/L and 42-48% respectively. In Table I, the mean values obtained for RBC, WBC the control and low-dose groups fell within the normal range whereas the mean values for the medium-dose and high-dose groups were higher than the normal range. On the other hand, the mean values of PCV were within the normal range in the control, low and medium-dose groups whereas the mean PCV for the high-dose group was slightly higher.

All the test groups showed significantly different mean values compared to the control group as shown in Tables I and II indicating considerable effects of the aqueous extracts of *T. occidentalis* in reducing the liver enzymes and increasing the haematological parameters in the experimental rats. However, these effects were observed to be dose-dependent considering the mean values obtained for individual groups of increasing doses. Further testing revealed that the effects were not significantly different for all the test groups in this study. Nonetheless, for the red blood cells (RBC) counts (Table I), the high-dose group had the highest RBC counts (13.3±0.2 × 10^{12}/L). This was followed by the mean value for the medium-dose group with RBC value of 11.7±0.2 × 10^{12}/L, and the lowest mean value was recorded in the low-dose group (9.1±0.1 × 10^{12}/L). A similar trend was observed for white blood cells (WBC) across the dose groups in comparison to the control group (6.9±0.3 × 10^{12}/L). The lowest WBC count was recorded in the low-dose group (8.7±0.2 × 10^{12}/L) and this increased to 10.4±0.1 × 10^{12}/L in the medium-dose group and 12.5±0.1 × 10^{12}/L in the high-dose group. The PCV also showed similar trends of progressively increasing mean values with increasing doses with 50.4±1.5%, 45.2±2.2%, and 40.4±2.1% for the high-dose, medium-dose, and low-dose group respectively.

Table I: The mean values of the haematological parameters in the different dose groups of rats treated with *T. occidentalis* and the control group

Groups	*RBC (×10¹²/L)*	*P values*	*WBC (×10⁹/L)*	*P values*	*PCV (%)*	*P values*
Control	7.5±0.2[a]		6.9±0.3[a]		36.6±1.0[a]	
Low-dose	9.1±0.1[b]	0.00	8.7±0.2[b]	0.04	40.4±2.1[b]	0.00
Medium-dose	11.7±0.2[b]	0.03	10.4±0.1[b]	0.02	45.2±2.2[b]	0.02
High-dose	13.3±0.2[b]	0.02	12.5±0.1[b]	0.03	50.4±1.5[b]	0.04

The values in the same column with the same superscripts (different doses) were significantly different at $p<0.05$ from the values with different superscript. The different doses were compared against the control group. The number of replicates, n= 6. RBC – Red Blood Cells count; WBC – White Blood Cells count; PCV – Packed Cell Volume

The reference ranges of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) are 50- 150 IU/L, 10-50 IU/L, 30- 130 IU/L respectively. The mean values of ALP for the control, low, medium and high-doses groups fell within the reference range but when the various dose groups were compared to the control group, there was a significant difference as shown in Table II. The mean values of AST were within the reference range in the various groups except for the high-dose group which was lower than the reference range but when the values for the various dose groups were significantly lower than the mean value for the control group.

Table II shows showed significant differences between the mean values of the blood levels of the enzymes between the control group and the test groups. The mean values of the enzymes decreased in the test groups with the lowest enzyme concentrations in the high-dose group. Specifically, the low-dose group had the highest mean ALP (62.5±3.4 IU/L), but the mean value for the medium-dose group reduced to 51.3±4.6 IU/L. Similarly, the mean values of ALT did not show any contrast to the mean values of ALP as the low-dose group had the highest mean ALT level (52.5±4.6 IU/L), and then progressively declined to 46.2±3.4 IU/L in the medium-dose group and 37.4±4.2 IU/L in the high-dose group. The mean values of AST also followed a similar trend among the dose groups. The high-dose group had the lowest mean AST (42.8±3.5 IU/L) whereas the medium-dose and the low-dose groups had mean AST levels of 53.7±5.7 IU/L and 68.5±3.5 IU/L respectively.

Table II: The mean values of selected liver enzymes (Alkaline Phosphatase, Alanine aminotransferase, and Aspartate aminotransferase) in the different dose groups of rats treated with *T. occidentalis* and the control group

Group	*ALP (IU/L)*	*P values*	*ALT (IU/L)*	*P values*	*AST (IU/L)*	*P values*
Control	78.8±5.4[a]		67.6±3.5[a]		88.6±2.5[a]	
Low-dose	62.5±3.4[b]	0.001	52.5±4.6[b]	0.036	68.5±3.5[b]	0.001
Medium-dose	51.3±4.6[b]	0.000	46.2±3.4[b]	0.044	53.7±5.7[b]	0.000
High-dose	45.6±3.5[b]	0.042	37.4±4.2[b]	0.025	42.8±3.5[b]	0.003

Values in the same column with the same superscripts (different doses) were significantly different at $p<0.05$ from values with different superscript. The different doses were compared against the control group. The number of replicates, n = 6. ALP – Alkaline Phosphatase; ALT – Alanine aminotransferase; AST – Aspartate aminotransferase.

Discussion

Telfairia occidentalis contains certain nutritional and phytochemical constituents which can cause different physiological effects. [4] Some of these chemicals are saponin, alkaloids, tannins, and flavonoids and also antioxidants which scavenge free radicals; thus, reducing oxidative stress. [2] The mineral components of this plant provide added nutritional benefits. Essentially, metabolically important amino acids such as leucine and vitamins are vital for the well-being of organisms, including humans. *T. occidentalis* has very good iron and phosphorus contents. Iron is the vital mineral required for red blood cell formation, for maintaining the integrity and functioning of the haem in transporting oxygen. Phosphorus is vital for bone formation, in nucleic acid, cell membrane, and in energy metabolism. Therefore, it may do humans a lot of good when it forms a part of their regular meal. Decreased incidence of Protein Energy Malnutrition such as kwashiorkor and marasmus among people who consume *T. occidentalis,* [7] is a known nutritional benefit.

This study has indicated that the aqueous extract of *T. occidentalis* caused significant increases in red blood cells, white blood cells, and packed cell volume. These increases in hematological indices may be attributed to the haemopoietic effect of iron, proteins, thiamine, riboflavin, and nicotinamide in the leaves of *T. occidentalis.* [6] The observed increases in the hematological parameters are consistent with the previously reported findings. [2] Therefore, the aqueous extract of *T. occidentalis* could be useful in the management of anaemia or conditions that put high demand on the haemopoietic system such as HIV/AIDS patients receiving antiretroviral medications.

The study also showed that the extract caused a significant decrease in the tested liver enzymes (ALP, ALT, and AST) and this is indicative of the hepatoprotective properties of *T. occidentalis*. The protection of the liver functions by the extract agreed with the findings of Kayode *et al* [17] who reported improvements in hepatic and brain functions in malnourished rats treated with *T. occidentalis*. The outcomes of this study also agree with the report of Ekpenyong *et al* [18] that aqueous leaves extract of the plant decreased the blood levels of ALT and ALP but caused a significant increase in blood AST levels in contrast to what was found in the present study. The same hepatoprotective effect of *T. occidentalis* was observed in oxidative stress-induced animals as reported by another researcher, though with ethanolic extract of the plant administered to the rats. [19]

Although the present study used animal models, the findings could be extrapolated to humans since the findings portend beneficial effects on man. As such, the findings could be applied to patients with anaemia or on drugs causing anemia. Also, the extract of the *T. occidentalis* may be used to treat patients receiving hepatotoxic medications. More studies are required to evaluate the effects of the extracts of *T. occidentalis* on drug-induced anaemia in man.

Conclusions

The present study has demonstrated the medicinal benefits of aqueous extract of *T. occidentalis* which showed improvements in haematological parameters and liver function enzymes.

Authors' Contributions: OIO conceived and designed the study and participated in data analysis. FAS conducted the literature review, participated in data analysis and drafted the manuscript. EEN participated in data collection and literature review, ATK participated in study design while AAA participated in data collection. All the authors approved the final version of the manuscript.
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