Hematological Effect and Toxicity study of Aqueous leaf extract of Telfairia occidentalis on

Albino Rats

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ABSTRACT

Aims: This study investigated the hematological effects and toxicological implications of the aqueous leaf extract of *T. occidentalis* on liver and kidney function indices.

Study Design: Toxicological study

Place and Duration of the study: Department of Biochemistry and Molecular Biology Federal University, Birnn Kebbi, Kebbi State Nigeia, Between July to October, 2024.

Methodology: Twenty (20) albino rats of both sexes were randomized into four (A, B, C and D) different groups of five (5) animals each. Group A was given distilled water, group B was administered with 200mg/kg body weight of aqueous leaf extract of T. occidentalis, Group C was administered 300mg/kg body weight of aqueous leaf extract of T. occidentalis and Group D was administered 400mg/kg body weight of aqueous leaf extract of T. occidentalis orally for 14 days. Theanimals were sacrificed and blood sample was collected for haematological and biochemical analyses. Results: Qualitative phytochemical screening of the aqueous leaf extract of T. occidentalis revealed the presence of Alkaloids, Flavonoids, Phenols, Saponins, Tannins, Terpenoids and Carbohydrate. A significant (p < 0.05) increased in theweight of the experimental rats compared to the control group was observed in all the groups treated with aqueous leaf extract of T. occidentalis. Oral administration of different doses of T. occidentalis to the experimental rats significantly (p<0.05) increased the concentration of RBC, HCT, GRA, PLT, LYM, MCV, MCH, Hb and WBC in a dose dependent manner when compared with the control.Administration of different doses of *T. occidentalis* causes significant decreased (P<0.05) in AST, ALT, ALP, TB and DB levels while significant increased (p<0.05) of TP and albumin concentration were observed when compared with control. Similarly, administration of different doses of T. occidentalis significantly (p<0.05) decreased the concentration of Urea, Creatinine, Na⁺ and K⁺ while the concentration of Cl⁻ was significantly (p<0.05) increased in a dose dependent manner when compared with the control. Conclusion: In conclusion, the findings showed that the aqueous leaf extract of Telfairia occidentalis is relatively non-toxic at acute exposure doses exceeding 5000 mg/kg and exhibits potential anti-anemic effects.

Key words: Haematological, Kidney, Liver, Talfairia occidentalis, Toxicity

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1.0 Introduction

Nigeria possesses a diverse array of genetic resources comprising cultivated, semi-wild, and wild species of crops utilized as traditional vegetables, with various ethnic groups consuming these for a multitude of reasons (Akinola, Olawuy, & Ogunmokunwa, 2020) (ARO & JAYEOLA, 2024). Edible foliage from vegetable plants is incorporated into both supplementary food items and primary dishes (Akindele, Oladimeji-Salami, Oyetola, & Osiagwu, 2018). These leaves may exhibit aromatic, bitter, or neutral flavor profiles; however, they represent the most economical and readily available sources of proteins, vitamins, minerals, and essential amino acids. Furthermore, they contain certain hormonal precursors alongside energy (Olorunfemi, Munavvar, & Hassaan, 2014).

Leafy vegetables confer significant health benefits and serve a crucial role in disease prevention. They provide an invaluable source of dietary components that can be effectively harnessed to enhance and fortify the human body (Mutiu & Abidemi, 2013). These vegetables are characterized by their substantial carbohydrate, vitamin, and mineral concentrations.

The plant *Telfairia occidentalis* Hook. f. (Cucurbitaceae), commonly designated as "fluted gourd" or "fluted pumpkin," is cultivated in West Africa primarily for its leaves and edible seeds (Mutiu & Abidemi, 2013). Within Nigeria, the leaves are consumed across various regions due to their nutritional and medicinal properties (OJO, et al., 2012)(ARO & JAYEOLA, 2024). It is recognized by distinct traditional names: "Ugu" among the Igbos, "Iroko" among the Yoruba, "Ubong" among the Efik, and "Umeke" among the Edo (Saalu, Kpela, Benebo, Oyewopo, Anifowope, & Oguntola, 2010). In traditional medicine, the fresh leaves are employed in the treatment of conditions such as anemia, acute convulsions, and malaria (Mutiu & Abidemi,

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2013). Prior research has documented the hypoglycemic, antinociceptive, and anti-inflammatory properties of extracts derived from *T. occidentals* (Mutiu & Abidemi, 2013). It have anxiolytic and sedatives properties (Mutiu & Abidemi, 2013), blood coagulation (Thomas, et al., 2013), immunomodulatory (Okokon, Dar Farooq, Choudhary, & Antia, 2012), testiculoprotective (Saalu, Kpela, Benebo, Oyewopo, Anifowope, & Oguntola, 2010). Hence, this study aims to investigate the hematological effects and toxicological implications of the aqueous leaves extract of *T. occidentalis* on liver and kidney function indices.

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2.0 Materials and Methods

2.1. Chemicals and Reagents

Chemicals and reagents of analytically grade were used for the experiment.

2.2. Collection of Plant Material and Aqueous Extraction

The fresh foliage of *Telfairia occidentalis* was procured from the vegetable section of the Birnin Kebbi Central Market, situated in Kebbi State. The leaves underwent a rigorous washing process and were subsequently chopped into diminutive fragments, followed by air-drying until a stable weight was achieved. The dry leave was then subjected to milling into a fine powder utilizing an electric blender, resulting in 500 g of the pulverized substance. A quantity of one hundred grams (100 g) of the plant powder was subjected to maceration in 500ml of distilled water for duration of 72 hours. The resultant extract was then decanted and subjected to filtration twice employing Whatman filter paper. The filtrate was concentrated to dryness utilizing a rotary evaporator set at 40°C.

2.3 Experimental Animals

Twenty (20) albino rats of both sexes weighing 100 - 200 g were used for the study. The rats were kept at the animals' house under normal environmental conditions and maintained with free

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access to pelletized growers' feed and access to water ad libitum. The albino rats were allowed to acclimatize for 14 days. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health and the guidelines of the Animal Welfare Act, 1999.

2.4 Experimental Design

Albino rats were used for this study. After acclimatization, all animals were housed in cages at regulated room temperature, allowed daily 12 hour light and dark cycle, feed with pelletized growers feed and allowed free access to water during the experiment.

The animals were grouped into four (4) groups of five (5) rats each.

List 1- treatment details

Groupings	treaments
Group A	Control (Administered distilled water and feeds only)
Group B	Administered aqueous extract of Telfairia occidentalis leaves extract at
	200mg/kg body weight
Group C	Administered aqueous extract of <i>Telfairia occidentalis</i> leaves extract at 300mg/kg body weight
Group D	Administered aqueous extract of <i>Telfairia occidentalis</i> leaves extract at 400mg/kg body weight

2.5 Qualitative phytochemical screening

Five grams (5g) of crude extracts was dissolved in 40 ml of distilled water and thereafter subjected to phytochemical screening through established methodologies (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993).

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2.6 Oral Acute Toxicity Study of Telfairia occidentalis

The median lethal dose (LD50) of the aqueous extract derived from the foliage of *Telfairia occidentalis* was assessed to determine appropriate safe dosages for the appraisal of the aqueous extract's effects. This assessment was executed utilizing the protocol delineated by Lorke (1983). During the preliminary phase, rats were categorized into three cohorts of three individuals each and were administered 10 mg, 100 mg, and 1000 mg of aqueous extract per kg of body weight via oral ingestion. They were monitored for 24 hours for manifestations of toxicity, encompassing alterations in behavior and mortality.

In phase II, three rats were allocated into three separate groups, each consisting of one rat, and were treated with the aqueous extract in accordance with the findings obtained from the initial phase. In alignment with the survivorship observed in phase I, three rats were individually administered 1600, 2900, and 5000 mg/kg body weight of the aqueous extract fraction in the second phase, respectively, and the incidence of death within 24 hours were meticulously documented. The LD50 was derived from the outcomes of the final phase as the square root of the product of the minimal lethal dose and the maximal non-lethal dose, specifically, the geometric mean of the successive doses with recorded survival rates of 0% and 100%(Lorke, 1983).

The mathematical expression of Geometric mean (LD₅₀) = $\sqrt{X} * Y$

Where X and Y represents lowest lethal dose and the highest non-lethal dose respectively.

2.7Hepato and Renal toxicity Effect

Twenty albino rats were divided into four groups of five rats each. The first group received distilled water (0.2ml) as control while group 1 to group 3 received 200mg/kg bw, 300mg/kg bw and 400mg/kg bw of the extract respectively. The animals were sacrificed two weeks after administration to assess the effects of aqueous leaves extract of T. occidentals on biochemical parameters.

2.8 Collection of Blood Sample

After duration of two weeks during which various concentrations of the extracts were administered, the albino rats underwent an overnight fasting period. The rats were subjected to anesthesia by being placed within a sealed container filled with cotton wool that had been saturated with diethyl ether in an inhalation apparatus. The albino rats were euthanized via decapitation, and subsequently, blood specimens were collected and subjected to centrifugation at 4000 ×g for duration of 10 minutes at a temperature of 4°C. The resulting supernatant was preserved at a temperature of 37°C for subsequent biochemical analyses.

2.9 Haematological Analysis

Blood was collected in an EDTA blood sample container. It was gently swirled to mix with the EDTA so as to prevent it from coagulating. The sample container was placed at the seeping inlet of the automated haematological analyser. The automated haematological analyser (Medonic M32S Cell Counter, India) seeped in the blood sample and then analysed haematological parameters like Parked Cell Volume (PCV), Haemoglobin concentration, Red Blood Cells count (RBC), White Blood Cells count (WBC) (neutrophils, lymphocytes, eosinophils, monocytes, T. occedentalisphiles), Haematocrit (Hct), Platelets, Mean Cell Haemoglobin Concentration (MCHC) and Mean Cell Haemoglobin (MCH) was analysed using an automated haematological analyser (Medonic M32S Cell Counter, India).

2.10 Determination of Liver Function Indices

Alkaline phosphatase (ALP) activity was evaluated by the Para-Nitrophenyl phosphate (PNPP) method (Kind & King, 1954) Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) according to the methods (Reitman & Frankel, 1957) Serum Total Protein (TP), Albumin

Total and Direct bilirubin as described by (Tietz, 1976). Assay kits used were obtained from the Randox

2.11 Determination of Renal Function Indices

Urea and creatinine were estimated using the diagnostic kits supplied by Randox. In addition, blood electrolytes levels (sodium, potassium and chloride) were also measured using the Randox kit. The procedure described in each user's manual of the kit was followed while estimating the biomarker parameters.

2.12 Data Analysis

The results were presented as Mean and Standard error means (SEM), and n represents the number of animals used. The differences between means were carried out using one-way analyses of variance (ANOVA) using the statistical software SPSS version 20. The Duncan post Hoc comparison test was used to check differences between individual groups, and mean differences were considered significant when P<0.05.

3.0 Results and Discussion

3.1 Extraction

A 40.6g of dark brownish solid extract with 20% yield was obtained after 72h of extraction with aqueous. It was then labeled as crude extract.

3.2 Phytochemical composition of the crude extract

Table 1 showed the qualitative phytochemical screening of the aqueous extract of T. occidentalis. Alkaloids, Flavonoids, Phenols, Saponins, Tannins and Terpenoids and Carbohydrate were detected in the aqueous leave extract

Table 1: Qualitative Phytochemical Content of Crude Aqueous Extract of *T. occidentalis* Test Crude Extract

Test	Crude Extract
Alkaloids	
Anthraquinones	
Flavonoids	+
Phenols	+
Saponins	+
Steroids	+
Tannins	+
Anthracyanins	-

+

Key: + = detected, - = not detected



3.3. Oral Acute Toxicity Studies

The Median Lethal Dose (LD50) of aqueous extract (leaves) on the tested *albino* rats was presented in Table 2. There was no mortality recorded at 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight respectively. The LD50 of aqueous extract(leaves) was taken to be >5000 mg/kg body weight.

Table 2: LD₅₀ of the aqueous extracts of T. *occedentalis*

	Dosage(mg/kg	g)bw Number	of rats Motality	% Lethality
		used		
Phase 1	10	3	0/3	0
	100	3	0/3	0
	1000	3	0/3	0
Phase 2	1600	1	0/1	0
	2900	1	0/1	0
	5000	1	0/1	0

The LD₅₀ of the extracts was determine to be greater than 5000 mg/kg body weight

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3.4 Effect of T. occidentalis on Body Weight

Figures 1 and 2 display the weight gain observed across the groups in phase I and II. There was an increase in body weight among treated groups (B,C and D) when compared with the control group (A).

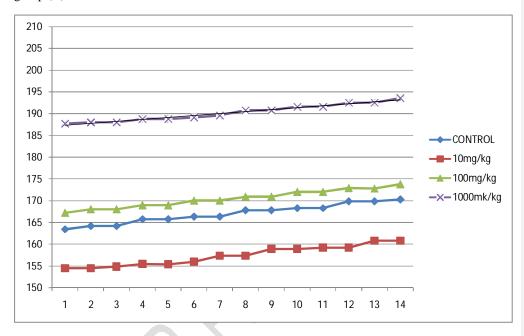


Figure 1: Change in body weight of rats during acute toxicity study of *T. occidentalis* (Phase I)

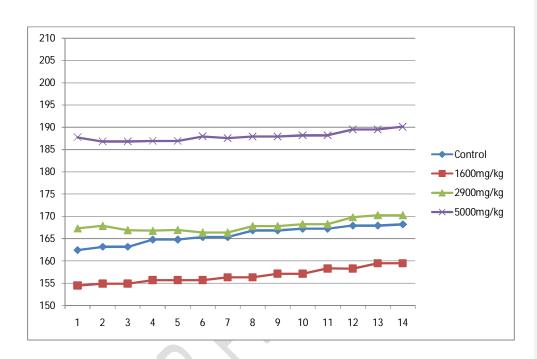


Figure 2: Change in body weight of rats during acute toxicity study of *T. occidentalis* (Phase II)

3.5 Effect of *T. occedentalis* Haematological Indices

Table 3 shows the effect of oral administration of different doses of *T. occidentales* on haematological indices. *T. occidentales* significantly (p<0.05) increased the concentration of RBC, HCT, GRA, PLT, LYM, MCV, MCH, Hb and WBC in a dose dependent manner when compared with control

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Table 3: Effect of Administration of Different doses of T. occidentales on Haematological Indices.

Doses (mg/kg)	RBC (10 ¹² /L)	HCT (g/dL)	GRA (pg)	PLT (fL)	MCHC (g/dL)	LYM (%)	MCV (x10 ⁹ /L)	MCH (x10 ⁹ /L)	Hb (g/dl)	WBC (x10 ⁹ /L)
Distilled Water (0.2ml)	5.9±0.3 ^a	33.4±0.5 ^a	0.3±0.2 ^a	67.4±0.5 ^a	41.3±0.1 ^a	75.4±0.5 ^a	53.6±0.6 ^a	15.2±0.3 ^a	10.6±0.1 ^a	8.3±0.3 ^a
200mg/kg	6.4 ± 0.6^{b}	35.5±0.4 ^b	0.4 ± 0.3^{b}	70.4 ± 0.7^{b}	42.6±0.3 ^b	78.5±0.5 ^b	55.2±0.4 ^b	16.4 ± 0.9^{b}	11.1±0.2 ^b	8.4 ± 0.6^{ab}
300mg/kg	6.6±0.5°	39.7±0.6°	0.5 ± 0.2^{c}	73.4±0.3°	44.3±0.1°	80.6±0.6°	58.3±0.3°	17.4±0.4°	12.2±0.3°	8.6±0.1 ^b
400mg/kg	7.6±0.3 ^d	43.4±0.3 ^d	0.7 ± 0.1^{d}	76.3±0.2 ^d	45.8±0.2 ^d	83.3±0.4 ^d	60.2±0.3 ^d	19.1±0.1 ^d	13.4±0.2 ^d	9.2±0.2 ^c

Values were expressed as mean \pm standard error of mean, n = 3. Mean values having common superscript letters in a column are not significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test). RBC- Red Blood Count, MCHC-Mean Cell Haemoglobin Concentration, MCH- Mean Cell Haemoglobin, MCV- Mean Corpuscular Volume, HGB- Haemoglobin, PCV-Packed Cell Volume, PLT- Platelets, WBC- White Blood Count.

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3.6 Effect of T. occedentalis on Liver Function Indices.

Table 4 shows the effect of administration of different doses of T. occedentalis on liver function indices. T. occedentalis causes significant decreased (P<0.05) in AST, ALT, ALP, TB and DB levels while significant increased (p<0.05) of TP and albumin concentrations were observed when compared with control.

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Table 4: Effect of Oral administration of different doses of *T.occidentales* on liver function indices

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TREATMENT	AST (U/l)	ALT (U/l)	ALP (U/l)	TP (g/dl)	ALB (g/dl)	TB (mg/dl)	DB (mg/dl)
					•		
Distilled Water (2ml/kg)	13.50±0.11 ^c	38.67±1.76 ^b	70.67±0.88°	7.29±0.12 ^a	5.50±0.01 ^a	1.05±0.08°	0.32±0.03 ^b
200mg/kg	12.59±0.12 ^b	35.33±0.88 ^b	68.00±1.73°	7.77±0.13 ^b	5.17±0.17 ^a	0.92±0.04 ^b	0.26±0.01 ^a
300mg/kg	12.35±0.04 ^b	30.33±0.88 ^a	60.33±2.03 ^b	8.00±0.06 ^b	6.53±0.20 ^b	0.86±0.01 ^{ab}	0.25 ± 0.01^{a}
400mg/kg	11.170±0.23 ^a	26.67±0.88 ^a	46.67±1.45 ^a	7.93±0.09 ^b	7.33±0.09°	0.74±0.01 ^a	0.22±0.01 ^a

Values were expressed as mean \pm standard error of mean, n = 3. Mean values having common superscript letters in a column are not significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test). ALP-Alkaline Phosphatase, AST-Aspartate Amino Transferase, ALT-Alanine Amino Transferase, ALB-Albumin, TP-Total Protein, DB-Direct Bilirubin and TB-Total Bilirubin,

3.7 Effect of T. occidentalis on Kidney Function Indices.

Table 5 shows the effect of oral administration of different doses of *T. occidentalis* on kidney indices. *T. occidentales* significantly (p<0.05) decreased the concentration of Urea, Creatinine, Na⁺ and K⁺ while the concentration of Cl⁻ was significantly (p<0.05) increased in a dose dependent manner when compared with the control.

Table 5: Effect of Administration of Different Doses of PLANT on Kidney Function Indices

Doses (mg/kg)	Urea (mg/dl)	Cr (mg/dl)	Na ⁺ (meq/l)	K ⁺ (meq/l)	Cl ⁻ (meq/l)
Distilled Water	27.35±0.04 ^d	6.51±0.04°	147.28±0.03 ^d	4.63 ± 0.03^{d}	104.32±0.01 ^a
(0.2ml) 200mg/kg	24.15±0.02°	6.51±0.02°	146.43±0.06°	4.51±0.03°	104.51±0.07 ^b
300mg/kg	23.20 ± 0.03^{b}	6.31 ± 0.03^{b}	145.57 ± 0.05^{b}	4.30 ± 0.02^{b}	104.54±0.01 ^b
400mg/kg	20.28 ± 0.04^{a}	6.15 ± 0.02^{a}	142.27 ± 0.03^a	4.19 ± 0.02^{a}	104.71 ± 0.03^{c}

Values were expressed as mean \pm standard error of mean (n = 5). Mean values having common superscript letters in a column are not significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test). Cr-Creatinine, Na- Sodium, K- Potassium and CI- Chloride

3.8Discussion

Plants are widely recognized in the pharmaceutical industry for their phytochemical content and diverse pharmacological activities, which can be utilized in the treatment of both chronic and infectious diseases (Junejo, Zaman, Rudrapal, Celik, & Attah, 2021). The preliminary phytochemical screening of *Telfairia occidentalis* revealed the presence of tannins, flavonoids, glycosides, phenols, and terpenoids. These findings are consistent with previous studies conducted by (OJO, et al., 2012), (Ogbonnaya, Anthony, Monago, Comfort, & Chuka, 2010)(Mutiu & Abidemi, 2013). These phytochemicals are known to have various therapeutic applications (Shivashankar, Murali, & Sangeetha, 2019) (Ukwuani-Kwaja, Sani, & Kindzeka, 2021) (Ugwah, 2023). For example, tannins are diverse organic compounds with astringent properties that promote wound healing and soothe inflamed mucous membranes (Orlowski, et al., 2018). Additionally, tannins exhibit antibiotic properties (Motaharesadat, Lalehvash, Leonie, Silvia, Dietmar, & S., 2025). Alkaloids, another class of phytochemicals, are reported to possess analgesic, anti-inflammatory, and adaptogenic activities, which help alleviate pain, enhance disease resistance, and improve stress endurance (Mukta, Naresh, Monica, Reena, Kalvatala, & Bhupinder, 2021).

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In the present study, administering an aqueous leaf extract of *T. occidentalis* significantly enhanced the percentage weight gain in treated rats compared to the normal control group. This effect is likely attributed to the nutrient-rich composition of *T. occidentalis* leaves. These results align with the findings of (Akinola, Olawuy, & Ogunmokunwa, 2020). Furthermore, (Olorunfemi, Munavvar, & Hassaan, 2014) reported that *T. occidentalis* exhibits inhibitory effects on intestinal microbes such as *E. coli* and *S. typhi*, which could enhance gastrointestinal tract efficiency by improving the absorption of digested nutrients.

The acute toxicity of the aqueous leaf extract of T. occidentalis was evaluated through the calculation of its median lethal dose (LD₅₀), defined as the dose required to kill 50% of a test population. The LD $\Box\Box$ was found to be greater than 5000 mg/kg, indicating a wide safety margin in acute toxicity tests conducted on albino rats (Lorke, A new approach to practical acute toxicity testing, 1983). This finding aligns with the study by (Adisa, Nwankwo,, & Osim, 2024) but contradicts the results of (Mutiu & Abidemi, 2013). The observed discrepancies may be attributed to geographical and environmental factors, as well as variations in plant age and harvesting time. Hayes (Hayes, 1989) reported that no dose-related toxicity should be considered above 5000 mg/kg body weight while the Hodge and Sterner Scale of toxicity classes categorized products with LD50 value > 5000 mg/kg as practically non-toxic (DAWOUD, SHAYOUB, & SHAYOUB, 2015)

Results of studies of haematological properties of *T. occidentalis* leave showed a significant of hematological parameters i.e., RBC, Hb concentration, MCV, Mean Corpuscular Haemoglobin MCHC and PLT. This finding agreed with the report of (UDOSEN & OSU, 2022) (Suleiman, Salahuddeen, & Sabiu, 2022) (ALADA, 2000) (Ochokwu, Taiwo, & Bashir, 2021). The increases in the heamatological parameters could be due to the chemical composition of the leaves of T. occidentalis. According to (Ochokwu, Taiwo, & Bashir, 2021) and (OKONWU, AKONYE, & MENSAH, 2018) the nutrient composition of the *T. occidentalis* includes protein, fat, carbohydrate, calcium, iron, vitamin A, vitamin E, vitamin K, vitamin C, thiamine and riboflavin. Most of these constituents are well-known haematological factors that have direct influence on the production of blood from the bone marrow (UDOSEN & OSU, 2022). For instance, iron is a well-established haemopoetic factor and deficiency of it produces anaemia. The haematological activity of *T. occidentalis* lead extracts is thought to be enhanced by these

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compounds. Many bioactive components of *T. occidentalis* such as vitamin C and E, phenolic and flavonoids have been reported to have natural antioxidant potential. According to the (OKONWU, AKONYE, & MENSAH, 2018), vitamin C is a major antioxidant in the human body and possesses the capacity to participate in enzymatic and hydroxylation reactions, participates in the oxidation-reduction processes. It also promotes the absorption of microelements such as iron and copper, involves in trace element metabolism and protects red blood cells from damage caused by free radicals and environmental pollution.

ALT, AST and ALP activities are commonly measured to monitor potential of plant in drug induced hepatic injury in both pre-clinical studies and human patients and thus, they serve as biomarkers of liver toxicity (Meunier & Larrey, 2019). Non-significant increase of serum ALT, AST and ALP activities in albino rats administered with *T. occidentalis* is an indication that *T. occidentalis* has no significant effect on cellular integrity of the liver. A dose dependent decrease in ALT, AST and ALP activities following administration with crude extract of *T. occidentalis* suggests that *T. occidentalis* might possess hepatoprotective potentials. This finding was in line with report of (Osonuga, Faponle, Ezima, Adenowo, & Adelegan, 2020). The outcomes of this study also agree with the report of (Oboh, 2005)(Ekpenyong, Akpan, & Udoh, 2012) (Agada, Odama, & Kenechukwu, 2024) but contradict the report the report of (Ogunmoyole, Oladele, Aderibigbe, & Johnson, 2019) (Eze, et al., 2020).

Albumin and total proteins are globular proteins synthesized by the liver and found in the serum. A decrease in their levels often indicates a reduced synthetic capacity of the liver or impaired hepatocellular function (Yakubu, Bilbis, Lawal, & Akanji, 2003). In the present study, the absence of alterations in serum total protein levels suggests that liver physiology remained unaffected. Bilirubin, a byproduct of hemoglobin breakdown, is processed and excreted by the liver. Elevated bilirubin levels can indicate liver dysfunction or an increased rate of red blood cell (RBC) destruction. This study observed no significant differences in serum total and direct bilirubin concentrations between the control group and the groups treated with *Telfairia occidentalis*. These findings indicate that *T. occidentalis* did not interfere with bilirubin metabolism in the liver.

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The kidneys play a vital role in the elimination of metabolic waste products and toxic substances. When exposed to toxic substances, kidney function can be compromised, leading to the leakage of biochemical substances into the bloodstream (Frąk, et al., 2024). Urea and uric acid, the primary nitrogenous end-products of protein catabolism, along with creatinine, a byproduct of muscle energy metabolism, are transported to the kidneys for excretion. Healthy kidneys effectively remove these compounds from the bloodstream, ensuring their elimination in the urine. In this study, the serum levels of urea and creatinine were unaffected across all groups treated with *T. occidentalis*. This indicates that *T. occidentalis* did not exert any adverse effects on kidney function, suggesting its potential safety for consumption. The outcome of this study agreed with finding of (Akinola, Olawuy, & Ogunmokunwa, 2020).

3.9 Conclusion

The findings indicate that the aqueous leave extract of *Telfairia occidentalis* is relatively nontoxic at acute exposure doses exceeding 5000 mg/kg and exhibits potential anti-anemic effects.

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Comment [DK28]: correct the letters in lower case

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Comment [DK29]: Justify the references, use APA referencing style and write the names of the journals in full