

Liver synthetic ability and hematological profile changes by *Telfairia* occidentalis in carbontetrachloride-induced toxicity in rats

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Abstract

Background: *Telfairia occidentalis* is among the most popular vegetable crops propagated in the West African rainforest zone for its green leafy vegetable and ellipsoidal fruit, which are highly nutritious. This study investigated the liver's synthetic ability and hematological profile potential of T. occidentalis against carbon tetrachloride-induced toxicity in Wistar rats.

Methods: Five experimental groups of rats were used in this study. One group received distilled water and served as the normal control. The second group received carbon tetrachloride (CCl_4) alone for four days. The third and fourth groups received CCl_4 for four days prior to treatment with 200 mg/kg and 400 mg/kg *T. occidentalis* aqueous extract for six days, respectively. The last group received CCl_4 for four days prior to treatment with silymarin (100 mg/kg) for six days. With the exception of normal control rats, all rats received a mixture of freshly prepared CCl_4 in olive oil (1 ml/kg, 1:1 intraperitoneally) for four days. The activities of liver synthetic molecules, such as total protein, albumin, and total bilirubin, as well as hematological parameters, were measured in the blood.

Results: CCl_4 exposure and toxicity caused a significant (P < 0.05) increase in total bilirubin and white blood cells and a significant decrease in total protein, albumin, hemoglobin, hematocrit, red blood cells, and platelets. However, treatment with *T. occidentalis* aqueous extract significantly (P < 0.05) ameliorated the levels of these markers toward normal values.

Conclusion: *T. occidentalis* aqueous extract exhibited enhancement of liver synthetic ability and hematological profile in CCl₄-induced toxicity.

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Introduction

Leafy vegetables, including *Telfairia occidentalis*, are rich sources of phytochemicals that can contribute to improved human health. *T. occidentalis*, popularly called fluted pumpkin or ugwu leaf, remains one of the most consumed leafy vegetables used by nearly all ethnic groups and regions in West Africa, especially Nigeria, for various dishes and side dishes. *T. occidentalis* is consumed in different parts of Nigeria because of its numerous nutritional and medicinal attributes (1), including its traditional usage in the treatment of diseases such as convulsions, gastrointestinal disorders, malaria, and anemia (2,3). *T. occidentalis* is also useful in the management of hypercholesterolemia, liver problems, and impaired immune defense system, as well as in treating heart disease, hypertension, diabetes, and cases of meningitis (4).

CCl₄ treatment is known to invigorate lipid peroxidation, reactive oxygen species generation, and centrilobular necrosis and steatosis (5,6). The toxicity of CCl₄ is dependent on the formation of the trichloromethyl radical (CCl₃) which, in the presence of oxygen, is converted to the trichloromethyl peroxyl radical (CCl₃O₂) which is more lethal than trichloromethyl radicals (7). By interacting with lipids, proteins, and DNA, these radicals cause peroxidative degeneration in a variety of tissues. Therefore, this study was carried out to ascertain the effect of *T. occidentalis* aqueous extract on liver synthetic ability and hematological index in CCl₄-induced toxicity in Wistar albino rats.

Methods

Silymarin, hydrogen peroxide, KMnO₄, epinephrine, thiobarbituric acid, and carbon tetrachloride were purchased from Sigma-Aldrich (USA). Biochemical assay kits were obtained from Randox Diagnostics (Randox, United Kingdom). All other chemicals and reagents were of analytical grade.

Fresh leaves of *Telfairia occidentalis* will be purchased in Benin City, Edo State, Nigeria, and identified. The fresh leaves will be thoroughly rinsed and airdried at room temperature (24°C) and then pulverized, crushed into a fine powder using a manual blender, and weighed. An aqueous extract of the plant will be prepared by soaking 1000 g of the dry powdered plant material in 5 liters of double-distilled water and then keeping it at room temperature for 48 hours (For thorough extraction). At the end of the 48 hours, the extracts will be filtered first through Whatman filter paper No. 42 (125 mm) and then through cotton wool. The filtrate will be concentrated using a rotary evaporator with the water bath set at 40°C to one-tenth of its original volume and then finally with a freeze-drier.

The dried residue (Crude extract) will then be stored at 4°C. Aliquot portions of the crude plant extract residue will be weighed and dissolved in normal saline for use on each day of the experiments.

Adult male albino rats were purchased and allowed to acclimatize for seven days and were maintained under standard conditions, provided with pelleted grower's mash (Containing 18% crude protein and 2600 Kcal/kg metabolizable energy, Guinea Feed, Nigeria PLC) and drinking water *ad libitum*. A daily cycle of 12 hours of light and 12 hours of darkness was provided for the animals. The study was conducted on forty healthy Wistar male albino rats weighing 190-200 g, randomly assigned to five treatment groups of eight (8) rats each. The study was carried out in accordance with the guidelines for ethical conduct in the care and use of nonhuman animals in research (8).

One group received distilled water and served as the normal control. The second group received carbon tetrachloride (CCl₄) alone for four days. The third and fourth groups received CCl₄ for four days prior to treatment with 200 mg/kg and 400 mg/kg *T. occidentalis* aqueous extract for six days, respectively. The last group received CCl₄ for four days prior to treatment with silymarin (100 mg/kg). With the exception of the normal control rats, all rats received a mixture of freshly prepared CCl₄ in olive oil (1 ml/kg, 1:1 intraperitoneally) for four days. *T. occidentalis* at doses of 200 mg/kg and 400 mg/kg was chosen based on previous studies (9,10).

Twenty-four hours after the last administration, rats from each group were sacrificed by cervical dislocation, and blood samples were obtained through heart puncture via a syringe into sample bottles containing no anticoagulant or into EDTA containers for hematology assessment. The blood samples collected in sample bottles were allowed to clot and were subsequently centrifuged at 5000 rpm for 20 minutes at room temperature to obtain serum for biochemical assays.

Total protein was determined using a Randox kit (United Kingdom) according to the method of Lowry *et al.* (1951) (11). Albumin was determined using a Randox kit (United Kingdom) according to the method of Doumas *et al.* (1971) (12), while total bilirubin was determined using a Randox kit (United Kingdom) according to the method of Jendrassik and Grof (1938) (13).

Hematological analyses of hemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), platelets (PLTs), and hematocrit (HCT) were carried out using a fully automated blood cell counter, PCE-210N, at the Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria.

Data obtained from this study were expressed as mean value \pm standard deviation. Differences between means of groups were determined by one-way ANOVA using the Statistical Package for Social Sciences. The mean differences

were compared using the Duncan multiple range test. A probability level of less than 5% (P < 0.05) was considered significant.

Results

The effect of the aqueous leaf extract of *T. occidentalis* on CCl₄ mediated alteration in serum protein, albumin, and total bilirubin levels in experimental rats is presented in Table 1. The results showed that following CCl₄ induction, there was a significant (p < 0.05) reduction in both serum albumin and total protein levels and a significant increase in total bilirubin when compared to the control and extract-treated groups. However, CCl₄-induced rats treated with 200 mg/kg, 400 mg/kg, and silymarin displayed a significant total protein when compared to untreated CCl₄ rats. Treatment with 400 mg/kg *T. occidentalis* extract or 100 mg/kg silymarin led to a further significant decline in total bilirubin and a significant increase in albumin and total protein extract rats given 200 mg/kg *T. occidentalis* extract.

Values are expressed as Mean \pm Standard Deviation. Values with different superscripts (a, b, c, d) down the column differ significantly at (p < 0.05). CCl₄: Carbon Tetrachloride.

The results of hematological parameters shown in Table 2 indicate that CCl_4 significantly decreased (p < 0.05) RBC, Hb, PLTs, and HCT levels and increased WBC in the CCl₄-alone treated rats compared to the control and extract-treated rats. However, *T. occidentalis* administration significantly increased and attenuated (p < 0.05) the RBC, Hb, PLTs, and HCT levels compared to the CCl₄-alone rats.

Discussion

The liver, apart from being the body's major detoxification organ-removing wastes and xenobiotics through metabolic conversion and biliary excretion-is also responsible for the metabolism, synthesis, storage, and redistribution of nutrients, carbohydrates, lipids, and vitamins, thereby playing a key role in metabolic homeostasis (14,15).

Liver cells synthesize various proteins like albumin, fibrinogen, haptoglobin, transferrin, and antitrypsin. The blood levels of these proteins decrease in cases of extensive liver damage. The liver is the main organ responsible for protein synthesis and the maintenance of protein homeostasis. Hence, liver injury results in impaired protein synthesis. In this study, we observed a significant decrease in total protein and albumin in CCl₄-alone rats compared to control and *T. occidentalis*-treated rats, indicating that CCl₄ toxicity compromised protein synthesis due to liver damage and/or impaired hepatic function. Albumin is important in reducing the bioavailability and toxicity of many substances by binding them. During hypoalbuminemia, the binding potential of albumin to xenobiotics is reduced, leading to the blockage of binding sites by various

metabolites (16). However, following T. occidentalis treatment in CCl4-induced rats, the observed increase in serum protein and albumin compared to CCl₄-alone rats suggests the restoration of hepatic function, stimulation of protein synthesis, and/or protection against CCl4-impaired protein synthesis. This effect can be attributed to bioactive agents, including flavonoids, previously reported in our studies (17). The ability of T. occidentalis to restore serum protein and albumin toward normal levels aligns with previous related works (5,18,19). Bilirubin, the end product of hemoglobin catabolism (20), is a biomarker of hepatic and blood disorders. The significant increase in serum bilirubin levels observed following CCl4 administration in rats compared to control and T. occidentalis-treated rats indicates the occurrence of liver disease. In this study, the increase in total bilirubin in CCl₄-alone rats suggests interference of CCl₄ toxicity with the liver's transport function. However, treatment with the aqueous extract of T. occidentalis at doses of 200 mg/kg and 400 mg/kg restored the abnormalities in total bilirubin levels, with 400 mg/kg T. occidentalis extract exhibiting higher activity, comparable to the standard drug silymarin. This finding is consistent with previous related works (5,18,19).

The blood contains several constituents and metabolites that, when assessed, can provide information on the toxicity of drugs and medicinal plants (21,22,23). Hematological measurement is a recognized method for assessing the health status of humans and animals (24). All vertebrate red blood cells and some invertebrate tissues contain hemoglobin, an iron-containing oxygen transport metalloprotein. It transports oxygen from the lungs to the rest of the body, where it is released to oxidize nutrients and supply energy to regulate the organism's functions (25). In the present study, HCT, PLTs, Hb, and RBC levels were found to be significantly lower (p < 0.05) in the CCl₄-alone group when compared to the normal and *T. occidentalis*-treated groups, similar to the findings of a previous related study (26). The decreased RBC, PLTs, HCT, and Hb levels following CCl4 induction may suggest free radical generation from CCl4 metabolism and toxicity, which affected the hematopoietic, erythropoietic, and thrombopoietic processes in the bone marrow (27). PCV, also known as HCT, is the volume percentage of red blood cells in the blood, and its value depends on the number and size of red blood cells. An abnormally low HCT may indicate anemia (28). However, rats treated with T. occidentalis extract following CCl4 induction showed an improved hematoprotective effect compared to the CCl4 alone group, similar to previously reported findings (29), as there was a significant difference (p < 0.05) in the values of RBC, PLTs, HCT, and Hb upon T. occidentalis treatment compared to the CCl₄ alone group. The reduction in red blood cell count and packed cell volume, also known as HCT, following CCl4 induction may be due to hematopoiesis impairment, RBC destruction, and shrinkage (30). Meanwhile, the increase in these parameters following treatment with 200 mg/kg and 400 mg/kg T. occidentalis indicates a restoration in the oxygen-carrying capacity of red blood cells (31).

Table 1. Effects of aqueous leaf	extract of telfairia occident	<i>talis</i> on liver synthetic molecules	in carbon tetrachloride (CCl4)-induced wistar rats

Treatment groups	Albumin (g/dl)	Tot. Protein (g/dl)	Tot. Bilirubin (mg/dl)
Control	$3.88^{a}\!\!\pm 0.04$	7.85 ^a ±0.19	0.67 ^a ±0.11
CCl4 alone	1.19 ^b ±0.08	2.11 ^b ±0.11	3.97 ^b ±0.32
T. occidentalis (200mg/kg) + CCl ₄	2.02°±0.08	4.97°±0.20	1.34°±0.10
T. occidentalis (400mg/kg) + CCl ₄	2.65 ^d ±0.10	5.43 ^d ±0.22	$1.06^{d}\pm 0.05$
Silymarin (100mg/kg) + CCl ₄	2.73 ^d ±0.11	5.78 ^d ±0.15	1.02 ^d ±0.09

Table 2. Effects of telfairia occidentalis aqueous leaf extract on Hb, WBC, HCT, RBC, and PLTs in carbon tetrachloride (CCl4)-induced wistar rats

Treatment groups	HB (g/dl)	WBC (x10 ³ /ul)	HCT (%)	RBC (x10 ⁹ /L)	PLTS (x10 ⁹ /L)
Control	14.05ª±0.48	9.91ª±0.39	48.10 ^a ±1.01	8.09ª±0.57	312.37ª±10.43
CCl4	10.01 ^b ±0.32	15.87 ^b ±0.26	31.32 ^b ±2.01	4.08 ^b ±0.21	165.74 ^b ±9.76
T. occidentalis (200mg/kg) + CCl ₄	12.11°±0.36	12.01°±0.20	38.45°±1.53	6.04°±0.32	208.81°±10.01
T. occidentalis (400mg/kg) + CCl ₄ Silymarin (100mg/kg) + CCl ₄	13.86 ^a ±0.44 13.79 ^a ±0.51	$\begin{array}{c} 10.12^{a}{\pm}0.38\\ 10.65^{a}{\pm}0.35\end{array}$	40.76 ^c ±1.50 40.07 ^c ±2.07	7.89ª±0.41 7.63ª±0.60	$\begin{array}{c} 241.01^{d}\!\!\pm\!\!9.64 \\ 259.43^{d}\!\!\pm\!\!8.65 \end{array}$

Values are expressed as Mean \pm Standard Deviation. Values with different superscripts down the column (a, b, c, d) differ significantly (p < 0.05). Hb: Hemoglobin; WBC: White Blood Cell; HCT: Hematocrit; RBC: Red Blood Cell; PLTs: Platelets

Conclusion

T. occidentalis administration enhanced liver synthetic ability and hematological index following CCl₄-induced toxicity and damage, which can be attributed to the bioactive agents present in *T. occidentalis*, such as flavonoids, saponins, tannins, and phenols

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Ethical statement

The study was carried out after approval by the EDSU Institutional IBR Research Committee and in accordance with the *American Psychological Association* (APA) guidelines for ethical conduct in the care and use of nonhuman animals in research.

Conflicts of interest

The authors declare that no conflict of interest exists regarding this work.

Author contributions

Prof. Usunobun carried out animal studies, laboratory analysis, manuscript writing, and proofreading, while Dr. Akpovona conducted statistical analysis, writing, and manuscript proofreading.

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