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Telfairia Occidentalis Hook f. Mitigates Carbon-Tetrachloride-Induced Nephrotoxicity in Wistar Rats

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Abstract

Telfairia occidentalis Hook f. is consumed in different parts of the Niger-Delta region of Nigeria due to its high nutritional and medicinal benefits. This study focused on the restorative potentials of Telfairia occidentalis aqueous leaf extract on Carbon-tetrachloride (CCl_4)-induced renal toxicity in wistar rats. Five experimental groups of rats were used in this study. One group received distilled water and serve as normal control. Second group received Carbon-tetrachloride (CCl_4) alone for four days. Third and fourth groups received CCl_4 for four days prior to treatment with 200mg/kg and 400mg/kg T. occidentalis aqueous extract for six days respectively. The last group received CCl_4 for four days prior to treatment with Silymarin (100mg/kg) for six days. With exception of normal control rats, all rats received a mixture of freshly prepared CCl_4 in olive oil (1ml/kg, 1:1 intraperitoneally) for 4 days. Activities of renal markers and lipid profile molecules in serum and histopathogical analysis were assessed. Results revealed that CCl_4 toxicity caused a significant increase ($P < 0.05$) in the level of serum kidney function markers (Creatinine and Urea) and in lipid profile molecules (Total Cholesterol and Triglycerides) whereas T. occidentalis administration showed a dose-dependent nephro-protection as it significantly mitigated the effects of CCl_4 on the kidney function markers and lipid profile molecules assessed. The observed CCl_4 toxicity and renal protection by T. occidentalis were corroborated by the results of histopathological analysis. T. occidentalis aqueous leaf extract mitigated the exacerbated effect of CCl_4 on renal functions which can be attributed to its bioactive agents.

1.0. Introduction

Plants have played very important therapeutic roles in maintaining and enhancing the quality of human health for thousands of years. For decades now, natural compounds have continued to be considered as one of the promising therapeutic agents against cancer, cardiovascular diseases, aging, diabetes, and neurodegenerative disorders due to their wide variety of modes of action, efficiency, accuracy, and fewer side effects [1-2]. *Telfairia occidentalis* Hook f. commonly called fluted pumpkin or ugwu, which occurs in the forest zone of West and Central Africa most especially in Nigeria, Benin and Cameroun is well grown as a leaf vegetable [3-4]. *Telfairia occidentalis* is a medicinal plant with a number of pharmacological activities attributed to its extracts. *T. occidentalis*, darkish-green leafy Vegetable of the Cucurbitaceae family is used in herbal preparations for the management of many diseases such as anaemia, hypertension, diabetes and heart diseases in Nigeria

[5-6]. *T. occidentalis* is rich in minerals such as iron, potassium, phosphorus, calcium and magnesium [7] as well as in antioxidants and phytochemical compounds such as phenols, cucubitacine, anthocynins, flavonoids, tannins, β-carotene, lycopene, vitamins A, C and E [8-9]. It is a popular medicinal plant known locally for its antidiabetic, antiplasmodial, hypoglyceamic, hypolipidemic and antibacterial properties with most of these benefits being reported in various parts and different preparations of the plant in the laboratories [10-13]. *T. occidentalis* leaves have been found to suppress or prevent the production of free radicals and scavenge already produced free radicals, lower lipid peroxidation status and elevate antioxidant enzymes such as superoxide dismutase and catalase *in vitro* and *in vivo* [13-15].

CCl_4 intoxication is associated with high free radical production in several organs, including the liver and kidney. CCl_4 activation by cytochrome P_{450} results in formation of trichloromethyl (CCl_3) and trichloromethylperoxy radical (CCl_3O_2) which reacts with lipids or proteins and subsequently cause lipid peroxidation, free radicals, reactive oxygen species generation and oxidative stress as well as necrosis and steatosis. Exposure to CCl_4 by humans can occur via oral, inhalation and dermal routes [16]. In view of the importance of evaluation in drug and standardized herbal remedy discovery and development as well as in view of the bioactive components of *T. occidentalis*, this study aimed at the restorative potentials of *T. occidentalis* aqueous leaf extract on Carbon-tetrachloride (CCl_4)-induced renal toxicity in wistar rats.

2. Materials and Methods

2.1 Chemicals

Silymarin, Hydrogen peroxide, $KMnO_4$, Epinephrine, thiobabituric acid, Carbon-tetrachloride, Absolute ethanol (99.8%) were purchased from Sigma-Aldrich (USA). Biochemical assays kits were obtained from Randox Diagnostics (Randox, United Kingdom). All other chemicals and reagents were of analytical grade.

2.2 Collection and Extraction of *Telfairia occidentalis* leaves

Fresh leaves of *T. occidentalis* were purchased from Ekiosa market, Benin City, Edo State, Nigeria and identified by a taxonomist. The Fresh leaves were thoroughly rinsed and air-dried at room temperature (24ºC) and then pulverized, crushed into fine powder using a manual blender and weighed. Aqueous extract of the plants was prepared by soaking 1000g of the dry powdered plant materials in 5 litres of double distilled water and then kept at room temperature for 48hours (for thorough extraction). At the end of the 48hours, the extract was filtered first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The filtrate was concentrated using a rotary evaporator with the water bath set 40ºC until the crude extract was obtained. The dried residue (crude extract) was then stored at 4ºC. Aliquot portions of the crude plant extract residue was weighed and dissolved in normal saline for use on each day of the experiments.

2.3 Experimental design/procedure

Adult male albino rats were purchased and allowed to acclimatize for 7 days and maintained under standard conditions, provided pelleted grower's mash (containing 18 % crude protein and 2600Kcal/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 were provided for the animals. The study were conducted on healthy forty (40) 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 [17].

One group received distilled water and serve as normal control. Second group received Carbon-tetrachloride (CCl_4) alone for 4 days. Third and fourth groups received CCl_4 for 4 days prior to treatment with 200 mg/kg and 400 mg/kg *T. occidentalis* aqueous extract for 6 days respectively. The last group received CCl_4 for 4 days prior to treatment with Silymarin (100mg/kg). With exception

of normal control rats, all rats received a mixture of freshly prepared CCl_4 in olive oil (1ml/kg, 1:1 intraperitoneally) for 4 days. *T. occidentalis* at a dose of 200mg/kg and 400mg/kg was chosen based on the previous studies of Saalu et al [18] and Akang et al [19].

Twenty-four (24) hours after last administration, rats from each group were sacrificed by cervical dislocation and blood samples obtained through heart puncture via a syringe into sample bottles containing no anticoagulant. The blood samples collected in sample bottles were allowed to clot and subsequently centrifuged at 5000rpm for 20mins at room temperature to obtain serum for biochemical assays.

2.4 Biochemical parameters

Serum urea was determined using the RANDOX Kit according to the manufacturer's instructions following the method of [20] while serum Creatinine was determined by using the Jaffe' method. The determination of serum Total Cholesterol (TC) was by method of [21] while serum Triglyceride (TG) was by method of [22].

2.5 Histopathological analysis

Immediately after sacrifice, the kidney of both the test and control rats were excised, dried with blotting paper, weighed and a portion instantly fixed in 10% phosphate buffered formalin. Fixed tissue samples were embedded in paraffin blocks and sections of 5 mm were prepared. Sections were stained with hematoxylin and eosin (H & E) and examined under Olympus/3H light microscope. Photomicrographs of the kidney were captured using a Moticam Images Plus 2.0 digital fitted to the light microscope.

2.6 Statistical analysis

Data obtained from this study were expressed as mean value ± standard deviation. Differences between means of groups were determined by One way ANOVA using Statistical Package for social scientist (SPSS). The mean differences were compared with the Duncan multiple range test. A probability level of less than 5% ($P<0.05$) was considered significant.

3. Results

Kidney functional markers in the serum of control and treated rats are provided in Table 1. The result of Kidney assessment showed that compared to control and extract treated groups, CCl_4 alone rats exhibited a significant increase in serum Creatinine and Urea. However, treatment of CCl_4-induced rats with *T. occidentalis* aqueous extract at a dose of 200 mg/kg and 400 mg/kg resulted in a significant reduction in Creatinine and Urea.

Table 1: Effects of *Telfairia occidentalis* aqueous leaf extract on Kidney function parameters in Carbon-tetrachloride (CCl_4) -induced wistar rats

Treatment groups	Creatinine (mg/dl)	Urea (mg/dl)
Control	0.61^{a}±0.05	15.21^{a}±1.17
CCl_4	1.98^{b}±0.11	65.87^{b}±2.02
T. occidentalis (200mg/kg) + CCl_4	1.09^{c}±0.09	31.43^{c}±2.01
T. occidentalis (400mg/kg) + CCl_4	1.03^{c}±0.05	24.63^{d}±2.30
Silymarin (100mg/kg) + CCl_4	0.99^{c}±0.07	24.98^{d}±2.51

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly ($p<0.05$).

The results presented in Table 2 showed the data obtained from the effects of *T. occidentalis* aqueous leaf extract on lipid profile in CCl_4-induced toxicity in rats. The result showed that CCl_4 induction elevated serum total Cholesterol and triglyceride levels when compared to control and extract treated groups. However, it was also revealed that treatment with *T. occidentalis* produced a dose dependent significant ($p<0.05$) reduction in serum total cholesterol and triglycerides levels compared to the CCl_4 alone group.

Table 2: Effects of *Telfairia occidentalis* aqueous leaf extract on Lipid Profile in Carbon-tetrachloride (CCl_4)-induced wistar rats

Treatment groups	Tot. Cholestrol (mg/dL)	Triglyceride (mg/dL)
Control	98.38^{a}±2.67	121.83±3.05
CCl_4	176.26^{b}±3.06	201.87±3.02
T. occidentalis (200mg/kg) + CCl_4	114.42^{c}±3.02	140.01±2.31
T. occidentalis (400mg/kg) + CCl_4	109.01^{c}±2.54	133.06±3.11
Silymarin (100mg/kg) + CCl_4	107.07^{c}±3.31	129.21±3.27

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly ($p<0.05$).

The result of histopathological analysis showed control rat kidney to express intact basement membranes, normal tubules and normal capillary tufts (Figure 1A) while the kidney of rat that received CCl_4 alone showed renal tubular nephrosis, discrete necrosis of some tubular cells and blood vessel congestion, severe vacuolar degenerated glomerulus and swelling of several renal epithelium (Figure 1B). The Photomicrograph of kidney of rat given CCl_4 and 200mg/kg *T. occidentalis* showed minimized necrosis in renal tubules (Figure 1C) whereas Photomicrograph of kidney of rat given CCl_4 and 400mg/kg *T. occidentalis* with no observed necrosis expression (Figure 1D). Meanwhile Photomicrograph of kidney of rat given CCl_4 and 100mg/kg Silymarin showing mild mild renal tissue vascular congestion (Figure 1E).

4. Discussion

In assessment of the kidney functionality, serum urea elevation is an indication of kidney failure which normally degenerates to severe kidney damage in prolonged cases [23-24]. More so, renal failure is believed to be linked to high serum levels of creatinine, which is a useful index in the diagnosis of chronic kidney disease. Creatinine is not reabsorbed at the Loop of Henle like urea; thus, this marker helps to understand the glomerular filtration rate of the kidney [25]. The result of this study showed that CCl_4 induction significantly increased Urea and Creatinine levels, thus indicating that the impact of CCl_4 metabolism triggers glomerular cell and kidney tubule toxicity similar to findings of [26-29]. The increased levels of blood Urea and Creatinine in CCl_4-induced rats implies inability of the kidneys to excrete these by-products leading to their elevated levels in the blood and decreased excretion in urine [30]. However, *T. occidentalis* at a dose of 200mg/kg and 400mg/kg similar to Silymarin at 100mg/kg showed a decrease in Urea and creatinine levels compared to CCl_4 alone rats, thus restoring the abnormalities in the levels of these biomarkers with 400mg/kg *T. occidentalis* extracts having higher activity and bringing the kidney parameters close to normal which also compared well with standard drug, silymarin similar to related findings of [28-29]. The renal maintenance following *T. occidentalis* treatment can be attributed to the phytoconstituents in *T. occidentalis* including flavonoids [9] which protected the kidney from free radical attacks and thus against oxidative stress and damage compared to the group that received CCl_4 alone. Lipids are known to play an important role in the incidence of liver disease. Increased levels of cholesterol and triglycerides are known to be associated with atherosclerosis and coronary heart disease [31].

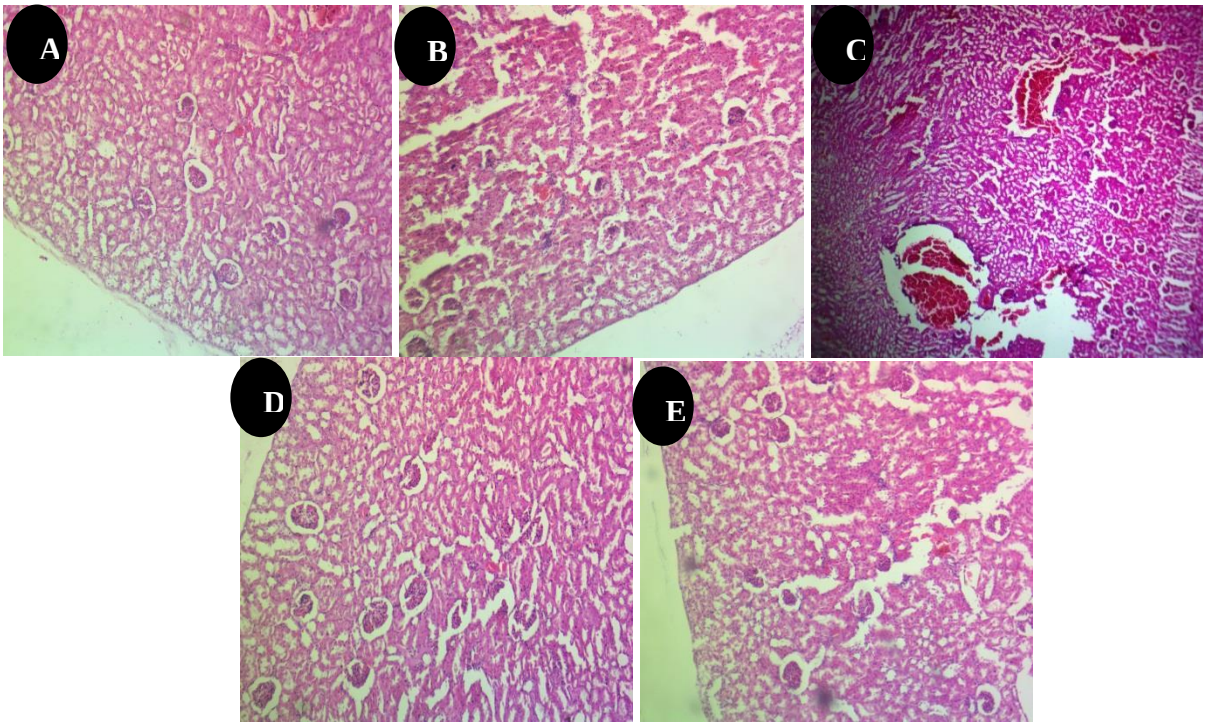

Figure 1A: Photomicrograph of rat kidney showing intact basement membranes, normal tubules and normal capillary tufts. **Figure 1B**: Photomicrograph of rat kidney given CCl_4 alone showing renal tubular nephrosis, discrete necrosis of some tubular cells and blood vessel congestion, severe vacuolar degenerated glomerulus and swelling of several renal epithelium. **Figure 1C**: Photomicrograph of rat kidney given CCl_4 and 200mg/kg *T. occidentalis* showing minimized necrosis in renal tubules. **Figure 1D**: Photomicrograph of rat kidney given CCl_4 and 400mg/kg *T. occidentalis* with no observed necrosis expression. **Figure 1E**: Photomicrograph of rat kidney given CCl_4 and 100mg/kg Silymarin showing mild mild renal tissue vascular congestion.

In this study, administration of CCl_4 resulted in significant increase in Total Cholesterol and Triglycerides in CCl_4-alone rats compared to the control rats and *T. occidentalis* treated rats, a result that was found similar to previous work of [32]. Thus, this study shows that CCl_4 treatment causes a disruption of lipid metabolism as seen in the increased total cholesterol and Triglyceride. The increase in total cholesterol and triglycerides can be attributed to free radicals generated from CCl_4 metabolism and toxicity which damage the endoplasmic reticulum, leading to reduced protein synthesis and lipid accumulation in the liver [33-34]. However, although in a higher dose dependent manner, administration of *T. occidentalis* at doses of 200mg/kg and 400mg/kg significantly lowered ($p < 0.05$) the levels of total cholesterol and triacylglycerol in comparison with CCl_4 alone administered rats. The result of our study on the ability of *T. occidentalis* to lower CCl_4 elevated blood lipids agrees with similar reported works of [35-38]. The ability to lower the lipid levels can be attributed to our previously reported phytoconstituents in *T. occidentalis* [9]. Specifically, flavonoids have been reported to lower lipid levels by inhibiting lipid absorption, lipogenesis and stimulating lipolysis [39].
The observed lesions seen in the photomicrograph of kidney of rats that received CCl_4 and the nephroprotection observed following administration of *T. occidentalis* corrobated the results of the observed biochemical parameters.

5. Conclusion

In conclusion, the results of our study showed that CCl_4 induction caused hepato-renal dysfunction characterized by altered biochemical, compromised cellular and structural integrity. However,

treatment with aqueous leaf extract of *T. occidentalis* significantly reversed these anomalies, suggesting its protective effect against CCl_4-induced hepato-renal damage. It can be exerted that *T. occidentalis* nephroprotective effect is associated with its ability to improve renal function parameters as it may have increased glomerular filtration rate resulting in less serum creatinine and urea level. The antioxidant properties and detoxification capacity of *T. occidentalis* may also be responsible for its protective effect against CCl_4 toxicity.

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Conflict of Interest
The authors declare that no conflict of interest exists with respect to this work.

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