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Research Paper

Telfairia Occidentalis Supplementation Attenuate Carbon-Tetrachloride-Induced Hepatic Damage and Oxidative Stress

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Abstract— This study determined hepatoprotective effect of *Telfairia occidentalis* aqueous leaf extract on Carbon-tetrachloride (CCl_4)-induced toxicity in male Wistar rats. Of the five experimental groups presented, first group was control and it received distilled water. Rats in second group received CCl_4 alone for four consecutive days. Rats in third and fourth groups were administered CCl_4 for four consecutive days prior to treatment with 200mg/kg and 400mg/kg *T. occidentalis* aqueous extract for six consecutive days respectively. The fifth group received CCl_4 for four consecutive days prior to treatment with Silymarin (100 mg/kg) for six consecutive days. All rats intraperitoneally received 1ml/kg, 1:1, a mixture of freshly prepared CCl_4 in olive oil with exception of normal control rats. Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Acid Phosphatase (ACP) were assayed in the serum while Superoxide Dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA), and Nitric Oxide (NO) in the liver homogenates were measured. From results obtained, exposure to CCl_4 orchestrated significant ($p < 0.05$) increases in AST, ALT, ALP, LDH, ACP, MDA and NO and significantly ($p < 0.05$) decreased SOD and CAT whereas *T. occidentalis* aqueous extract administration at a dose of 200mg/kg and 400mg/kg alleviated toxic effects of CCl_4. The result of toxicity of CCl_4 and hepatoprotection by *T. occidentalis* were corroborated by histopathological analysis. In conclusion, this study established that *T. occidentalis* possesses and enhanced antioxidant activity, thus attenuating CCl_4-induced hepatic oxidative damage and oxidative stress.

Keywords— Antioxidants, Carbon-tetrachloride, Hepatotoxicity, Liver, Oxidative stress, *Telfairia occidentalis*

1. Introduction

Hepatotoxicity is a frequent condition that can have catastrophic implications ranging from metabolic problems to death [1, 2]. Carbon-tetrachloride (CCl_4) is a manufactured chemical and does not occur naturally in the environment. CCl_4 is toxic to the mammalian liver and is hepatocarcinogenic in both rats and mice [3]. CCl_4 poisoning has been shown to be a substantial source of free radical generation in a variety of organs, including the liver, kidney, lungs, brain, and blood [4]. In the liver, CCl_4 is actively converted to the extremely reactive trichloromethyl free radical, CCl_3*. In the presence of molecular oxygen, CCl_3* interacts with cellular macromolecular protein and polyunsaturated fatty acids to create highly hazardous trichloromethyl peroxyl radicals CCl_3O, H_2O_2, O_2^-, OH, which cause liver damage [5, 6, 7].

Medicinal herbs have long been utilized in traditional medicine to treat liver problems in developing countries as well as other regions of the world. These plants have been suggested for *in vivo* pharmacological testing in order to identify good candidates [8]. Due to the bioactive phytochemicals present, decades of research has shown significant improvement from using a variety of plant parts, either natively or as extracts, in the treatment of a variety of illnesses [9, 10, 11]. With the global rise of liver diseases, tremendous scientific advancement in the field of medicinal plants and finding effective and safe natural hepatoprotective agents is one of the future directions [12]. Herbal medicine remain the most accepted and recognized form of medicine in today's society, and for the treatment and prevention of many diseases around the world, various medicinal plants would be the best sources to obtain quality herbal medicine drugs [13]. This present study seeks to evaluate the hepatoprotective and modulatory potential of *T. occidentalis* against CCl_4-induced hepatotoxicity in rats.

2. Related Work

Since ancient times, plants have been the source of agents that have therapeutic potentials and up till today plays important

role in the basic health care system of a large percentage of the world's population [14]. *Telfairia occidentalis* popularly called fluted pumpkin has many traditional names such as being called *ugwu* among the Igbos, *aporoko* among the Yorubas, *ubong* among the Efiks, and *umeke* among the Edos [15]. *T. occidentalis*, consumed in different parts of Nigeria because of the numerous nutritional and medicinal attributes ascribed to it [16], has been reportedly used for the treatment of various diseases including convulsion, gastrointestinal disorders, malaria and anaemia [17, 18]. Leaves of *T. occidentalis* serves often as vegetable in soup preparation just as its seeds are eaten raw or roasted and also ground into powder and used as soup thickening. *T. occidentalis*, in addition to being rich in protein, carbohydrate, vitamins minerals and fibre, also contain phytoagents including saponins, glycosides, flavonoids, alkaloids and resins [19, 20, 21, 22]. The leaves are very rich in Iron, Vitamin A, C, K, potassium, folic acid, copper, calcium, zinc and cobalt [16]. *T. occidentalis* is known to have antioxidant and free radical scavenger properties, which can be said to ascribe to why it is being used in management of oxidative damage and oxidative stress related ailments such as cancers, and liver diseases [23]. Antioxidants neutralize free radicals and stop the oxidation process which cause damage to cellular structures in the body. *T. occidentalis* consumption, moreover has been reported to elicit hypolipidemic effect and help to reduce the risk of cardiovascular disease [24, 25].

3. Materials and Method

3.1 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 at 40ºC until the crude extract was obtained. The obtained dried extract was stored at 4ºC and its aliquots weighed and dissolved in normal saline for use on each day of the experiments.

3.2 Experimental design/procedure

Purchased Adult male albino rats were 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 total of forty (40) Wistar male albino rats weighing 190 – 200g, randomly assigned to five treatment groups of eight (8) rats each were used for this study. This study followed ethical conduct guidelines and rules in care and use of nonhuman animals in research [26].

Of the five experimental groups presented, first group was control and it received distilled water. Rats in second group received CCl_4 alone for four consecutive days. Rats in third and fourth groups were administered CCl_4 for four consecutive days prior to treatment with 200 mg/kg and 400 mg/kg *T. occidentalis* aqueous extract for six consecutive days respectively. The fifth group received CCl_4 for four consecutive days prior to treatment with Silymarin (100 mg/kg) for six consecutive days. All rats intraperitoneally received 1ml/kg, 1:1, a mixture of freshly prepared CCl_4 in olive oil with exception of normal control rats. *T. occidentalis* at a dose of 200mg/kg and 400mg/kg were chosen based on the previous studies of [27, 28]. Silymarin was chosen as a hepatoprotection; thus, it was a positive control. At the end of administration (24hours after last treatment), all the rats were sacrificed and serum obtained from collected centrifuged (at 5000rpm for 20mins at room temperature) blood samples into sample bottles containing no anticoagulant for biochemical analysis.

3.3 Liver tissue Collection and preparation of tissue homogenates

The excised liver were rinsed with normal saline, weighed and a small portion fixed in 10% formalin (4% formaldehyde) for histopathological examinations while the remaining part were stored at –20°C for biochemical analysis. A 10% tissue homogenate of the stored tissues were prepared in physiological saline and the homogenates were centrifuged at 5000xg for 20 minutes and a clear supernatant obtained for determination of Malondialdehyde (MDA), Nitric oxide (NO), Superoxide dismutase (SOD) and Catalase (CAT).

3.4 Biochemical parameters

The colorimetric method of Reitman and Frankel [29] was used for determination of AST and ALT activities in serum while Randox kits (UK) according to the manufacturer`s instructions was used for determination of Alkaline Phosphatase (ALP). Serum Acid Phosphatase (ACP) activity was estimated using BIO-SYSTEMS ACP assay kit according to the procedure suggested by the manufacturer. The method of Spiegel et al [30] was used to determine Lactate dehydrogenase. Superoxide dismutase (SOD) was assayed according to the method of Misra and Fridovich [31] while Catalase was assayed according to the method of Cohen *et al*. [32]. MDA was estimated in a colorimetric reaction with thiobarbituric acid [33]. The levels of nitric oxides (NO) were measured by the simple reaction of Griess, described by Rios *et al* [34].

3.5 Histopathological analysis

A portion of the obtained liver tissues of all rats in the groups were instantly fixed in 10% phosphate buffered formalin and thereafter embedded in paraffin blocks and sections of 5 mm were prepared. Hematoxylin and Eosin (H & E) was then applied for staining and examination was carried out using Olympus/3H light microscope. Moticam Images Plus 2.0 digital fitted to the light microscope was used to capture photomicrograph of the liver tissues..

3.6 Statistical analysis

This study data were expressed as mean ± standard deviation. One way ANOVA using Statistical Package for social scientist (SPSS) was deployed to determine mean differences between groups. A probability level of less than 5% (P<0.05) was considered significant.

4. Results and Discussion

The hepatic toxicity following induction of CCl_4 significantly elevated levels of liver functional markers as presented in Table 1 below, serum AST, ALT, ALP, LDH and ACP compared to the extract treated and control groups. However, although in a higher dose dependent manner, *T. occidentalis* at a dose of 200mg/kg and 400mg/kg declined the elevated level of AST, ALT, ALP, LDH and ACP when compared to CCl_4 alone group. The group that received Silymarin and CCl_4 also showed reduction in AST, ALT, LDH and ACP compared to the group that received CCl_4 alone. The liver is a rich source of the aminotransferases, AST and ALT, enzymes known to be involved in transamination reactions [35, 36]. Liver enzymes including AST, ALT and ALP are known to enter the circulatory system after lesions of the hepatic parenchyma that alters membrane permeability [37]. Cytochrome P450-dependent monooxygenases is known to process accumulated CCl_4 to trichloromethyl (CCl_3) radicals in the hepatic parenchymal cells [38] and besides its role in the alkylation of cellular proteins, CCl_3 causes polyunsaturated fatty acids to produce lipid peroxides, which could induce hepatotoxicity and alter hepatic marker enzyme levels [39, 40]. In this study, following CCl_4 administration, ALT, AST, ALP, ACP and LDH levels were significantly increased in rats that received CCl_4 alone when compared to the normal control rats and *T. occidentalis* treated rats similar to previous studies of [41, 42, 43]. This implies hepatocellular damage by CCl_4, leading to the leakage of the enzymes into the blood stream[44]. Lactate dehydrogenase (LDH) is found in almost every tissue including skeletal muscle, heart, liver, kidney, brain, lungs and red blood cells. Serum LDH activity is an indicator of cell damage and increase in LDH level occur in association with a wide variety of diseases [45]. Thus the observed increase in serum LDH activity following CCl_4 induction in the rats indicates hepatocellular damage resulting in high level of serum LDH. Thus, following CCl_4 administration, the marked release of these biochemical markers into rat's blood circulation indicated severe damage in the hepatic tissue membranes. Alkaline phosphatases (ALPs), found in high concentrations in liver, bone and kidney tissues as well as placenta and intestinal wall [46] catalyze hydrolysis of organic phosphate in proteins, nucleotides and alkaloids at alkaline pH [47]. Thus the observed increase in serum ALP activity following CCl_4 induction in the rats that received CCl_4 alone compared to *T. occidentalis* treated rats connotes occurrence of liver and bone diseases [48] as well as an indication of pathological alteration of biliary flow. ALP and ACP are marker enzymes for plasma membrane stability and are required for proper liver functioning [49] and changes in ALP and ACP could indicate tissue damage or disease. Thus, in this study, the observed increased serum activities of ALP and ACP in CCl_4-alone rats is suggestive of CCl_4-induced tissue damage. With a pH-optimum in the acid zone, acid phosphatase (ACP) is a hydrolase enzyme that catalyzes the hydrolysis of different phosphate esters [50]. ACP is located in cellular lysosomes and lipid peroxidation that compromises the integrity of the membrane may cause an increase in ACP in blood. As seen in this study, rats exposed to CCl_4 had higher levels of ACP activity than those in the control group and *T. occidentalis* groups. The rupturing of lysosomal membrane and the enzyme liberation by CCl_4 that result in the enhanced activity of ACP may be the cause. The production of additional lysosomes as a result of lipid peroxidation may also be the cause of ACP increased activity [51]. However, in a higher dose dependent manner, *T. occidentalis* and Silymarin drastically reduced AST, ALT, ALP, ACP and LDH compared to the group that received CCl_4 alone, an indication of *T. occidentalis* hepatocellular efficiency/improvement and hepatic tissue restoration similar to previous findings of [52, 53, 42, 43]. The return towards normalcy of AST, ALT, ALP, ACP and LDH following *T. occidentalis* treatment corresponds to healing of the liver parenchyma and regeneration of hepatocytes. *T. occidentalis* at a dose of 200 mg/kg BW and 400 mg/kg BW can be said to have initiated the process of regeneration and repair of liver damage caused by CCl_4, thus the reduction observed in AST, ALT, ALP, ACP and LDH. The hepato-curative activity of *T. occidentalis* could be attributed to its phytoconstituents in particular, our previously reported flavonoids and other polyphenols which are known to have radical scavenging capacity [19].

Table 1: Effects of aqueous leaf extract of *Telfairia occidentalis* on Liver Function enzymes in Carbon-tetrachloride (CCl_4)-induced Wistar rats

Treatment groups	AST (U/L)	ALT (U/L)	ALP (U/l)	LDH (U/l)	ACP (U/l)
Control	34.12^{a} ± 4.27	36.34^{a}±5.05	32.25^{a}± 3.24	137.06^{a}± 15.21	25.12^{a}±1.65
CCl_4 alone	195.71^{b}± 3.29	173.88^{b}±4.32	176.32^{b}±5.84	626.53^{b}±20.96	152.54^{b}±4.02
T. occidentalis (200 mg/kg BW) + CCl_4	102.3^{d}c±3.80	105.54^{c}±3.01	99.23^{c}±3.88	274.04^{c}±9.01	83.07^{c}±3.16
T. occidentalis (400 mg/kg BW) + CCl_4	80.21^{d}±3.01	83.14^{d}±2.76	79.52^{d}±4.88	249.65^{d}±8.23	62.67^{d}±3.01
Silymarin (100 mg/kg BW) + CCl_4	69.43^{e}±3.99	66.18^{e}±2.65	74.73^{d}±4.12	238.10^{d}±10.09	51.67^{e}±3.28

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly at (p<0.05). AST-Aspartate Aminotransferase; ALT-Alanine Aminotransferase; ALP- Alkaline Phosphatase; LDH-Lactate Dehydrogenase; ACP-Acid Phosphatase, CCl_4- Carbon Tetrachloride.

In order to evaluate *in vivo* antioxidant status, the hepatic antioxidants (SOD and CAT) and peroxide product (MDA) as well as the inflammatory marker, NO were assessed as shown in Figure 2 below. Compared to the values in rats from control group and extract treated groups, rats in CCl_4 group showed significant decline in hepatic SOD and CAT activities ($P < 0.05$), an indication that CCl_4 induced inflammation, oxidative stress and lipid peroxidation. In addition, NO and MDA level in CCl_4 alone group was significantly higher than that in control and extract treated group ($P < 0.05$). However, while SOD and CAT activity were increased significantly, MDA and NO levels were decreased in 200 mg/kg BW and 400 mg/kg BW *T. occidentalis* treated groups and 100 mg/kg BW Silymarin treated group, when compared with that in CCl_4 alone group, an indication that *T. occidentalis* could improve CCl_4-induced oxidative stress and damage in rat. The reactive intermediate of CCl_4 toxicity is believed to cause lipid peroxidation and breakdown of cellular membranes [54]. In this study, CCl_4 administration significantly increased MDA and NO level and significantly decreased SOD, and CAT levels similar to those obtained by Hsiao et al [55] The observed decrease in the activity of SOD and CAT concentration in the rats induced with CCl_4 could be attributed to their excessive utilization in mopping up the free radicals generated due to the CCl_4 induced attack and oxidative stress. NO plays crucial roles in inflammation and liver injury [56]. High lipid peroxidation as seen in liver MDA and NO of CCl_4-induced rats in this study is indicative of oxidative stress, inflammation and oxidative damage. The observed high levels of MDA and NO in this study following CCl_4 induction suggest enhanced peroxidation leading to tissue damage and failure of the antioxidant-defense mechanisms to prevent excessive free radicals formation [57, 58].

A team of defense against reactive oxygen species (ROS) that includes antioxidant enzymes plays a crucial function in the detoxification of oxidative damages [59]. In this study, treatment of the CCl_4-induced rats with *T. occidentalis* extract at a dose of 200mg/kg and 400mg/kg significantly improved the oxidative stress biomarkers by significantly decreasing the MDA and NO level and increasing the SOD and CAT levels. NO, oxidant mediators possesses prominent cytotoxicity and stress-induced cell injury[60]. Such a chain of oxygen free radicals cascade generates lipid peroxidation in several biological systems with the formation of the final product: malondialdehyde (MDA) [61]. The SOD, a major enzyme provides protection by lowering intensities of oxidants mutated to hydrogen peroxide (H_2O_2) broken down to water and oxygen by other enzymes [62, 63]. Catalase converts H_2O_2 molecules to water, which aidstoxic effect neutralization in biological system [62, 64]. The attenuative potential of *T. occidentalis* to enhance SOD and CAT as well as to decrease MDA and NO agrees with previous similar works of [42, 43, 52, 53, 65]. There was a protective effect of *T. occidentalis* at 200 mg/kg BW and 400 mg/kg BW as it improved the endogenous antioxidant status of the rats as well as prevented oxidative stress and oxidative damage caused by the CCl_4 generated free radicals. This study revealed that the administration of CCl_4 led to an increase of hepatic MDA and NO level whereas treatment with *T. occidentalis* reversed the anomaly, especially at the dosage of 400 mg/kg BW. This protection by *T. occidentalis* could be attributed to its bioactive compounds present such as flavonoids.[17] The higher protective activities of the extracts at dose of 400 mg/kg BW could be due to increased concentrations of bioactive compounds at this dosage which in turn maximally prevent, protect or repair the tissues from CCl_4 induced oxidative damage.

Table 2: Effect of aqueous leaf extract of *Telfairia occidentalis* on hepatic Oxidative stress parameters in Carbon-tetrachloride (CCl_4)-induced Wistar rats

Treatment groups	SOD (U/mg wet tissue)	MDA (U/mg wet tissue)	CAT (U/mg wet tissue)	NO (μmol/g tissue)
Control	81.34^{a}±4.61	1.91^{a}±0.17	8.96^{a}±0.32	110.14^{a}±2.76
CCl_4 alone	16.97^{b}±3.01	9.37^{b}±0.28	1.03^{b}±0.10	215.37^{b}±4.01
T. occidentalis (200mg/kg BW) + CCl_4	51.02^{c}±4.03	3.86^{c}±0.21	4.16^{c}±0.13	145.21^{c}±3.04
T. occidentalis (400mg/kg BW) + CCl_4	55.58^{c}±3.11	3.32^{d}±0.12	5.09^{d}±0.20	188.52^{d}±3.17
Silymarin (100mg/kg BW) + CCl_4	53.41^{c}±3.06	3.01^{e}±0.10	5.18^{d}±0.21	192.90^{d}±3.21

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly at (p<0.05). SOD- Superoxide Dismutase
MDA- Malondialdehyde, CAT- Catalase, NO- Nitric oxide; CCl_4- Carbon-tetrachloride

From the result of Histopathological analysis shown in the photomicrograph below, the control group (figure 1) showed normal liver structures with well-preserved cellular structure with clear cytoplasm, well-formed central vein and sinusoid, indicating healthy functional liver cells. The photomicrograph of the CCl_4 alone rats (figure 2) revealed hepatocellular degeneration, and vacuolization, necrosis, inflammatory infiltration around the central vein whereas the photomicrographs of the CCl_4 induced rats treated with *T. occidentalis* at a dose of 200 mg/kg BW (figure 3) and 400 mg/kg BW (figure 4) as well as 100 mg/kg BW Silymarin

(figure 5) showed less necrosis, reduced inflammation and hepatic cell regeneration.

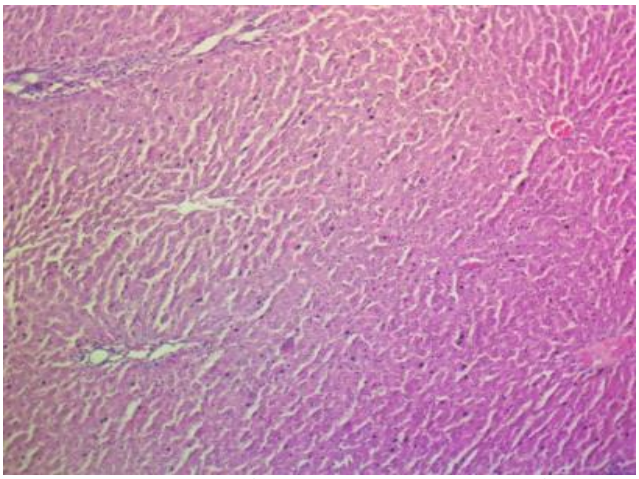

Figure 1: *Photomicrograph of control rat liver showing well defined hepatic lobules and radially arranged central vein, normal liver architecture, intact and normal hepatocytes with sinusoidal gaps and a normal parenchyma showing healthy liver function as seen in the well preserved cellular structure with clear cytoplasm.*

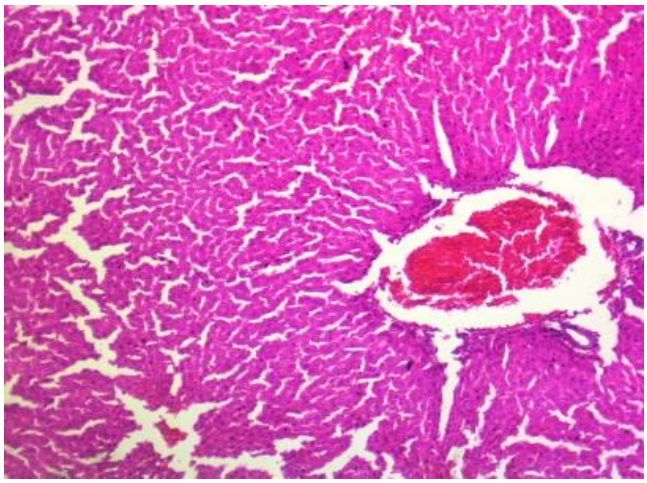

Figure 2: *Photomicrograph of rat liver that received CCl_4 alone showing hepatic lesions and intense hepatic necrosis, nuclear degeneration, ballooning Hepatocytes, lymphocyte cells infiltration*

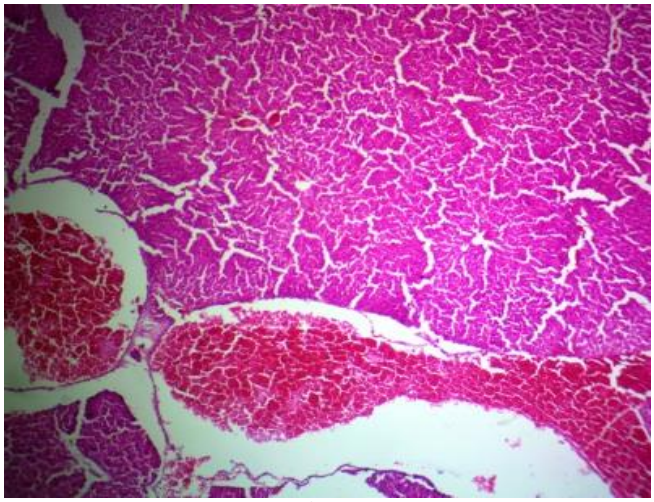

Figure 3: *Photomicrograph of rat liver that received CCl_4 and T. occidentalis at a dose of 200 mg/kg BW showing fairly hepatic lesion, mild infiltration of inflamed cells, few regenerative hepatocytes.*

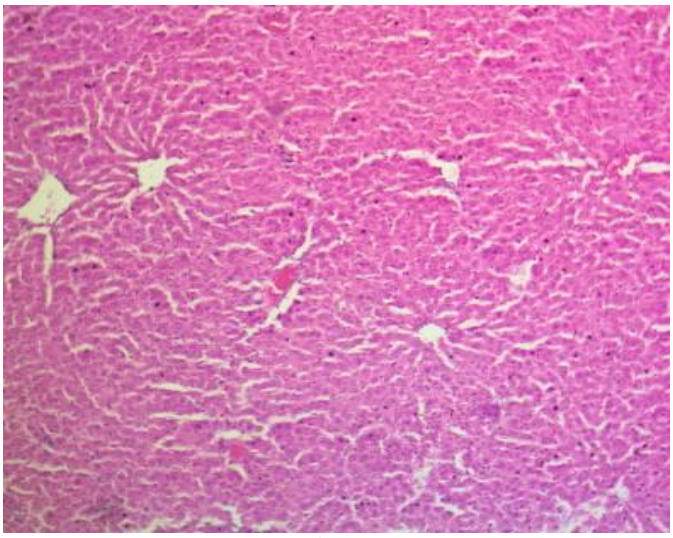

Figure 4: *Photomicrograph of rat liver that received CCl_4 and T. occidentalis at a dose of 400 mg/kg BW showing maintained hepatic architecture with minimal damage, no ballooning, recovery of hepatocytes, regenerative hepatocytes.*

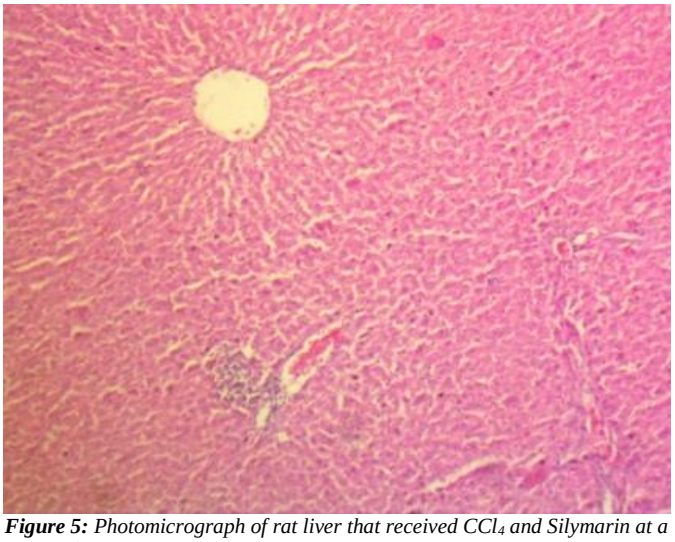

Figure 5: *Photomicrograph of rat liver that received CCl_4 and Silymarin at a dose of 100 mg/kg BW showing hepatocyte recovery, minimized lesions but no ballooning.*

The histological analysis of the liver tissues gave credence to our observed biochemical data that treatment of rats with *T. occidentalis* following CCl_4 induction exerts hepatoprotection and free radical scavenging potential against CCl_4 damage and attack. *T. occidentalis* administration at a dose of 200mg/kg and 400mg/kg brought about a substantial improvement in liver architecture by reducing hepatic damage and injuries, suppressing hepatocellular degeneration and necrosis and thus significantly improving liver structure and function.

5. Conclusion

In conclusion, *T. occidentalis* treatment showed the potential to ameliorate the cellular changes induced by CCl_4-intoxication as it efficiently restored toward normalcy all CCl_4-elevated rat liver function marker enzymes, resisted the CCl_4-induced reduction in SOD and CAT and significantly abrogated the CCl_4-induced increase in MDA and NO levels,

thus underscoring the reactive oxygen species (ROS) scavenging ability of *T. occidentalis*, which efficiently overcame the oxidative damage and oxidative stress elicited by CCl_4-intoxication in the rat. *T. occidentalis* treatment against CCl_4-induced damage demonstrated hepatoprotection ability against oxidative stress and damage in the liver, thus providing evidence that *T. occidentalis* popularly consumed as Ugwu leaf in Nigeria are excellent sources of antioxidants.

Competing interests
No conflict of interest exists with respect to this work.

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Authors' Contributions
Usunobun U.: involved in conceiving the study, protocol development, field work and drafted the manuscript.
Okpiabhele A.: researched literature and field work.
Adegbegi A. J.: participated in the field work and study conception.

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