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Protective effect of *Alchornea cordifolia* leaf extract on carbon tetrachloride-induced liver damage in Wistar rats

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Abstract

The effect of aqueous extract of the leaves of *Alchornea cordifolia* on Carbon tetrachloride (CCl₄)-induced liver damage was investigated in experimental rats. Treatment of separate groups of rats with 100mg/kg, 150mg/kg and 200mg/kg aqueous leaf extracts of *Alchornea cordifolia* for 2 weeks after establishment of CCl₄ induced liver damage, resulted in significantly (P<0.05) less hepatotoxicity when compared to the CCl₄-induced group, as measured by serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. The effect of extract was statistically significant (P<0.05) and was dose dependent. Histopathological study also showed significant reduction and even reversal of liver damage in the rats. The results of this study show that aqueous leaf extract of *Alchornea cordifolia* has a potent anti-hepatotoxic action against CCl₄ induced liver damage in rats.

Keywords: *Alchornea cordifolia*; Liver Damage; Carbon Tetrachloride (CCL₄); Antioxidants; Phytochemicals

1. Introduction

The liver is a key organ in the metabolism, detoxification, and elimination of a wide range of endogenous and exogenous substances, including xenobiotics. As a result of the liver's physiological activity, extremely reactive free radicals are produced, which form covalent bonds with membrane lipids, causing lipid peroxidation. Lipid peroxidation causes tissue injury by altering membrane permeability. Because the liver is engaged in so many metabolic activities, it is vulnerable to free radical damage and necrosis [1]. Furthermore, free radicals are implicated in a variety of clinical conditions, including heart disease, diabetes, gout, and cancer [2]. Antioxidant systems built into the tissues, such as superoxide dismutase (SOD), tissue glutathione (GSH), and others, defend the tissues from free radical attack. Excessive reactive oxygen species release overwhelms this mechanism, causing organ damage. Exogenous antioxidant administration or strengthening of the body's own defensive systems may be beneficial in protecting the organs [1]. In orthodox medical practice, however, there are no satisfactory liver protective medications for significant liver problems.

Herbal medications aid in the treatment of a variety of liver ailments, with the majority of them speeding up the liver's natural healing processes. Furthermore, there is a growing interest in antioxidants, particularly those designed to prevent the alleged harmful effects of free radicals in the human body and the deterioration of fats, with a preference for antioxidants derived from natural rather than synthetic sources due to the natural drugs' safety. In light of modern medicine, many plants have been studied for their hepatoprotective and antioxidant properties [3,4] *Alchornea cordifolia* (*A. cordifolia*) Mull. Arg (FAM: Euphorbiaceae) is a perennial shrub or small tree that grows up to 4 meters tall

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and reproduces from seeds. The plant is said to be used as a topical anti-inflammatory, antibacterial, and antifungal agent in African traditional medicine [5,6]. In experimental animals, the hepatoprotective activity of *A. cordifolia* against hepatotoxicity induced by paracetamol at high dosages has been studied and reported [7,8]. We use various free radical scavenging methods such as reduced glutathione, glutathione peroxidase, and S-transferase, lipid peroxidation, catalase peroxidation, superoxide dismutase, or in vitro techniques such as the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging models to investigate the efficacy of hepatoprotective and antioxidant substances. Drugs like acetaminophen, isoniazid, and rifampicin, as well as poisons like carbon tetrachloride, are commonly used to cause hepatic damage. The goal of this study is to see how an aqueous extract of *Alchornea cordifolia* leaf affects CCl₄-induced liver damage in Wistar rats and to seek alternative medications to treat liver illnesses.

2. Material and methods

2.1. Ethical Clearance

All procedures carried out during this research were done in accordance with the guiding principles of research involving animals as recommended by the Research Ethics Committee of the University of Port Harcourt. Animals were kept in standard metal cages and at normal room temperature.

2.2. Procurement of Animal

A total of twenty-four (24) adult male Wistar rats weighing 100 – 250g were obtained from the Animal House of the Department of Pharmacology, Faculty of Basic Clinical Science, and University of Port Harcourt. They were housed in stainless steel cages (4 rats per cage) and kept in a well-ventilated room. The rats were fed with standard diet (livestock feeds Nig. Ltd. Ikeja, Nigeria) and water *ad libitum*. The standard guidelines for the use of experimental animals were adhered to.

2.3. Plant collection

Fresh leaves of *Alchornea cordifolia* were obtained from Choba in Obio-Akpor Local Government Area of Rivers State, Nigeria. It was properly identified at the herbarium unit of Plant Science and Biotechnology Department of the University of Port Harcourt where the plant was identified by Dr. Ekeke Chimezie.

2.4. Plant Preparation and Extraction

The fresh leaves of *Alchornea cordifolia* were washed under running water to remove dirt and debris and other contaminants. The leaves were dried in open air, avoiding direct contact with sun for duration of three (3) weeks, after which these leaves were ground to fine powder using mortar and pestle. 20g of the ground powder was soaked in 200ml of distilled water, the mixture was shaken vigorously for 10mins and it was kept in the laboratory bench for 24hours. The mixture was sieved with a white cotton cloth and filtered with Whatman paper No.1. The extract was then separated into different concentrations and diluted in different volume of water in plastic bottles as follows: 10mls of extract was diluted in 90ml of distilled water, 15ml of extract was diluted in 85ml of distilled water, and 20ml of extract was diluted in 80ml of distilled water.

2.5. Induction of Carbon-Tetrachloride Liver Damage

Liver damage was induced by the oral administration of 10ml of CCl₄ diluted with 10ml of olive oil in a ratio of 1:1 at 0.5mg/kg body weight except for the control animals [9].

2.6. Experimental Design

The rats were randomized into 6 experimental groups with each group consisting of four rats per cage. The Group 1 (positive control) rats were fed with commercially formulated feed and water only. The Group 2 (Olive oil Group) received vegetable oil 0.5mg/kg i.p., Group 3 (CCl₄ control) received CCl₄ and 0.5mg/kg vegetable oil i.p at a ratio of 1:1, was induced with CCl₄, Group 4 received CCl₄: vegetable oil (1:1) 0.5mg/kg and 100mg/kg aqueous leaves extract of *A. cordifolia*, Group 5 received CCl₄: vegetable oil (1:1) 0.5mg/kg and 150mg/kg aqueous leaves extract of *A. cordifolia*, Group 6 received CCl₄: vegetable oil (1:1) 0.5ml/kg and 200mg/kg aqueous leaves extract of *A. cordifolia*. After fourteen (14) days of treatment, the animals were anaesthetized using cotton wool soaked in chloroform in a desiccator. The anaesthetized animals were placed on a dissecting slab and blood sample for biochemical assay was collected into lithium-heparin bottles from the jugular vein and liver tissues of the rats were collected and stored in 10% formalin for histological examination. The blood and tissue samples were taken to the Chemical Pathology Laboratory of University of Port Harcourt Teaching Hospital (UPTH) for various analyses.

2.7. Biochemical Assays

Biochemical analysis of the serum enzymes for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was by the method of Reitman and Frankel [10]. Alkaline phosphatase (ALP) was assayed according to the method of REC [11].

2.8. Histopathology

The sections of the preserved liver and cardiac muscle slices obtained with the use of a tissue slicer were fixed on microscopic slides and stained before observing them under the microscope following the method described by Baker and Silverton [12].

2.9. Method of Data Analysis

Data were analyzed using SPSS version 23.0. All data obtained were expressed as Mean \pm SD. One-way analysis of variance (ANOVA) was used to compare the means between and within the groups and a p -value <0.05 was considered significant. A Tuckey's post-hoc test was also applied to assess significant differences between groups.

3. Results

Table 1 Effect of *Alchornea cordifolia* aqueous leaves extract on ALT, AST and ALP liver enzyme parameters of CCL₄-induced liver damage in Wistar rats

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Positive Control	36.12 \pm 1.58	121.20 \pm 2.67	244.16 \pm 4.12
0.5mg/kg Olive oil	36.51 \pm 3.40	124.16 \pm 4.10	231.17 \pm 4.85
CCL ₄ + 0.5mg/kg Olive Oil	70.24 \pm 2.21*	164.89 \pm 3.16*	366.51 \pm 3.46*
CCL ₄ + Olive Oil + 100mg/kg AC	48.27 \pm 2.53*#	137.18 \pm 2.14*#	328.48 \pm 3.76*#
CCL ₄ + Olive Oil + 150mg/kg AC	41.95 \pm 1.94 *#	130.05 \pm 1.42*#	268.40 \pm 4.82*#
CCL ₄ + Olive Oil + 200mg/kg AC	37.32 \pm 2.06*#	123.15 \pm 1.23 *#	251.90 \pm 3.22*#

Each value represents mean \pm SD, Values marked with (*) differ significantly from positive control (1ml of Water) value (* p <0.05) while those marked with (#) differ significantly from CCL₄ + Olive oil group (# p <0.05). CCL₄= Carbon-Tetrachloride, AC = *Alchornea cordifolia* Extract

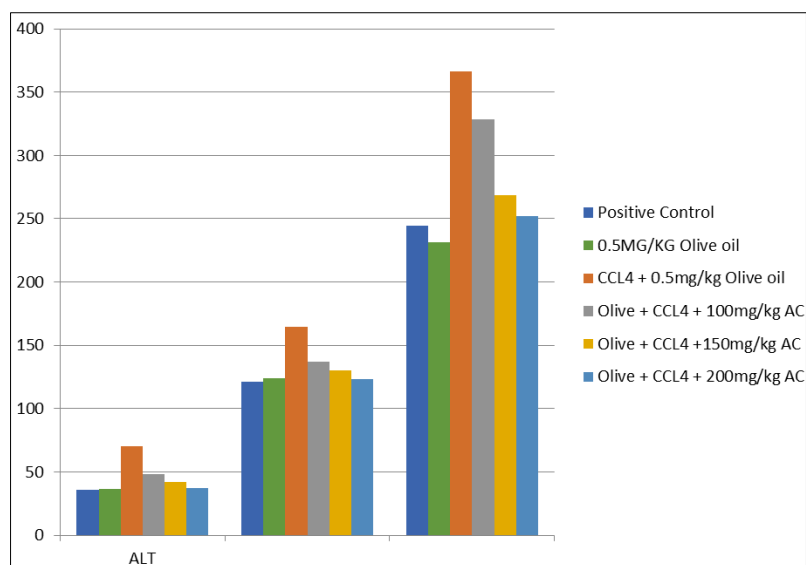


Figure 1 Effect of *Alchornea cordifolia* aqueous leaves extract on ALT, AST and ALP liver enzyme parameters of CCL₄-induced liver damage in Wistar rats

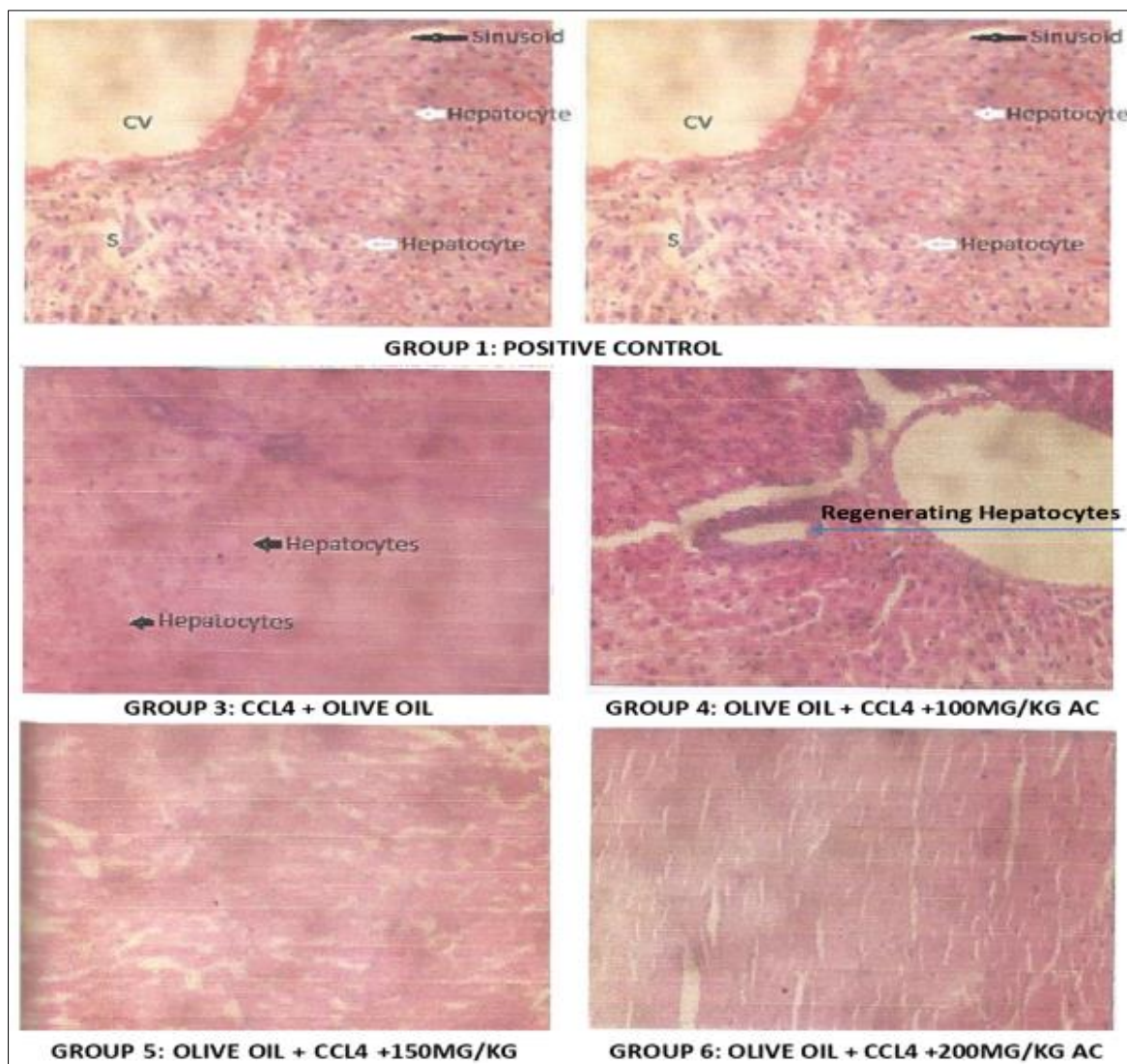


Figure 2 Photomicrograph of liver tissue. Group1: Positive control group showing a normal liver architecture, normal sinusoids and hepatocytes; Group 3 (CCL₄ + 0.5mg/kg Olive oil): Showing necrosis of the liver; Group 4 (0.5mg/kg Olive oil + CCL₄ + 100mg/kg): Showing some degree of reversal of induced liver lesion evident by regenerating hepatocytes; Group 5 (0.5mg/kg Olive oil + CCL₄ + 150mg/kg): Showing improved sinusoids; Group 5 (0.5mg/kg Olive oil + CCL₄ + 200mg/kg): Improved liver architecture showing improved structure of hepatocytes and normal sinusoids. Magnification: x200

4. Discussion

Hepatic damage caused by carbon tetrachloride is a typical experimental approach for studying the hepatoprotective effects of medicinal plants and medicines [13]. The activities of serum ALT, AST, and ALP, which are enzymes that were initially present in high concentration in the cytoplasm, can be used to assess liver function [13]. When the liver is damaged, these enzymes seep into the bloodstream in proportion to the severity of the damage [14]. When rats were given carbon tetrachloride, it caused hepatotoxicity by activating metabolic pathways; as a result, it produces toxicity only in liver cells with a semi-normal metabolic activity. The endoplasmic reticulum's cytochrome P-450 dependent mixed oxidase converts carbon tetrachloride to trichloromethyl free radical (CCL₃), which reacts with cellular lipids and proteins in the presence of oxygen to cause lipid peroxidation. This causes alterations in the endoplasmic reticulum and other membranes, as well as a loss of metabolic enzyme activation, protein synthesis, and glucose-6-phosphatase activation, all of which lead to liver injury. This could explain what happened in the current study's CCL₄-treated groups (Groups 3-6).

The experimental groups treated with different doses of aqueous extract doses of *Alchornea cordifolia* showed a significant ($p < 0.05$) dose dependent reduction in blood serum levels of AST, ALT, and ALP as a revealed from this investigation. The reduction of increased serum enzymes (particularly ALT and ALP) could be related to the inhibition of intracellular enzyme leakage by their membrane stabilizing activity, which is mediated by the administration of *Alchornea cordifolia* aqueous extract dosages, which have antioxidant properties. Reduced ALT levels suggest that the liver cells' secretory system is improving quickly. Any hepatoprotective drug's efficacy is determined by its ability to either reduce the detrimental effect or restore normal liver physiology after a hepatotoxin has disrupted it. The current findings show that the plant's antioxidant properties lowered the generation of the trichloromethyl peroxide radical, hence minimizing tissue damage. As a result, the plant's hepatoprotective properties could be attributable to its antioxidant capability. This finding is consistent with research by Osadebe *et al.* [15], Jacob *et al.* [16] and Etienne *et al.* [17].

Phytochemicals found in *A. cordifolia* leaves include tannins, phenolic acids, flavonoids, and alkaloids, according to other studies [15, 16, 18]. The presence of flavonoids and tannins in the current study may have contributed to the antioxidant effect observed. Foliage tannins contain powerful antioxidant and anti-inflammatory properties, according to Perchellet *et al.* [19]. Flavonoids have also been found to have antioxidant and anti-inflammatory properties [20, 21]. Tannins and similar substances, according to Okuda *et al.* [22], may protect liver cells from the damaging effects of lipid peroxide by reducing lipid peroxide levels. Plants that contain saponins are also said to have antioxidant qualities [21, 23]. Furthermore, phenols, lignans, essential oils, terpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids, and xanthenes have been found in liver-protecting herbal medicines [23]. As a result, it's probable that additional secondary metabolites of the plant are responsible for the plant's antioxidant and thus hepatoprotective effect. In the CCL₄-induced toxic group, histological examination of the liver revealed hepatocellular disintegration and inflammation in the liver (group 3). However, in CCL₄ pretreated rats, treatment of aqueous leaf extracts at dosages of 100-200mg/kg reduced the production of histopathological damage, as seen in figure 2. This is in line with Jacob *et al.* [16] research. The ability of *Alchornea cordifolia* to restore damaged hepatocytes in CCL₄-induced Wistar rats was demonstrated in this study. As a result, *Alchornea cordifolia* leaf extract is an excellent hepatoprotector.

5. Conclusion

Our findings showed that after CCL₄-induced liver damage was established, treatment with *Alchornea cordifolia* extracts considerably decreased and even reversed the damage in rats. Because the model of CCL₄-induced liver damage in rats mimics many of the hallmarks of human liver fibrosis, leaf extracts of *Alchornea cordifolia* may be useful hepatoprotectors in the diets of patients with hepatopathies.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest exist.

Statement of ethical approval

All procedures carried out during this research were done in accordance with the guiding principles of research involving animals as recommended by the Research Ethics Committee of the University of Port Harcourt.

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