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Evaluation of the Biochemical and Morphological Effects of *Annona muricata* Leaf Extract Against Carbon Tetrachloride-Induced Toxicity on the liver of Male Wistar Rats

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ABSTRACT

Carbon tetrachloride (CCl₄) is a known toxicant capable of inducing oxidative liver damage via free-radical generation, with concurrent haematological disturbances arising from oxidative haemolysis and bone marrow suppression. *Annona muricata* Linn. a medicinal plant with documented antioxidant and anti-inflammatory properties could have therapeutic efficacy against established CCl₄-induced injury. However, there is a dearth of information on the basis of its hepato and haemato-protective potential. To evaluate the hepatoprotective and haematoprotective effects of graded doses of *A. muricata* leaf extract (AMLE) in CCl₄-induced male Wistar rats. Twenty-five male Wistar rats (n = 5/group) received a single intraperitoneal CCl₄ dose (1 mL/kg, 1:1 v/v) on day 1, followed by oral treatment with 0, 50, 100, or 200 mg/kg/day AMLE for 14 days while the control received distilled water only. The biochemical markers determined were alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, albumin, aspartate aminotransferase (AST), catalase (CAT), malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and lipid profile. The full blood count was performed and liver architectural structure were also examined. CCl₄ induced significant (p < .05) elevated levels of ALT, AST, ALP, albumin and bilirubin with reduced total protein alongside induced oxidative stress (elevated MDA levels); depleted antioxidant parameters (SOD, CAT, GSH) and haematotoxicity (leucocytosis, neutrophilia, anaemia, thrombocytopenia, lymphopenia, monocytosis). Post-treatment with 100 and 200 mg/kg AMLE significantly reversed biochemical and haematological perturbations in a dose-dependent manner. However, H&E histopathology revealed that only the 50 mg/kg group exhibited partial hepatocellular recovery; the 100 and 200 mg/kg groups retained marked hepatic lesions comparable to the CCl₄-only group, suggesting dose-dependent divergence between biochemical and morphological endpoints. AMLE at 100–200 mg/kg significantly ameliorates CCl₄-induced biochemical and haematological toxicity but does not appear to restore hepatic histoarchitecture at these doses over a 14-day post-treatment period.

1. INTRODUCTION

The liver is the principal site of xenobiotic biotransformation in mammals, and its unique metabolic burden renders it highly vulnerable to chemically induced damage. Carbon tetrachloride (CCl₄) is a toxicant well researched in preclinical pharmacology due to its hepatotoxic effect. CCl₄

is bio-activated by cytochrome P₄₅₀ 2E1 to trichloromethyl radical (•CCl₃) after uptake by the hepatocytes and this spontaneously reacts with available molecular oxygen to generate trichloromethylperoxyl radical (CCl₃OO•). Hence, the reactive species generated initiate



a self-propagating cascade of lipid peroxidation that culminates in hepatocyte necrosis, depletion of antioxidant, and cytosolic transaminases leaked into the blood (Weber et al., 2003; Allameh et al., 2023). This lipid peroxidation thus affect the lipid profile of the system.

Also, CCl₄ exerts significant haematotoxicity via oxidative haemolysis from free-radical attack on erythrocyte membranes leading to the decline in the concentration of haemoglobin (Hb), red blood cell (RBC) count, and packed cell volume (PCV) that initiate normocytic anaemia (Ifeoluwa et al., 2012, Mustafa & Qaid Hadwan (2020), Orrico et al., 2023). Consequently, a suppression of haematopoietic progenitor activity is triggered by reactive oxygen species (ROS) in the bone marrow, contributing to thrombocytopenia (Asmaa et al., 2018). Moreover, hepatic dysfunction triggers leucocytosis alongside neutrophilia and a reduction in relative lymphocyte count by oxidative lymphocyte apoptosis (Ujowundu et al., 2021).

Annona muricata Linn. (*A. muricata*) belong to Annonaceae family, popularly called soursop is a pan-tropical medicinal tree found in the Caribbean and widely grown across sub-Saharan Africa, Southeast Asia and South America. Numerous traditional use of the leaves have been documented in ethnopharmacological surveys this includes management of liver dysfunctions, cancer, hypertension, and diabetes mellitus (Moghadamtousi et al., 2015). *A. muricata* systematic phytochemical investigation revealed that the leaves is rich in

Annonaceous acetogenins a key bioactive component, flavonoids, phenolic acids, alkaloids, tannins, and terpenoids (Mutakin et al., 2022). Also, polyphenolic fraction of *A. muricata* is a potent free-radical scavenger identified by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) as well as upregulating Nrf2-mediated antioxidant gene expression, while acetogenins distrupts mitochondrial complex I and regulates superoxide production (Sanni et al., 2014).

Though, there are reports on the hepatoprotective potential of *A. muricata* extracts but there is a dearth of information on its dual role as both hepato- and haemato- protective agent.

2. Materials and Methods

2.1 Plant Material and Preparation

Fresh, mature leaves of *Annona muricata* Linn. were gotten from a garden in Osogbo, Osun State, Nigeria. It was authenticated and certified in Department of Plant Biology herbarium by a plant taxonomist, and deposited with a voucher number 2026040005. The leaves were washed thoroughly with water and air-dried until a constant weight was obtained. The dried material was then pulverised with a blender to get a fine powder. *A. muricata* leaves powder was then soaked and macerated with 80% ethanol (1:10 w/v) with intermittent agitation for 72 h. It was filtered and concentrated in a rotary evaporator under reduced pressure at 45°C while the resulting concentrate was freeze-dried. The percentage yield was calculated to 39.4% and the stock suspensions prepared using 5% dimethyl sulphoxide (DMSO) and diluted distilled to required doses when needed.

2.3 Experimental Design

The experimental rats were randomly grouped into five ($n = 5$) as illustrated in the table below

Groups	Treatments
Group 1	positive control group, received only distilled water
Group 2	negative control group, received CCl ₄ -only (1 ml/kg/day, i.p.)
Group 3	received CCl ₄ + 50 mg/kg received CCl ₄ (day 1) + AMLE 50 mg/kg (p.o., days 2–15)
Group 4	received CCl ₄ + 100 mg/kg received CCl ₄ (day 1) + AMLE 100 mg/kg (p.o., days 2–15)
Group 5	received CCl ₄ + 200 mg/kg received CCl ₄ (day 1) + AMLE 200 mg/kg (p.o., days 2–15)

All animals were sacrificed under light diethylether anaesthesia 24 h after the last AMLE treatment while the blood was collected via cardiac puncture into EDTA test tubes for haematology and plain tubes with centrifugation at $3,000 \times g$ for 10 min for serum collection. The liver tissue was excised for biochemical assays, and part fixed in formal saline for histopathological studies.

2.4 Hepatic Function Tests

The activities of ALT and AST (Reitman and Frankel, 1967), and ALP (Statland et al., 1972) in the serum were spectrophotometrically determined using Randox Laboratories kits. Albumin by dye-binding techniques (Doumas et al. 1971), Total bilirubin by the diazonium salt method (Pearlman et al., 1974), and total protein by the Biuret colorimetric method, all using Randox reagent kits following the manufacturer instructions.

2.5 Hepatic Oxidative Stress Assays

Liver tissue was homogenized in ice-cold 0.25M sucrose buffer and the homogenates obtained were

assayed for malondialdehyde (MDA) concentration using Ohkawa et al. (1979) method. The protocol of Misra and Fridovich (1972) was used to determine superoxide dismutase (SOD) activity by epinephrine autoxidation method. Catalase (CAT) activity was assessed by Aebi (1984) protocol via decomposition of hydrogen peroxide while reduced glutathione (GSH) was measured following Ellman (1959) method.

2.6 Haematological Analysis

The blood was collected into EDTA tubes and analyzed for total red blood cell count (RBC, $\times 10^{12}/L$), packed cell volume (PCV, %), white blood cell count (WBC, $\times 10^9/L$), haemoglobin concentration (Hb, g/dL), platelet count ($\times 10^9/L$), and differential leucocyte count (neutrophil%, lymphocyte%, monocyte%) using the automated haematology analyzer. The measurement was performed in duplicate and averaged.

2.7 Liver Histopathology

The liver tissue fixed with formalin was processed using alcohol, xylene, paraffin, and then sectioned using a rotary microtome (Leica RM2235, Germany). Thereafter, the sections were processed via dewaxing, rehydration, and staining with haematoxylin and eosin (H&E) and then viewed under a microscope. Lesions were evaluated semi-quantitatively and scored on a four-point scale: 1 = normal; 2 = mild lesions; 3 = moderate lesions; 4 = severe lesions, based on hepatocyte necrosis, vascular degeneration, Kupffer cell activation, and overall lobular architecture.

2.8 Statistical Analysis

Data were expressed as mean $\pm$ SEM (n=5). Group comparisons were done by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test and statistical significance set at $p < 0.05$.

3. Results

3.1 Effect of AMLE on Serum Liver Function Markers

Intraperitoneal CCl₄ administration triggers a significant ($p < 0.05$) elevation in serum ALT (17.21 ± 2.00 U/L), AST (24.71 ± 1.60 U/L), ALP (9.45 ± 0.54 U/L), and total bilirubin (3.06 ± 0.23 mg/dL) compared with the control (ALT: 9.55 ± 1.22 U/L; AST: 13.74 ± 1.14 U/L; ALP: 3.70 ± 0.26 U/L; bilirubin: 2.26 ± 0.12 μ mol/L). A significant reduction (2.65 ± 0.23 g/dL) in total protein was recorded in CCl₄-only group when compared with the control (3.62 ± 0.70 g/dL). Although, 200 mg/kg treated group was able to drastically reduce the activity of ALT to 10.42 ± 0.76 U/L, AST to 18.67 ± 0.46 U/L, ALP to 5.75 ± 0.66 U/L as well as the concentration of bilirubin to 2.07 ± 0.20 mg/dL, and total protein to 3.84 ± 0.35 g/dL at significant difference ($p < .05$) from the CCl₄-only group (Table 1). Also, a drastic reduction was recorded in the albumin of CCl₄-only group (2.20 ± 0.10 g/dL) when compared with the control (2.99 ± 0.48 g/dL) while treatment with 200 mg/kg AMLE caused significant ($p < 0.05$) elevation in albumin to 3.70 ± 0.03 g/dL when compared with the CCl₄-only group.

Table 1. Effect of AMLE Post-treatment on Serum Biochemical Markers in CCl₄-induced Male Wistar Rats

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
ALT (U/L)	9.55 ± 1.22	17.21 ± 2.00 ^a	16.70 ± 0.73 ^a	12.94 ± 1.46 ^{ab}	10.42 ± 0.76 ^b
AST (U/L)	13.74 ± 1.14	24.71 ± 1.60 ^a	20.77 ± 1.27 ^a	22.21 ± 1.53 ^{ab}	18.67 ± 0.46 ^b
ALP (U/L)	3.70 ± 0.26	9.45 ± 0.54 ^a	6.19 ± 0.32 ^a	5.58 ± 0.15 ^{ab}	5.75 ± 0.66 ^b
Total Bilirubin (mg/dL)	2.26 ± 0.12	3.06 ± 0.23 ^a	2.00 ± 0.50 ^a	2.12 ± 0.13 ^{ab}	2.07 ± 0.20 ^b
Total Protein (g/dL)	3.62 ± 0.70	2.65 ± 0.23 ^a	2.96 ± 0.23 ^a	3.48 ± 0.70 ^{ab}	3.84 ± 0.35 ^b
Albumin (g/dL)	2.99 ± 0.48	2.20 ± 0.10 ^a	2.76 ± 0.05 ^a	2.96 ± 0.35 ^b	3.70 ± 0.03 ^b

(Mean ± SEM, n = 5), while ^a*p* < 0.05 vs. Group 1; ^b*p* < .05 vs. Group 2; ^{ab}*p* < 0.05 vs. Group 1 and Group 2.

3.2 Effect of AMLE on Hepatic Oxidative Stress Biomarkers

CCl₄ induction trigger significant (*p* < 0.05) elevation in the concentration of MDA (2.10 ± 0.21 µM) when compared with the control (1.60 ± 0.02 µM) with concurrent depletion of the antioxidants markers activities SOD (20.54 ± 0.97 U/L), CAT (7.67 ± 0.48 µmoles/min/ml), and GSH concentration (17.60 ± 0.70 mM) when compared with the control 32.07 ± 3.00 U/L (SOD), 8.43 ± 0.01 µmoles/min/ml (CAT), and 27.53 ± 2.72 mM (GSH) while AMLE post-treatment caused a reversal with increase in dose. However, 200 mg/kg treatment significantly (*p* < 0.05) decrease the concentration of MDA to 1.60 ± 0.03 µM, while SOD (31.59 ± 1.88 U/L), CAT (8.46 ± 0.01 µmoles/min/ml), and GSH (31.67 ± 0.99 mM) were significantly elevated when compared with the CCl₄-only group. Though, the 50 mg/kg treatment significantly reduced MDA and restored the antioxidant enzyme activities when compared with the CCl₄-only group (Table 2).

Table 2. Effect of AMLE Post-treatment on Hepatic Oxidative Stress Biomarkers in CCl₄-induced Male Wistar Rats

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
MDA (µM)	1.60 ± 0.02	2.10 ± 0.21 ^a	1.61 ± 0.04 ^a	1.59 ± 0.04 ^b	1.60 ± 0.03 ^b
SOD (U/L)	32.07 ± 3.00	20.54 ± 0.97 ^a	24.44 ± 1.05 ^a	24.77 ± 2.31 ^{ab}	31.59 ± 1.88 ^b
CAT (µmoles/min/ml)	8.43 ± 0.01	7.67 ± 0.48 ^a	8.43 ± 0.02 ^a	8.42 ± 0.01 ^{ab}	8.46 ± 0.01 ^b
GSH (mol/g tissue)	27.53 ± 2.72	17.60 ± 0.70 ^a	19.51 ± 0.34 ^a	19.51 ± 1.13 ^{ab}	31.67 ± 0.99 ^b

Values are Mean ± SEM (n = 5). ^a*p* < 0.05 vs. Group 1; ^b*p* < .05 vs. Group 2; ^{ab}*p* < 0.05 vs. Group 1 and Group 2.

3.3 Effect of AMLE on Haematological Parameters

In CCl₄-induced Wistar rat, there is a significant ($p < .05$) increase in WBC ($9.15 \pm 0.10 \times 10^9/L$ vs. $7.35 \pm 0.17 \times 10^9/L$ in controls), neutrophils ($46.00 \pm 2.45\%$ vs. $32.50 \pm 2.63\%$), monocytes ($5.50 \pm 0.50\%$ vs. $2.50 \pm 0.50\%$), but caused a drastic decrease in lymphocytes ($46.00 \pm 1.96\%$ vs. $54.25 \pm 5.45\%$), Hb (9.55 ± 0.21 g/dL vs. 11.81 ± 0.19 in control), RBC (1.55 ± 0.03 vs. $1.71 \pm 0.04 \times 10^{12}/L$ in control), and PCV ($31.25 \pm 0.95\%$ vs. $37.25 \pm 1.80\%$ in control). Also, 200 mg/kg dose significantly ($p < 0.05$) decreased WBC $7.23 \pm 0.13 \times 10^9/L$, neutrophils ($35.33 \pm 7.42\%$), monocyte percentage ($2.67 \pm 0.67\%$) when compared with CCl₄-only group. Moreover, the lymphocytes ($62.00 \pm 7.02\%$) and platelets ($145.53 \pm 0.13 \times 10^9/L$) revealed a significant ($p < 0.05$) increase when compared with the CCl₄-only group. Though the PCV at 200 mg/kg ($29.67 \pm 0.33\%$) showed no significant difference from the CCl₄-only group (29.67 ± 0.33) at $p < .05$ (Table 3).

Table 3. Effect of AMLE Post-treatment on Haematological Parameters in CCl₄-induced Male Wistar Rats

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
WBC ($\times 10^9/L$)	7.35 ± 0.17	9.15 ± 0.10^a	8.95 ± 0.28^a	7.98 ± 0.11^{ab}	7.23 ± 0.13^b
RBC ($\times 10^{12}/L$)	1.71 ± 0.04	1.55 ± 0.03^a	1.53 ± 0.12^a	1.49 ± 0.05^a	1.67 ± 0.09^b
Platelets ($\times 10^9/L$)	154.33 ± 1.33	121.08 ± 0.61^a	126.88 ± 0.91^a	132.88 ± 1.73^{ab}	145.53 ± 0.32^b
PCV (%)	37.25 ± 1.80	31.25 ± 0.95^a	32.75 ± 0.95^a	30.50 ± 1.55^a	29.67 ± 0.33^a
Hb (g/dL)	11.81 ± 0.19	9.55 ± 0.21^a	9.68 ± 0.28^a	9.45 ± 0.17^a	10.37 ± 0.32^{ab}
Neutrophils (%)	32.50 ± 2.63	46.00 ± 2.45^a	44.00 ± 2.94^a	44.75 ± 2.87^a	35.33 ± 7.42^b
Lymphocytes (%)	54.25 ± 5.45	46.00 ± 1.96^a	51.50 ± 4.03	52.50 ± 2.87	62.00 ± 7.02^b
Monocytes (%)	2.50 ± 0.50	5.50 ± 0.50^a	3.00 ± 0.58^b	3.25 ± 0.48^b	2.67 ± 0.67^b

Values are Mean $\pm$ SEM ($n = 5$). ^a $p < 0.05$ vs. Group 1; ^b $p < .05$ vs. Group 2; ^{ab} $p < 0.05$ vs. Group 1 and Group 2.

3.4 Liver Histopathology

The H&E-stained liver sections show distinct histopathological profiles across groups (Figures 1A– E; Table 4). The normal control revealed intact hepatic lobular architecture, with normal central veins surrounded by well-preserved hepatocytes arranged in regular cords (Figure 1A). Hepatic sinusoids were patent, and no inflammatory infiltrates, necrosis, or vascular changes were observed.

However, the CCl₄-only showed marked hepatocyte necrosis with nuclear pyknosis, extensive vascular degeneration (VC), activation and enlargement of Kupffer cells (K),

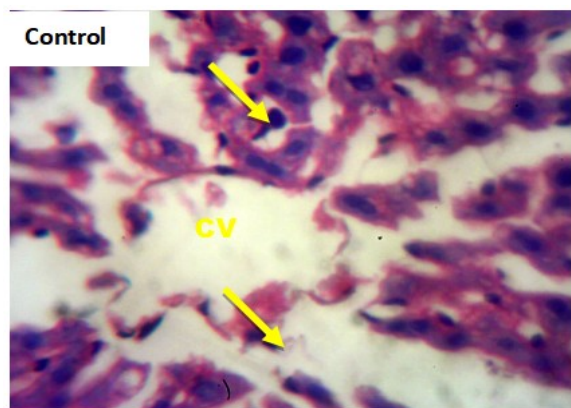
dilated central veins (DCV) with erythrocytic congestion, necrotic foci (N) scattered throughout the parenchyma, and engorged blood sinusoids (Figure 1B).

Also, groups treated with 50 mg/kg AMLE showed partial histological recovery with mild dilated and blended central veins surrounding hepatocytes, mild residual necrotic foci, and a generally near-normal architecture (Figure 1C). Furthermore, treatment with 100 and 200 mg/kg AMLE caused histopathological changes comparable to the CCl₄-only, displaying marked hepatocyte necrosis, vascular degeneration, dilated central veins with erythrocytic congestion, activated Kupffer cells, and necrotic foci (Figures 1D and 1E).

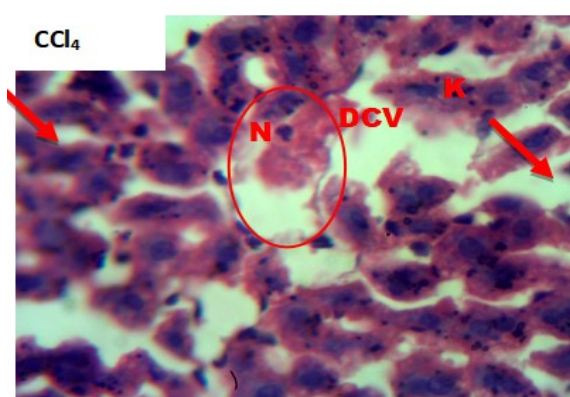
Table 4. Semi-Quantitative Histopathological Scoring of Liver Sections from CCl₄-induced Rats Following AMLE Post-treatment

Groups	Hepatocyte Necrosis	Vascular Degeneration	Kupffer Cell Activation	Overall Architecture	Score (1–4)
Group 1	None	None	None	Normal	1
Group 2	Marked	Marked	Marked	Abnormal	4
Group 3	Mild	Mild	Absent	Near-normal	2
Group 4	Marked	Marked	Present	Abnormal	3
Group 5	Marked	Marked	Present	Abnormal	3

Histopathological score: 1 = normal; 2 = mild lesions; 3 = moderate lesions; 4 = severe lesions.



A: Photomicrographs of liver sections from the Control showed normal globular architecture is preserved. The central vein (CV) is of normal calibre, surrounded by well-organised hepatocyte cords (yellow arrows). No necrosis, inflammation, or vascular degeneration is observed (x400; H&E)



B: Photomicrographs of liver sections from the CCl₄-only group showed severe hepatic injury, widespread hepatocyte necrosis (red circles), vascular degeneration (VC), activated Kupffer cells (K), dilated central veins (DCV) with erythrocytic congestion, necrotic foci (N), and congested blood sinusoids (x400; H&E)

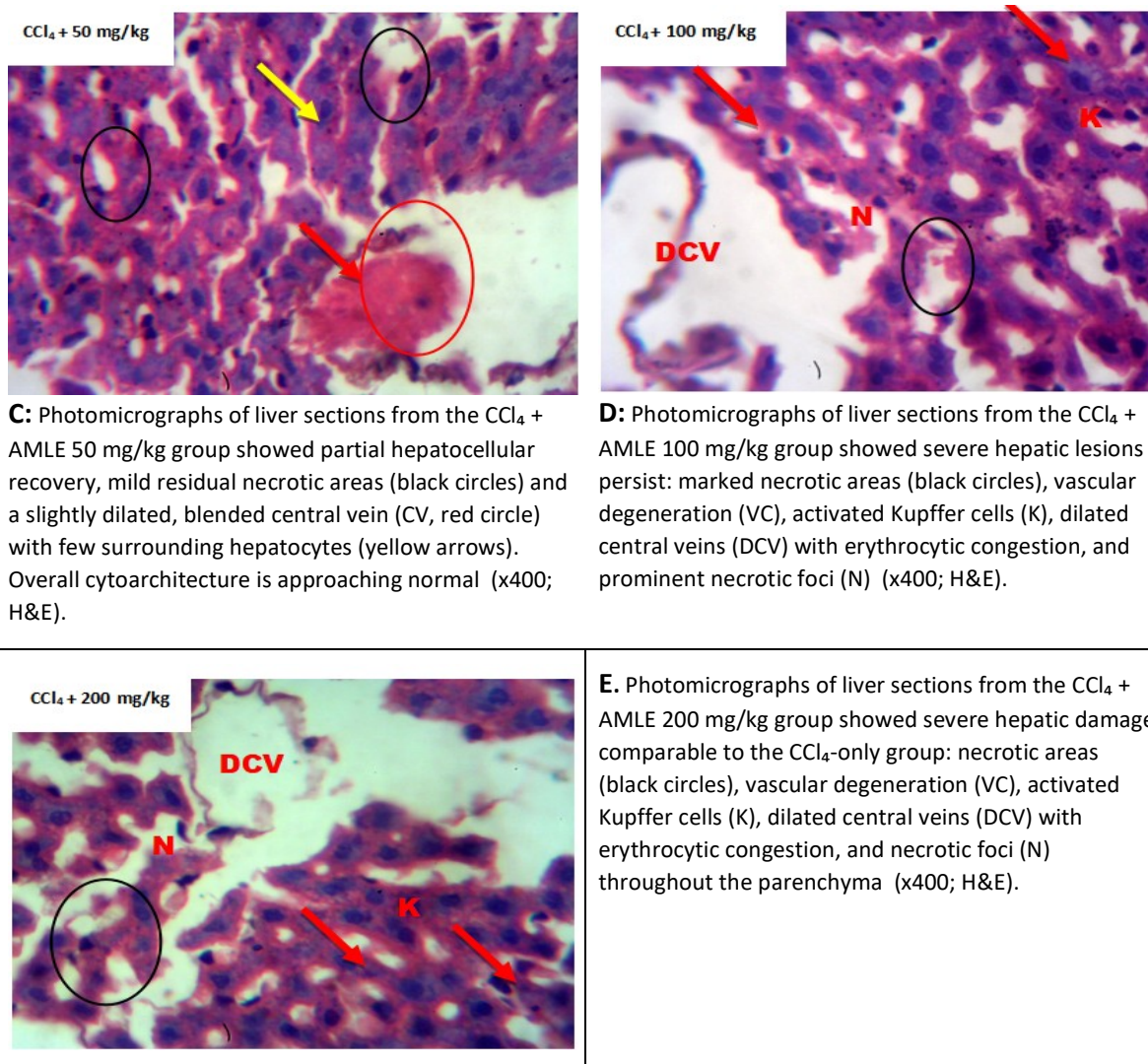


Figure 1(A-E): Photomicrographs of liver sections stained with H&E at x400 from the Control, CCl₄ and CCl₄ + AMLE treated groups

3.5 Effect of AMLE on Serum Lipid Profile

CCl₄ induction caused a significant ($p < 0.05$) elevation in total cholesterol (59.48 ± 3.89 vs. 43.22 ± 2.75 mg/dL in controls), triglycerides (37.09 ± 0.72 vs. 28.89 ± 0.65 mg/dL), LDL-cholesterol (40.46 ± 0.52 vs. 23.91 ± 1.38 mg/dL), and vice versa in HDL-cholesterol (30.59 ± 1.13 vs. 47.54 ± 3.01 mg/dL) all compared with the controls. The results also revealed that 200 mg/kg reduced the total cholesterol to 48.16 ± 3.33 mg/dL, triglycerides to 33.20 ± 3.34 mg/dL, and LDL to 24.12 ± 2.48 mg/dL while partially restoring HDL to 34.07 ± 2.28 mg/dL when compared with the CCl₄-only group (Table 5).

Table 5. Effect of AMLE Post-treatment on Serum Lipid Profile and Albumin in CCl₄-Challenged Male Wistar Rats

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Cholesterol (mg/dL)	43.22 ± 2.75	59.48 ± 3.89 ^a	57.87 ± 4.34 ^a	59.95 ± 4.70 ^a	48.16 ± 3.33 ^b
Triglycerides (mg/dL)	28.89 ± 0.65	37.09 ± 0.72 ^a	30.94 ± 2.14 ^a	38.11 ± 2.19 ^a	33.20 ± 3.34 ^b
HDL-Cholesterol (mg/dL)	47.54 ± 3.01	30.59 ± 1.13 ^a	29.26 ± 1.97 ^a	32.56 ± 3.12 ^a	34.07 ± 2.28 ^b
LDL-Cholesterol (mg/dL)	23.91 ± 1.38	40.46 ± 0.52 ^a	24.67 ± 0.70 ^b	26.74 ± 1.88 ^{ab}	24.12 ± 2.48 ^b

Values are Mean ± SEM (n = 5). ^a*p* < 0.05 vs. Group 1; ^b*p* < .05 vs. Group 2; ^{ab}*p* < 0.05 vs. Group 1 and Group 2.

4. Discussion

In this study, the ameliorative potential of AMLE oral post-treatment on CCl₄-induced hepatotoxicity and haematotoxicity in male Wistar rats was examined. The elevation of serum ALT, AST, ALP, and total bilirubin following CCl₄ induction revealed the disruption caused on hepatocyte membrane integrity, thereby enabling cytosolic enzyme leakage, and impairment of hepatobiliary transport function (Weber et al., 2003, Thkur et al., 2024). Moreover, the subsequent decline in total protein also confirms diminished hepatic synthetic capacity. However, the dose-dependent normalization of these parameters by AMLE post-treatment is consistent with findings by Quassou et al. (2021) and Johra et al., (2023), who reported that *C. europaea* stem extract and whole *G. lucidum* reversed CCl₄-elevated AST, ALT, and ALP in Wistar rats, and with dose-dependent hepatoprotection against CCl₄ and acetaminophen reported by Kuswinarti et al. (2021). The potential of AMLE could be attributed to its polyphenolic fraction like quercetin and chlorogenic acid derivatives which have been reported to directly

scavenge CCl₃OO• radicals, while flavone-mediated upregulation of Nrf2 transcription enhances hepatocellular expression of glutathione S-transferase, haem oxygenase-1, and NADH:quinone oxidoreductase — enzymes central to electrophile detoxification (Mutakin et al., 2022).

Also, MDA is a principal end product of lipid peroxidation that is generated through the decomposition of arachidonic acid and polyunsaturated fatty acids (Pomi et al., 2025). Hence the disruption on the polyunsaturated fatty acids results in excessive production of oxidizing molecules or caused an unstable antioxidant systems. Antioxidants are molecules that neutralizes the effect of harmful substances. The depletion of SOD, CAT, and GSH in the CCl₄-only group revealed both direct inactivation of these enzymes by ROS and consumption of non-enzymatic antioxidant reserves. Hence, the restoration of hepatic MDA, SOD, CAT, and GSH by AMLE treatment further corroborates its antioxidant mechanism of action. Interestingly, the dose-dependent reversal caused by 100 and 200 mg/kg AMLE treatment can be linked with the report

of Adewole and Ojewole (2009) documented on *A. muricata* leaf aqueous extract in streptozotocin-treated diabetic rats, and it is also consistent with the documented report on annonaceous acetogenins to suppress mitochondrial complex I-mediated superoxide ion generation (Moghadamtousi et al., 2015).

The haematotoxicity induced by toxicant could trigger leucocytosis, neutrophilia, lymphopenia, monocytosis, anaemia, and thrombocytopenia through multi-target toxicity of reactive oxygen and nitrogen species on blood cells and haematopoietic tissue. Moreover, Ifeoluwa et al. (2012) reported that oxidative haemolysis arising from peroxidative attack on erythrocyte phospholipid membranes reduces RBC count, Hb, and PCV, which is consistent with the observed normocytic anaemia produced by CCl₄-haematotoxicity. Leucocytosis with neutrophilia reflects the acute hepatic inflammatory response; the concomitant lymphopenia might likely caused an inability in the antioxidant systems leading to oxidative T-cell apoptosis (Ujowundu et al., 2021). Interestingly, thrombocytopenia may arise from both reduced thrombopoiesis secondary to bone marrow oxidative stress and increased platelet consumption at sites of vascular injury (Asmaa et al., 2018). In this study, the 200 mg/kg AMLE administration effectively normalized the leucocyte differential and reversed monocytosis, consistent with suppression of hepatic NF- κ B-mediated inflammatory cytokine (TNF- α , IL-6) production reported for flavonoid-rich plant extracts (Moghadamtousi et al., 2015; Jomova et al.,

2025). Interestingly, erythroid parameters (RBC, Hb, PCV) showed incomplete normalization at all doses administered, suggesting that erythropoietic recovery requires a longer post-treatment interval than the two weeks duration.

The study also revealed that biochemical, haematological and histopathological recovery at AMLE dose 100 and 200 mg/kg restores the serum transaminases (ALP, AST and ALP), antioxidant indices, and haematological parameters. However, the H&E histopathology revealed persistent severe hepatocyte necrosis, vascular degeneration, Kupffer cell activation, and sinusoidal congestion comparable to the CCl₄-only group. Though, 50 mg/kg dose shows the best histological outcome near-normal lobular cytoarchitecture despite producing smaller biochemical improvements. Dzhemileva et al. (2023) documented that annonaceous acetogenins at higher concentrations have documented inhibitory effects on mitochondrial complex I in normal hepatocytes and this may impair hepatocyte ATP production, thereby leading to delay in hepatocellular regeneration despite adequate antioxidant defence. Also, cytotoxic acetogenins that demonstrate broad-spectrum selectivity towards rapidly proliferating cells could theoretically suppress hepatocyte regeneration at doses that suppresses inflammation and oxidative injury markers. Interestingly, the biochemical parameters measured reflected a cellular leakage and residual enzymatic activity which may improve through mechanisms that do not require histological resolution within the treatment period.

5. Conclusion

This study provides evidence that oral post-treatment with *Annona muricata* leaf extract at 50–200 mg/kg ameliorates CCl₄-induced hepatotoxicity and haematotoxicity in male Wistar rats in a dose-dependent manner across biochemical and haematological endpoints. At the morphological level, however, only the lowest dose (50 mg/kg) produced an observable improvement in hepatic histoarchitecture, while the 100 and 200 mg/kg doses maintained hepatic lesions comparable in severity to the CCl₄-only group despite the biochemical profiles. These unparallel biochemical histological outcomes highlight a critical limitation of relying exclusively on only biochemical markers to affirm the hepatoprotective efficacy, and implicate a possible interference of higher AMLE doses with hepatocellular regeneration. Hence, there is the need for researcher to isolate the main bioactive in AMLE and examine its efficacy.

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