The Journal of Phytopharmacology 2025; 14(1):14-22
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2025; 14(1): 14-22
January- February
Received: 21-12-2024
Accepted: 23-02-2025
Published: 23-03-2025

doi: 10.31254/phyto.2025.14103

Akeem Adewale Yusuff
Department of Medical Biochemistry, Faculty of Science, University of Ilesa, Ilesa, Osun State, Nigeria

Temidayo Ogunmoyole
Department of Medical Biochemistry, Faculty of Basic Medical Science, College of Medicine, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria

Olaitan Daniel Johnson
Department of Biochemistry, School of Applied Sciences and Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria

Correspondence:
Akeem Adewale Yusuff
Department of Medical Biochemistry, Faculty of Science, University of Ilesa, Ilesa, Osun State, Nigeria
Email: yusufflife@gmail.com

Protective effects of *Amaranthus spinosus* leaf extract against CCl_4-induced hepatorenal injury in rats: Insights from GC-MS analysis

Akeem Adewale Yusuff, Temidayo Ogunmoyole, Olaitan Daniel Johnson

ABSTRACT

Liver and kidney diseases present significant public health challenges due to limited treatment options, high management costs, and elevated mortality risks. This study evaluates the therapeutic effects of *Amaranthus spinosus* (*A. spinosus*) in mitigating CCl_4-induced hepatorenal injury in rats. Twenty-five adult male albino rats were divided into five groups: controls (distilled water and CCl_4), two treatment groups receiving *A. spinosus* extract (100 mg/kg and 200 mg/kg), and a standard drug group treated with silymarin (100 mg/kg). Biochemical evaluations, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, urea, and creatinine, were analyzed using standard protocols. Antioxidant markers, including reduced glutathione (GSH) levels, along with the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and lactate dehydrogenase (LDH) were also evaluated. Treatment with ethanolic *A. spinosus* extract significantly improved biochemical markers, restored antioxidant enzyme activity, and reduced CCl_4-induced hepatorenal damage. Histopathological analysis revealed restored liver and kidney architecture with reduced inflammation. Administration of the extract resulted in a reduction of pro-inflammatory cytokine IL-6 and LDH levels, alongside an increase in anti-inflammatory IL-10 levels, highlighting its anti-inflammatory potential. GC-MS analysis identified 16 bioactive compounds, including fatty acids, esters, phytol, β-panasinsene, squalene, and vitamin E, which likely contribute to its protective effects. These findings suggest that *A. spinosus* exhibits significant hepatoprotective, nephroprotective, antioxidant, and anti-inflammatory properties, highlighting its potential as a candidate for novel therapies targeting liver and kidney diseases. Further research is needed to elucidate its molecular mechanisms and clinical applications.

Keywords: *Amaranthus spinosus*, Hepatoprotective, Nephroprotective, Antioxidants, CCl_4-induced toxicity, GC-MS.

INTRODUCTION

Unintentional or negligent exposure to toxic chemicals from industrial, domestic, or environmental sources can impair physiological functions in living organisms. The severity of these effects depends on the chemical type, dose, length of exposure, and individual susceptibility [1]. These exposures often lead to severe organ impairment through mechanisms such as oxidative stress, inflammatory responses, and cellular damage, which, if unmitigated, can culminate in chronic diseases or acute organ failure [2].

The liver, a vital organ responsible for plasma protein synthesis, intermediary metabolism, energy regulation, and the excretion of nitrogen-containing compounds, is highly vulnerable to chemically induced injuries [3]. Toxicant induced-hepatotoxicity is primarily driven by intrinsic hepatocyte membrane damage caused by the metabolic biotransformation of xenobiotics into reactive metabolites and intermediates [4]. These metabolites trigger free radical production, resulting in oxidative stress, mitochondrial dysfunction, and disruptions in bile acid homeostasis [5,6]. The threat posed by chemically induced liver toxicity underscores the necessity for understanding and mitigating exposure to harmful substances.

Carbon tetrachloride (CCl_4) is a highly volatile and hazardous industrial chemical widely used as an experimental model for studying xenobiotic-induced liver injury due to its well-documented effectuality in reliably simulating liver damage in animal studies [7]. Humans are exposed to carbon tetrachloride (CCl_4) through oral, inhalation, and dermal pathways, which is known to cause pronounced hepatotoxicity primarily due to the metabolic activation by cytochrome P450 enzymes in hepatocytes [8]. These enzymes catalyze the reductive dehalogenation of CCl_4, producing trichloromethyl (CCl_3•) and peroxy trichloromethyl (CCl_3O_2•) radicals [9]. The highly reactive metabolites bind covalently to plasma

membrane proteins or induce lipid peroxidation by interacting with unsaturated fatty acids, initiating a cascade of deleterious mechanisms that disrupt the permeability of the mitochondria, endoplasmic reticulum, and plasma membrane, thus initiating cell death [10]. The excessive production of free radicals depletes antioxidant enzymes, exacerbates oxidative damage and contributing to pathological conditions [11,12]. Beyond its hepatotoxic effects, CCl_4 toxicity impacts multiple organs, including the lungs, heart, testes, brain, and kidneys, leading to systemic health complications [3]. Its nephrotoxic effects may result from direct toxic exposure or as a consequence of hepatotoxicity, underscoring its broad multi-organ harmful impact [3].

The use of plants for healing predates history with most conventional drugs originally derived from plant sources [13]. *A. spinosus* Linn. (Family: Amaranthaceae), widely cultivated in India, Sri Lanka, and other tropical regions is widely used in Ayurveda and is reported to have diverse medicinal properties, including, antioxidants [14-15], anti-inflammatory, antimalarial, antibacterial, antidiuretic, antiviral, anticancer, and hepatoprotective effects [16]. The whole plant's water extract is reported to enhances immunity [17], while its stem extract exhibits antimalarial activity [18,19]. *A. spinosus* contains bioactive compounds such as alkaloids, flavonoids, glycosides, phenolic acids, steroids, betalains, and carotenoids, which exhibit antioxidant, anticancer, antiviral, and antiparasitic properties [20]. Unique compounds such as amaranthoside and amaricin, along with significant bioactive components—including rutin, quercetin, 7-p-coumaroyl apigenin 4-O-β-D-glucopyranoside, α-xylofuranosyl uracil, β-sitosterol glucoside, β-D-ribofuranosyl adenine, and stigmasterol glycoside—were identified in the ethanolic whole-plant extract of *A. spinosus*, collectively enhancing its therapeutic potential [21].

Globally, approximately 2 million people die each year due to liver-related pathologies [23]. The increasing prevalence of hepatorenal diseases, coupled with the limitations of conventional treatments, underscore the urgent need for alternative therapeutic approaches.

It has been reported that the highest concentrations of phytochemicals are typically found in the mature leaves of the plants, as metabolic activity peaks during this stage of growth [22]. Hence, this study investigates the hepatorenal protective potential of *A. spinosus* leaf extract against *CCl_4*-induced toxicity in albino rats, aiming to characterize its bioactive compounds and provide pharmacological insights for improved therapeutic strategies.

MATERIALS AND METHODS

Plant Collection

A. spinosus leaves were obtained from a local farm in Ado Ekiti and authenticated at the Department of Plant Science, Ekiti State University, Ado Ekiti, Nigeria. A voucher specimen (herbarium number UHAE2021037) was deposited at the University herbarium.

Reagents and Chemicals

Adrenaline, malondialdehyde (MDA), phosphotungstic acid, magnesium acetate, creatine phosphate, potassium phosphate, hydrogen peroxide, ethylene diamine tetraacetate (EDTA), Ellman's reagent, reduced glutathione (GSH), and other chemicals and reagents used were of analytical grade, obtained from standard commercial suppliers. All diagnostic kits were products of Randox Chemical Ltd., England.

Preparation of Extract

Fresh *A. spinosus* leaves were rinsed with distilled water and air-dried. The dried leaves were pulverized using a Waring blender. Five hundred grams (500 g) of the powdered leaves were extracted with 3000 ml of 80% ethanol for 72 hours. The supernatant was carefully decanted and filtered using cheesecloth. The clear supernatant was then freeze-dried to obtain a crude extract, which was kept airtight for reconstitution with distilled water.

Animal Protocol

All experimental animals were used in accordance with established guidelines (Revised NIH Publications 2008, No. 8023) and the ethical approval of the Committee on Care and Use of Experimental Animal Resources, College of Medicine, Ekiti State University, Ado Ekiti, Nigeria. Twenty-five Wistar albino rats with an average weight of 170 g were purchased from the animal breeding colony of the College of Medicine, Ekiti State University, Ado Ekiti.

Animal Housing and Treatment

Five groups of five animals each were created, as described in Table 1. Experimental animals were housed in separate iron-meshed cages under standard conditions of temperature (24 ± 1°C), relative humidity, and a 12-hour light/dark cycle. The animals were provided unrestricted daily access to food and drinking water ad libitum. Rat bedding was changed daily to maintain good hygiene.

Table 1: Experimental group and treatment

Groups	Treatment
I: Negative Control (PC)	Distilled water only for 14 days
II: Positive Control (NC)	3 ml CCl_4 single administration without extract treatment
III	3 ml CCl_4 + 100 mg/kg b.w *A. spinosus*
IV	3 ml CCl_4 + 200 mg/kg b.w *A. spinosus*
V	3 ml CCl_4 + 100 mg/kg b.w Silymarin

Preparation of homogenates and serum

Rats were dissected and portion of whole blood was collected in plain sample bottles and allowed to stand for 1 h. Serum was prepared by centrifugation at 3000 rpm for 15 min at 25ºC. The clear supernatant was collected by decantation and used for the estimation of serum biochemical parameters. The liver, heart and kidney were excised using surgical scissors and forceps. They were trimmed of fatty tissue, washed in distilled water, blotted with filter paper and weighed. They were then chopped into bits and homogenized in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged at 3000 rpm at 4 °C for 30 min. The supernatant obtained was collected and stored under 4 °C and then used for biochemical analyses.

Serum Enzyme Biomarkers

Aspartate Aminotransferase (AST) Activity

AST activity was determined using the method described by Reitman and Frankel [24]. One hundred microliters of serum, liver, kidney, or heart homogenates were mixed with potassium phosphate buffer, L-aspartate, and α-oxoglutarate. The reaction was incubated at 37°C for 30 minutes. Dinitrophenylhydrazine was then added and re-incubated. Absorbance was measured at 546 nm.

Alanine Aminotransferase (ALT) Activity

ALT activity was determined by the method of Reitman and Frankel [24]. A reagent mixture containing potassium phosphate buffer, L-alanine, and α-oxoglutarate was added to 0.1 ml of sample and incubated at 37°C for 30 minutes. Dinitrophenylhydrazine was added, and the absorbance was read at 546 nm.

Alkaline Phosphatase (ALP) Activity

ALP activity was measured using commercial kits (Randox laboratories, UK), following the manufacturer's instructions [25]. Absorbance at 405 nm was measured for 3 minutes at 1-minute intervals.

Antioxidant Assays

Catalase Activity

Catalase activity was measured according to Sinha [26]. Two hundred microliters of serum or organ homogenates were mixed with hydrogen peroxide and potassium phosphate buffer. The reaction was monitored by withdrawal into dichromate/acetate reagent. Hydrogen peroxide content was determined.

Superoxide Dismutase (SOD) Activity

SOD activity was measured using the method of Misra and Fridovich [27]. Serum or organ homogenates were added to a carbonate buffer, and the reaction was initiated by adding adrenaline. The absorbance at 480 nm was monitored for 150 seconds.

Reduced Glutathione (GSH) Level

GSH levels were measured according to Beutler et al. [28]. The samples were mixed with precipitant, incubated, and filtered. The filtrate was combined with potassium phosphate buffer and Ellman's reagent, and absorbance was measured at 412 nm.

Lactate Dehydrogenase (LDH) Activity

LDH activity was determined according to the method of Sulaiman et al. [29]. The reaction of lactate with NAD was measured spectrophotometrically at 340 nm.

Markers of Kidney Function

Urea Determination

Serum urea concentration was measured using the method of Tietz [30] with Randox kits (UK).

Creatinine Determination

Serum creatinine concentration was determined using the method of Tietz [30] with Randox kits (UK).

Histopathological Examination

Formalin-preserved pancreatic tissues were processed for paraffin embedding. Tissue sections were cut, deparaffinized in p-xylene, rehydrated, stained with hematoxylin, counterstained with eosin, mounted, and viewed under a Leica slide scanner (SCN 4000, Leica Biosystems, Wetzlar, Germany).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Bioactive Compounds

GC-MS analysis was conducted using a Varian 3800/4000 gas chromatograph interfaced to a mass spectrometer. Samples were analyzed for qualitative and quantitative components using a VF-5MS capillary column. Mass spectra were compared with NIST Library spectra for identification.

Statistical Analysis

Data were expressed as mean ± SEM. One-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) was used for data analysis using SPSS 11.09 for Windows. Statistical significance was set at $p < 0.05$.

RESULTS

Effect of *A. spinosus* leaf extract on selected serum Liver function parameters in rat exposed to CCl_4 toxicity

As presented in Table 2, exposure to CCl_4 caused a notable increase in serum AST, ALT, and ALP activities, along with a higher total bilirubin level, compared to the control group ($p < 0.05$). However, administration of *A. spinosus* leaf extract and the standard drug silymarin significantly mitigated these effects when compared to the CCl_4-treated group ($p < 0.05$).

Effect of *A. spinosus* leaf extract on selected kidney parameters in rat exposed to CCl_4 toxicity

As shown in Table 3. exposure to CCl_4 led to a significant increase in the concentration of urea and creatinine in the serum and kidney tissue homogenate compared to the control group ($p<0.05$). However, treatment with *A. spinosus* leaf extract and the standard drug silymarin significantly reversed these changes when compare to the CCl_4-treated group in a concentration dependent manner ($p<0.05$)

Effect of *A. spinosus* leaf extracts on selected antioxidant parameters in rat exposed to CCl_4 toxicity

The study examined the impact of ethanolic leaf extract of *Amaranthus spinosus* on the antioxidant system in CCl_4-induced hepato-renal toxicity, with results detailed in Table 4. Administration of CCl_4 significantly depleted GSH levels and reduced the activities of antioxidant enzymes, including SOD, GPx, and CAT ($p < 0.05$). Treatment with *A. spinosus* demonstrated a significant protective effect in a concentration-dependent manner ($p < 0.05$), except for CAT, when compared to the CCl_4-treated group.

Effect of leaf extracts on selected pro and anti-inflammatory markers in rat exposed to CCl_4 toxicity

Table 5 shows a significant increase in LDH activity (a marker of cellular damage) and IL-6 levels (a pro-inflammatory mediator), along with a decrease in IL-10 levels (an anti-inflammatory mediator) in the liver, kidneys, and serum of CCL_4-induced animals compared to the control group ($p<0.05$). However, treatment with A. spinosus (200 mg/kg) significantly restored this marker compared to the CCL_4 group ($p<0.05$).

Effect of leaf extracts of *A. spinosus* on MDA production in rat exposed to CCl_4 toxicity

MDA, a byproduct of polyunsaturated fatty acid peroxidation, serves as a marker of oxidative stress. Figure 1 shows the effect of *A. spinosus* leaf extracts on MDA levels in the liver and kidney of rats exposed to CCl4 toxicity. CCl_4 significantly increased MDA levels ($p<0.05$) compared to the control. However, treatment with A. spinosus leaf extracts significantly reduced MDA production in a concentration-dependent manner ($p<0.05$).

Gas chromatography-mass spectroscopy profiling of ethanolic fraction of *A. spinosus.*

The GC-MS analysis of the ethanolic leaf extract of *A. spinosus* revealed 16 prominent peaks within a retention time range of 5.60 to 26.68 minutes (Figure 2). The identified active compounds, along with their retention times (RT), molecular formulas, molecular weights (MW), abundance (peak area in %) are summarized in Table 6. The major bioactive compounds include 9-hexadecenoic acid (26.21 %), n-Hexadecanoic acid (17.21%), phytol (9.78%), β-Panasinsene (9.39%), Squalene (9.00%), 7-Octen-2-one (7.43%), 9,12-Octadecadienoic acid (Z, Z)-(5.09%), and others.

Table 2: Effect of *A. spinosus* leaf extract on selected serum Liver function parameters in rat exposed to CCl_4 toxicity

Parameters	Control	CCl_4 only	CCl_4+100 mg/kg b.w of *A. spinosus*	CCl_4+200 mg/kg b.w of *A. spinosus*	CCl_4+100 mg/kg b.w of Silymarin
ALT U/L	50.66± 047	67.51± 0.97^{a}	56.12± 0.10^{b}	48.16± 0.19^{c}	45.49± 0.12^{a,b}
ALP U/L	51.5± 0.28	67.59± 0.08^{a}	55.47± 0.03^{b}	48.06± 0.06^{c}	48.62± 0.28^{c}
AST U/L	55.79± 1.61	76.42± 0.38^{a}	67.08± 0.63^{b}	58.64± 0.13^{c}	57.46± 1.47^{c}
T. BIL mg/dl	44± 1.14	56.1± 0.14^{a}	47.3± 0.14^{b}	41± 0.42^{c}	41.41± 1.06^{c}

Data represents mean ± S.D of two independent experiment performed in triplicate. Values with different superscript are significantly different (P<0.05)

Table 3: Effect of *A. spinosus* leaf extract on selected kidney parameters in rat exposed to CCl_4 toxicity

Parameter	Tissue	Control	CCl_4 only	CCl_4+100 mg/kg b.w of *A. spinosus*	CCl_4+200 mg/kg b.w of *A. spinosus*	CCl_4+100 mg/kg b.w of Silymarin
Urea	Kidney	48.06 ± 1.88	64.84 ± 1.49^{a}	50.96 ± 0.79^{b}	47.64 ± 0.22^{c}	50.41 ± 1.25^{b}
mg/dl	Serum	57.7 ± 1.27	79.2 ± 0.28^{a}	69.15 ± 0.77^{b}	62.45 ± 0.91^{c}	60.3 ± 0.28^{a,b}
Creatinine	Kidney	43.41 ± 2.70	59.86 ± 1.64^{a}	49.91 ± 2.00^{b}	46.65 ± 0.35^{c}	45.8 ± 1.83^{c}
mmol/1	Serum	46.65 ± 1.90	60.49 ± 0.15^{a}	53.8 ± 0.28^{b}	49.3 ± 0.98^{b}	44.65 ± 1.62^{c}

Data represents mean ± S.D of two independent experiment performed in triplicate. Values with different superscript are significantly different (P<0.05)

Table 4: Effect of *A. spinosus* leaf extracts on selected antioxidant parameters in rat exposed to CCl_4 toxicity

Parameter	Tissue	Control	CCl_4 only	CCl_4+100 mg/kg b.w of *A. spinosus*	CCl_4+200 mg/kg b.w of *A. spinosus*	CCl_4+100 mg/kg b.w of Silymarin
GSH	Kidney	89.85 ± 0.35	48.8± 1.69^{a}	57.55± 2.89^{b}	87.35 ± 0.49^{c}	84.1 ± 1.27^{c}
	Liver	75.25 ± 0.21	50.5 ± 0.14^{a}	54.11 ± 0.82	65.13 ± 0.09^{b}	74.56 ± 0.23^{c}
SOD	Kidney	98.33 ± 3.91	60.15 ± 0.49^{a}	73.85 ± 0.21^{b}	79.2 ± 1.55^{c}	108.95±2.05^{a,b,c}
	Liver	90.10 ±0.58	54,34 ± 0.96^{a}	67.06 ± 0.33^{b}	69.39 ± 0.87^{b}	82.28 ± 0.31^{a,b}
GPX	Kidney	55.21 ± 1.71	34.75 ± 0.91^{a}	49.75 ± 2.47^{a}	59.35 ± 1.34^{b}	56.44 ± 1.01^{b}
	Liver	60.48 ± 0.16	41.5 ± 1.83^{a}	48.31 ± 0.98^{b}	53.62 ± 0.24^{b}	59.22 ±0.55^{b}
CAT	Kidney	0.06 ± 0.04	0.03 ± 0.02^{a}	0.04 ± 0.02^{b}	0.05 ±0.07`	0.05 ± 0.01
	Liver	0.06 ± 0.02	0.04 ± 0.01^{a}	0.05 ± 0.01	0.06 ± 0.2	0.06 ± 0.01

Data represents mean ± S.D of two independent experiment performed in triplicate. Values with different superscript are significantly different (P<0.05) represents mean ± S.D of two independent experiment per triplicate

Table 5: Effect of *A. spinosus* leaf extracts on selected pro and anti-inflammatory markers in rat exposed to CCl_4 toxicity

Parameter	Tissue	Control	CCl_4 only	CCl_4+100 mg/kg b.w of *A. spinosus*	CCl_4+200 mg/kg b.w of *A. spinosus*	CCl_4+100 mg/kg b.w of Silymarin
LDH U/L	Serum	42.15 ± 0.21	58.5 ± 1.14^{a}	52.95 ± 1.06	41.37± 0.17^{b}	42.75 ± 0.74^{b}
	Kidney	41.37 ± 0.17	57.35 ± 0.91^{a}	46.4 ± 0.08^{b}	45 ± 0.81^{b}	48.1± 0.20^{b}
	Liver	44.85 ± 0.21	58.65 ± 1.20^{a}	52.85 ± 0.95^{b}	40.25 ± 1.20^{c}	43.92 ± 1.88^{c}
IL-6 pg/ml	Serum	38.2 ± 1.41	49.4 ± 1.97^{a}	44.49 ±1.18^{b}	42± 0.93^{c}	38.35±0.35^{a,b,c}
IL-10 pg/ml	Serum	53.5 ± 2.68	37.8 ± 2.26^{a}	40 ± 1.13^{b}	41.2 ± 0.28^{b}	48.4 ± 0.14^{a,b}

Data represents mean ± S.D of two independent experiment performed in triplicate. Values with different are significantly different (P<0.05)

Table 6: Gas Chromatography-Mass Spectrometry (G C-MS) Analysis of Bioactive Compounds in *A. spinosus* leaf results

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp. wt%	m/z
1	5.60	7-Octen-2-one	$C_8H_{14}O$	126	7.43	0.88	43, 58. 126
2	6.00	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	$C_{15}H_{24}$	204	3.91	0.41	41, 133, 204
3	6.98	9-hexadecenoic acid	$C_{16}H_{30}O_2$	254	26.21	34.31	43, 73, 254
4	7.19	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	3.52	0.85	43, 73, 228
5	7.75	(7Z,11Z)-hexadecadienal	$C_{16}H_{28}O$	236	2.35	0.40	41, 74, 236
6	8.31	β-Panasinsene	$C_{15}H_{24}$	204	9.39	4.50	41, 161, 204
7	9.50	1-Tetradecanamine	$C_{14}H_{31}N$	213	0.78	0.41	41, 44, 213
8	11.00	n-Hexadecanoic acid	$C_{14}H_{28}O_2$	256	17.21	26.11	60, 73, 256
9	12.50	9-Octadecenal	$C_{18}H_{34}O$	266	2.74	3.17	41, 55, 266
10	13.58	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	5.09	2.57	65, 67, 280
11	19.00	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.39	0.15	43, 73, 284
12	20.42	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264	0.71	1.44	41, 79, 264
13	24.48	Vitamin E	$C_{29}H_{50}O_2$	430	1.17	4.24	43, 165, 430
14	26.72	Phytol	$C_{20}H_{40}O$	296	9.78	10.10	43, 71, 296
15	29.91	Squalene	$C_{30}H_{50}$	410	9.00	10.08	69, 81, 410
16	26.68	β-Sitosterol	$C_{29}H_{50}O$	414	0.31	0.12	43, 81, 414

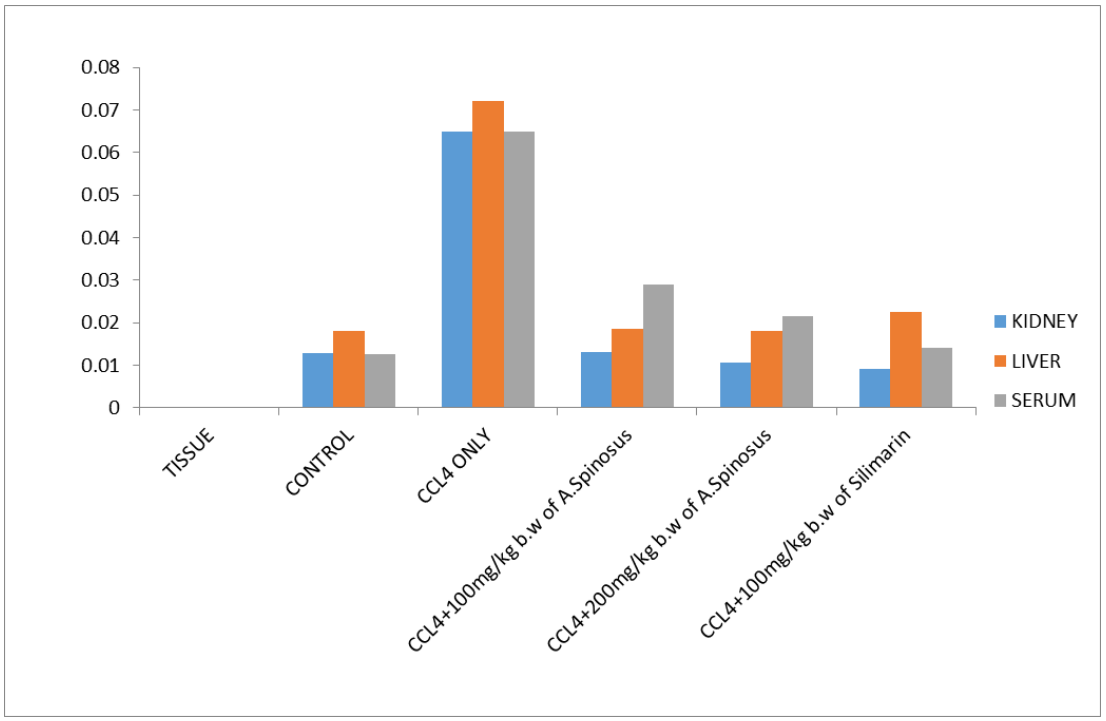

Figure 1: Effect of *A. spinosus* on serum, liver and kidney MDA level in animals exposed to CCl_4

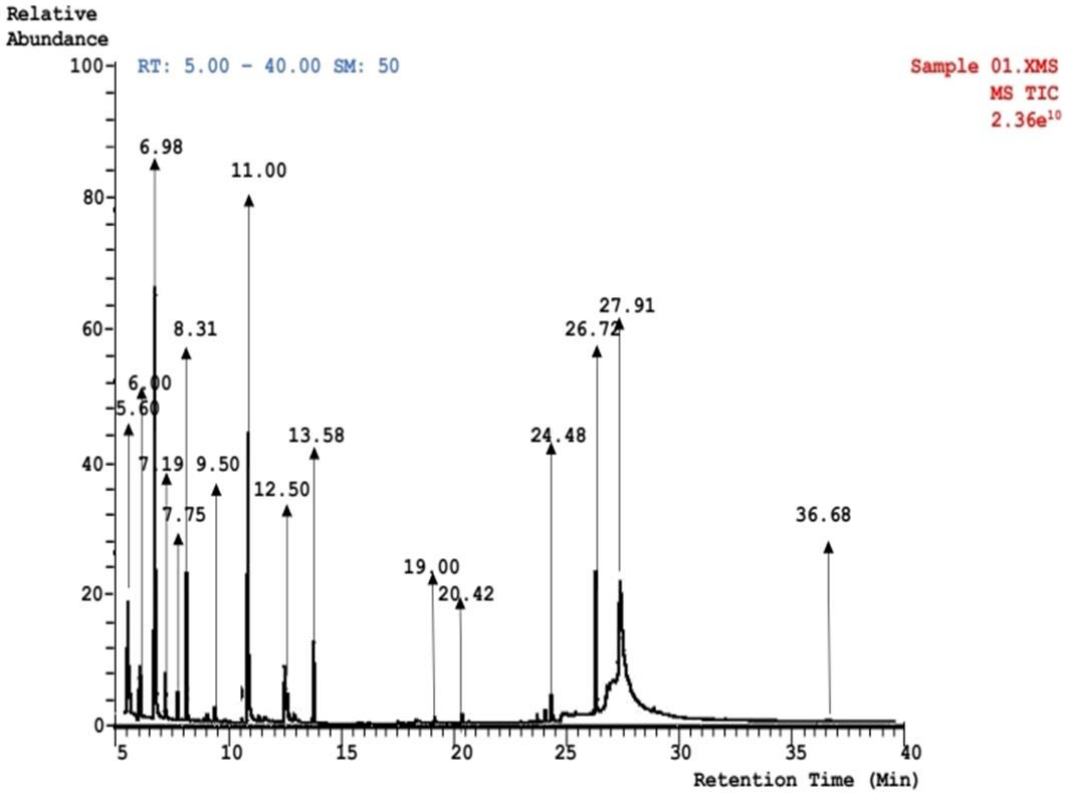

Figure 2: GC-MS Chromatogram of ethanolic extract of *A. spinosus* leaf

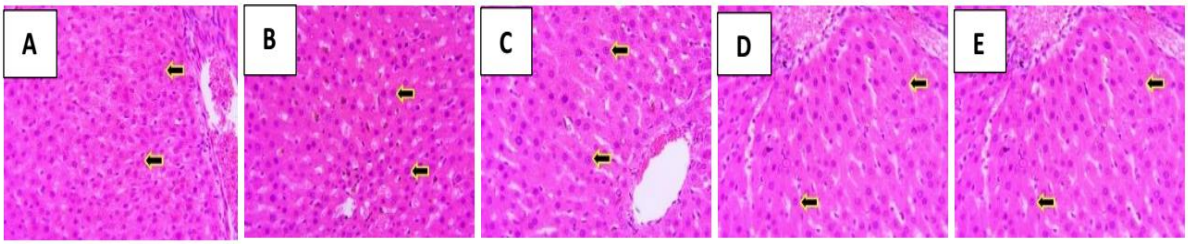

Figure 3: (A-E). Histopathological Analysis of Hepatic Tissues of Experimental Rats

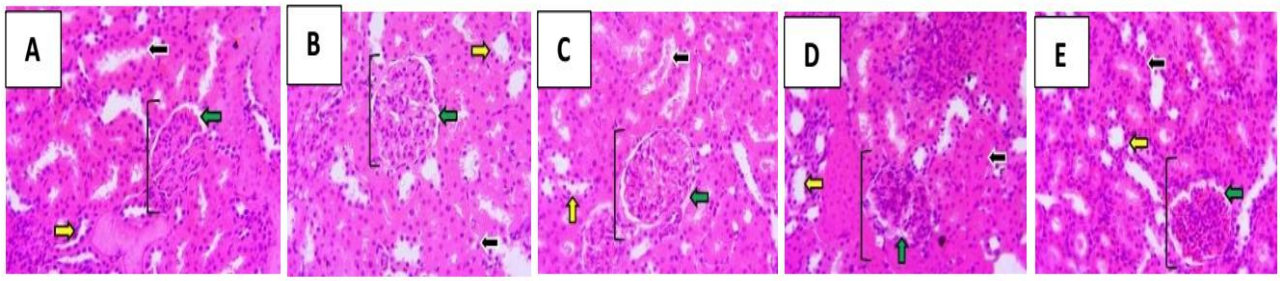

Figure 4: (A-E). Histopathological Analysis of Renal Tissues of Experimental Rats

Histopathological Analysis of Hepatic Tissues of Experimental Rats

GROUP A. (Negative Control): Showed normal histoarchitecture of liver cells in group. Yellow arrow represents hepatocytes (liver cells). (Stained with H&E, magnification: ×800).

GROUP B**.** (Positive control): Showed moderate loss of liver cells. Yellow arrow represents hepatocytes (liver cells). (Stained with H&E, magnification: ×800).

GROUP C**. (**Treated with *A. spinosus* at 100 mg/kg b.w.): Showed normal histoarchitecture of liver cells and moderate congestion. Yellow arrow represents hepatocytes (liver cells). (Stained with H&E, magnification: ×800).

GROUP D**. (**Treated with *A. spinosus* at 200 mg/kg b.w.): Showed normal histoarchitecture of liver cells and moderate congestion. Yellow arrow represents hepatocytes (liver cells). (Stained with H&E, magnification: ×800).

GROUP E**. (**Treated with *silymarin* at 100mg/kg b.w.): Showed normal histoarchitecture of liver cells and moderate congestion. Yellow arrow represents hepatocytes (liver cells). (Stained with H&E, magnification: ×800).

Histopathological Analysis of Renal Tissues of Experimental Rats

GROUP A (Negative control): Showed normal histology of the glomeruli and renal tubules. Green arrow represents urinary space; Green arrow represents urinary space; Black arrow represents

proximal convoluted tubules; Yellow arrow represents distal convoluted tubules. (Stained with H&E, magnification: ×800).

GROUP B. (Positive Control): Showed constriction of urinary space, degeneration of proximal convoluted tubules, shrunken urinary space, and loss of renal tubules. Green arrow represents urinary space; Black arrow represents proximal convoluted tubules; Yellow arrow represents distal convoluted tubules. (Stained with H&E, magnification: ×800).

GROUP C. (Treated with *A. spinosus* at 100 mg/kg b.w.): Showed normal renal histoarchitecture. Green arrow represents urinary space; Green arrow represents urinary space; Black arrow represents proximal convoluted tubules; Yellow arrow represents distal convoluted tubules. (Stained with H&E, magnification: ×800).

GROUP D. (Treated with *A. spinosus* at 200 mg/kg b.w.): Showed normal glomerulus and distal tubules. Green arrow represents urinary space; Green arrow represents urinary space; Black arrow represents proximal convoluted tubules; Yellow arrow represents distal convoluted tubules. (Stained with H&E, magnification: ×800).

GROUP E. (Treated with silymarin at 100 mg/kg b.w). showed normal renal histoarchitecture. Green arrow represents urinary space; Black arrow represents proximal convoluted tubules; Yellow arrow represents distal convoluted tubules. (Stained with H&E, magnification: ×800).

DISCUSSION

Liver and kidney diseases present significant challenges due to limited safe treatment options, high management costs, and the risk of progression to chronic or fatal conditions. Despite advances in care, many therapies have side effects, emphasizing the need for safer alternatives. Medicinal plant phytochemicals are valued for their availability, minimal side effects, and potential to cure, primarily by inhibiting oxidative injuries—a key factor in chemically induced hepato-renal damage [31-33].

This study investigated the protective effects of the ethanolic leaf extract of *A. spinosus* against CCl_4-induced acute hepato-renal injury in rats. CCl_4 exposure caused significant hepatic damage, as evidenced by elevated serum levels of ALT, AST, ALP, and bilirubin ($P \leq 0.05$) compared to the negative control group as presented in table 2.0. These findings are consistent with previous studies showing that a single dose of CCl_4 significantly increases these enzyme markers [31-32]. The elevated enzyme activity is likely attributed to cellular disruption, leading to the leakage of intracellular enzymes into the bloodstream [31]. Treatment with *A. spinosus* extract significantly ameliorated these enzymatic disturbances in a concentration-dependent manner, comparable to the standard drug silymarin. While ALT and AST are critical markers of hepatic damage, ALT is predominantly liver-specific, and AST plays a vital role in biomolecule synthesis and Krebs cycle replenishment, both of which show high sensitivity to hepatic injury and recovery [23]. Similarly, elevated ALP is a well-established indicator of cholestatic hepatotoxicity, suggesting biliary obstruction, dehydration, or reduced renal blood flow [34-35]. Increased plasma bile acids and bilirubin has been ascribed to impaired liver function, reflecting the liver's roles in protein synthesis, bile acid metabolism, and waste excretion. Collectively, the positive modulation of these biomarkers by *A. spinosus* underscores its potential hepatoprotective properties. These findings are consistent with the observations of Zeashan et al. [36], who reported that a 50% ethanolic extract of the whole plant of *A. spinosus* exhibited significant hepatoprotective activity based on the evauation of similar markers. Previous studies have linked the *in vitro* bioactivity of *A. spinosus* to its diverse phytochemical constituents, including alkaloids [37, 38], tannins [37,39], flavonoids [38,40], and saponins [37-39].

The nephrotoxic mechanism of CCl_4 is recursive to its hepatotoxicity, with the renal cortex's cytochrome P-450 system also being a target of CCl_4 metabolism [41]. In this study, acute CCl_4 exposure significantly impaired renal function, evidenced by elevated serum creatinine and urea levels in the exposed group compared to the control (Table 3). Creatinine, a breakdown product of creatine phosphate in muscle, is produced at a constant rate depending on muscle mass [42] while urea, the primary nitrogenous end product of protein metabolism, is synthesized in the liver, filtered by the kidneys, and partially reabsorbed with water [43], both are key clinical indices for evaluating renal function.

Furthermore, a single intraperitoneal exposure to CCl_4 significantly depleted ($p < 0.05$) glutathione (GSH) levels and the activities of key antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx), in liver and kidney tissue homogenates compared to the control group (Table 4). This aligns with the well-established mechanism of CCl_4 toxicity, where cytochrome P-450 metabolism produces reactive intermediates, such as trichloromethyl radicals, which induce lipid peroxidation and oxidative stress, depleting antioxidants like GSH and inhibiting antioxidant enzymes in serum, liver, and kidney tissues [8]. However, treatment with *A. spinosus* extract significantly ($p < 0.05$) restored GSH levels and enzyme activities in a concentration-dependent manner compared to both the CCl_4-treated and standard drug (silymarin) groups. This highlights the potent antioxidant properties of *A. spinosus*, in mitigating oxidative damage and protect against CCl_4-induced hepatotoxicity and nephrotoxicity. Previous studies have demonstrated its antioxidant activity through assays, including non-enzymatic haemoglycosylation, where rutin and quercetin inhibited haemoglycosylation by 42% and 52%, respectively, and through its free radical scavenging system [44-45]. A similar trend was observed with MDA generation, a marker of lipid peroxidation, which was positively modulated by *A. spinosus* administration (Figure 2). The anti-lipid peroxidation and antioxidant potential of the plant have also been evaluated through linoleic acid oxidation [36] and various scavenging assays [20].

Diseases with inflammatory origin, identified by the World Health Organization as a major health threat [46], play a central role in acute and chronic pathological processes, driving enzymatic and biochemical disruptions linked to liver and kidney dysfunction. These processes are mediated by inflammatory molecules such as vasoactive amines, eicosanoids, proteolytic cascade products, chemokines, and cytokines [47]. The effects of *Amaranthus spinosus* treatment on interleukin-6 (IL-6), a pro-inflammatory cytokine, and interleukin-10 (IL-10), an anti-inflammatory cytokine, were examined in a model of CCl_4-induced hepatorenal toxicity. Administration of *A. spinosus* crude extract (100 and 200 mg/kg) significantly reduced IL-6 levels, while concurrently increasing IL-10 levels, compared to the CCl_4 control group (Table 5). Baral et al [48] and Olajide et al [49], demonstrated the anti-inflammatory activity of *A. spinosus* methanolic extract, which inhibited carrageenan-induced paw edema (25–100 mg/kg) and reduced acetic acid-induced vascular permeability in animal models.

Building upon the ant-inflammatory effects previously discussed, histopathological examination further supports the potential therapeutic effect of *A. spinosus* in ameliorating liver and kidney dysfunction associated with CCl_4-induced toxicity. In the liver tissue, the normal control group exhibited a well-preserved histoarchitecture (Figure 3A), while CCl_4 exposure alone led to moderate loss of liver cells (Figure 3B). Treatment with *A. spinosus* resulted in significant improvements, showing normal histoarchitecture with moderate congestion. The higher dose (200 mg/kg b.w.) exhibited even better results, with less congestion compared to both the 100 mg/kg and CCl_4 groups. Silymarin-treated rats at 100 mg/kg b.w. also showed normal liver histology with mild congestion.

Similarly, renal tissue examination revealed normal glomeruli, proximal tubules, and distal tubules in the normal control group

(Figure 4A), while CCl_4 treatment alone led to significant renal injury, including constriction of the urinary space, degeneration of proximal convoluted tubules, and loss of renal tubules (Figure 4B). Treatment with *A. spinosus* crude extract (100 and 200 mg/kg b.w.) improved renal histoarchitecture, with mild to moderate improvements in the 100 mg/kg group (Figure 4C) and more substantial improvements in the 200 mg/kg group (Figure 4D). The higher dose showed patchy interstitial inflammatory cell infiltrates, mild congestion, and minimal focal tubular cell necrosis, suggesting a greater potential for tissue recovery. Silymarin-treated rats exhibited normal renal histoarchitecture, reinforcing the possible anti-inflammatory effects observed through cytokine modulation, particularly with the reduction in IL-6 and the increase in IL-10 levels. Together, these findings suggest that *A. spinosus* not only modifies inflammatory cytokine profiles but also improves both liver and kidney tissue architecture in the context of CCl_4-induced toxicity.

Expanding on the previous exploration of the therapeutic potential of *A. spinosus*, GC-MS analysis was employed to explore the diverse pharmacological mechanisms inherent in its crude leaf extract. This advanced analytical tool facilitates both qualitative and quantitative identification of biologically active compounds in medicinal plants, thus aiding in the discovery of potential therapeutic leads across species [50-51].

This study explored the therapeutic potential of *Amaranthus spinosus* leaf extract using GC-MS analysis, revealing a diverse profile of bioactive compounds. Fatty acids and their esters accounted for 57.51% of the total phytoconstituents, with major compounds identified include 9-hexadecenoic acid (26.21%) and n-hexadecanoic acid (17.21%), tetradecanoic acid (3.52%), and 9,12-octadecadienoic acid (5.09%). These fatty acids are well-documented for their antibacterial, antioxidant, anti-inflammatory, and hypocholesterolemic effects, highlighting the extract's broad therapeutic potential [52].

The analysis also identified Vitamin E (1.17%), a potent antioxidant and anti-inflammatory agent known to prevent lipid peroxidation, scavenge free radicals, and modulate inflammatory pathways. Additionally, phytol (9.78%), a monounsaturated diterpene alcohol, exhibited antioxidant, neuroprotective, and cytotoxic activities, particularly against breast cancer cell lines, while serving as a precursor to vitamins E and K [53-54]. These findings provide a mechanistic basis for the extract's hepatorenal protective effects, emphasizing its antioxidant, anti-inflammatory, and cytoprotective properties.

CONCLUSION

The therapeutic promise of *A. spinosus* lies in its bioactive compounds, such as fatty acids, Vitamin E, and Phytol, which contribute to its hepatoprotective, renal-protective, and anti-inflammatory effects. These results underscore its potential as a natural candidate for managing liver and kidney diseases, as well as inflammation and oxidative stress. Future research should focus on isolating these compounds, elucidating their molecular mechanisms, and evaluating synergistic effects for pharmaceutical applications.

Acknowledgement

The authors extend their appreciation to the Department of Medical Biochemistry, Ekiti State University, Ado Ekiti, and the Department of Biochemistry, University of Ilesa, Osun State, for their support.

Conflict of interest

The authors declared no conflict of interest.

Financial Support

None declared.

ORCID ID

Akeem Adewale Yusuff: https://orcid.org/0000-0001-7929-2111

Temidayo Ogunmoyole: https://orcid.org/0000-0002-6185-0602

Olaitan Daniel Johnson: https://orcid.org/0000-0002-3492-0986

REFERENCES

1. Alzahrani SA, Bekhet GM, Ammar RB, Abdallah BM, Ali EM, Al-Ramadan SY. The inhibitory effect of geraniol on CCl_4-induced hepatorenal toxicity in pregnant mice through the PI3K/AKT signaling pathway. Saudi J Med Med Sci. 2024;12:17-26.
2. DElsawy H, Badr GM, Sedky A, Abdallah BM, Alzahrani AM, Abdel-Moneim AM. Rutin ameliorates carbon tetrachloride (CCl_4)-induced hepatorenal toxicity and hypogonadism in male rats. PeerJ. 2019;7:e7011.
3. Fahmy MA, Diab KA, Abdel-Samie NS, Omara EA, Hassan ZM. Carbon tetrachloride-induced hepato/renal toxicity in experimental mice: Antioxidant potential of Egyptian *Salvia officinalis* L. essential oil. Environ Sci Pollut Res. 2018;25:27858–27876.
4. Zangar RC, Benson JM, Burnett VL. Cytochrome P450 2E1 is the primary enzyme responsible for low-dose carbon tetrachloride metabolism in human liver microsomes. Chem Biol Interact. 2000;125:233–243.
5. Bryant AE, Dreifuss FE. Valproic acid hepatic fatalities. III. U.S. experience since 1986. Neurology. 1996;46:465–469.
6. Young RA, Mehendale HM. Carbon tetrachloride metabolism in partially hepatectomized and sham-operated rats pre-exposed to chlordecone (kepone). J Biochem Toxicol. 1989;4:211–219.
7. Wu S, Fang Z, Zhou S. Saturated hydrogen alleviates CCl4-induced acute kidney injury via JAK2/STAT3/p65 signaling. J Int Med Res. 2020;48:300060519895353.
8. Abdel-Kader MS, Abulhamd AT, Hamad AM, Alanazi AH, Ali R, Alqasoumi SI. Evaluation of the hepatoprotective effect of a combination between hinokiflavone and glycyrrhizin against CCl_4-induced toxicity in rats. Saudi Pharm J. 2018;26(4):496-503.
9. Rahman MM, Muse AY, Khan DMIO, Ahmed IH, Subhan N, Reza HM, et al. Apocynin prevented inflammation and oxidative stress in carbon tetrachloride-induced hepatic dysfunction in rats. Biomed Pharmacother. 2017;92:421-428.
10. Manibusan MK, Odin M, Eastmond DA. Postulated carbon tetrachloride mode of action: a review. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2007;25:185-209.
11. Shi J, Aisaki K, Ikawa Y, Wake K. Evidence of hepatocyte apoptosis in rat liver after the administration of carbon tetrachloride. Am J Pathol. 1998;153:515-25.
12. Abir M, Ahmad M. Phytochemical, nutritional, and pharmacological potentialities of *Amaranthus spinosus* Linn.: A review. Arch Ecotoxicol. 2021;3(2):49-59.
13. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Vol. I, Oriental Enterprises, New Connaught Place, Dehradun, Uttranchal, India. 2001:2832-2836.
14. Odhav B, Beekrum S, Akula US, Baijnath H. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. J Food Comp Anal. 2000;13:430-435.
15. Saravanan R. Evaluation of In-Vitro and In-Vivo Anticancer Activity of Leaf Extracts of *Amaranthus spinosus* Linn. Master's thesis, College of Pharmacy Madras Medical College, Chennai. 2016;21-22.
16. Lin JY, Li CY, Lin BF. *Amaranthus spinosus* L. inhibits spontaneous and dexamethasone-induced apoptosis in murine primary splenocytes. J Food Drug Anal. 2008;16(4):52–61.

17. Hilou A, Nacoulma OG, Guiguemde TR. *In vivo* antimalarial activities of extracts from *Amaranthus spinosus* L. and *Boerhaavia erecta* L. in mice. J Ethnopharmacol. 2006;103:236–240.
18. Ahmed N, Wahab S. Plant-Derived Bioactive Compounds in the Management of Neurodegenerative Disorders: Challenges, Future Directions, and Molecular Mechanisms. Pharmaceuticals. 2021;15(3):749.
19. Sangameswaran B, Jayakar B. Anti-diabetic, antihyperlipidemic and spermatogenic effects of *Amaranthus spinosus* Linn. on streptozotocin-induced diabetic rats. J Nat Med. 2008;62(1):79–82.
20. Kumar BSA, Lakshman KN, Jayaveera KN, Shekar DS, Kumar AA, Manoj B. Antioxidant and antipyretic properties of methanolic extract of *Amaranthus spinosus* leaves. Asian Pac J Trop Med. 2010;3(9):702–706.
21. Abir MH, Ahmad M. Phytochemical, Nutritional and Pharmacological Potentialities of *Amaranthus spinosus* Linn. A review. Arch Ecotoxicol. 2021;49-59.
22. Gillessen A, Hartmut H, Schmidt J, Silymarin as supportive treatment in liver diseases: A narrative review. Adv Ther. 2020;37:1279–1301.
23. Reitman S, Frankel S. Glutamic–pyruvate transaminase assay by colorimetric method. Am J Clin Pathol. 1957;28(1):56-63.
24. Englehardt A. Measurement of alkaline phosphatase. Aerztl Labor. 1970;16(42):1.
25. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47(2):389-394.
26. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170–3175.
27. Beutler E. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882-888.
28. Sulaiman ST, Al-Najafi TS, Hamdon HS. Sensitive method for measuring lactate dehydrogenase activity in human serum by differential-pulse polarography. Analyst. 1994;119(10):2199-2200.
29. Tietz NW. Clinical guide to laboratory tests. 3rd ed. Philadelphia: Saunders and Co. Publishers; 1995. p. 1096.
30. Ullah H, Khan A, Baig MW, Ullah N, Ahmed N, Tipu MK. Poncirin attenuates CCl_4-induced liver injury through inhibition of oxidative stress and inflammatory cytokines in mice. BMC Complement Med Ther. 2020;20(115):1-14.
31. Bellassoued K, Hsouna AB, Athmouni K. Protective effects of *Mentha piperita* L. leaf essential oil against CCl_4-induced hepatic oxidative damage and renal failure in rats. Lipids Health Dis. 2018;17(9):1-14.
32. Hikal AH, Abd El-Fatta HM, El-Sheik NM. Comparative study of marjoram (*Origanum majorana* L.) and silymarin (*Silybum marianum* L.) extract against carbon tetrachloride induced hepatic injury. World J Pharm Pharm Sci. 2018;7(8):1969-92.
33. Björnsson E, Kalaitzakis E, Olsson R. The impact of eosinophilia and hepatic necrosis on prognosis in patients with drug-induced liver injury. Aliment Pharmacol Ther. 2007;25(12):1411–1421.
34. Whitby LG, Percy-Robb IW, Smith AF. Enzymes test in diagnosis. Lecture Notes on Clinical Chemistry, 3rd ed. London: Blackwell Sci. Publication; 1984. p. 138-168.
35. Zeashan H, Amresh G, Singh S, Rao CV. Hepatoprotective and antioxidant activity of *Amaranthus spinosus* against CCl_4 induced toxicity. J Ethnopharmacol. 2009;125(2):364–366.
36. Amabye TG. Evaluation of Physiochemical, Phytochemical, Antioxidant and Antimicrobial Screening Parameters of Amaranthus spinosus Leaves. Nat Prod Chem Res. 2016;4(01):1-8.
37. Khanal DP, Raut B, Dangol KS. Phytochemical Screening, Pharmacognostic Evaluation and Biological Activity of *Amaranthus spinosus* L. J Manmohan Mem Inst Health Sci. 2015;1(4):29–34.
38. Maiyo ZC, Ngure RM, Matasyoh JC, Chepkorir R. Phytochemical constituents and antimicrobial activity of leaf extracts of three Amaranthus plant species. Afr J Biotechnol. 2010;9(21):3178–3182.
39. Jiménez-Aguilar DM, Grusak MA. Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of Amaranthus leafy vegetables. J Food Comp Anal. 2017;58:33–39.
40. Akram MA, Tembhre M, Jabeen R, Defensive role of *Rosmarinus officinalis* in carbon tetrachloride-induced nephrotoxicity and oxidative stress in rats. Bull Natl Res Cent. 2019;43(50):1-10.
41. Baliga R, Zhang Z, Baliga M, Ueda N, Shah SV. Role of cytochrome P-450 as a source of catalytic iron in cisplatin-induced nephrotoxicity. Kidney international. 1998;54(5):1562-9.
42. Yuegang Z, Chengjun W. Simultaneous determination of creatinine and uric acid in human urine by high performance liquid chromatography. Anal Sci. 2008;24:1589-1592.
43. Corbett JV. Laboratory tests and diagnostic procedures with nursing diagnoses. 7th ed. 2008;90–107.
44. Tanmoy G, Arijit M, Tanushree S. Pharmacological actions and phytoconstituents of *Amaranthus spinosus* Linn: A review. Int J Pharmacogn Phytochem Res. 2014;6(2):405–413.
45. Jhade D, Ahirwar D, Sharma NK. Antifertility activity of ethanolic and aqueous root extract of *Amaranthus spinosus* Linn. in rats. Pharmacol Online. 2011;2(January 2011):959–967.
46. Soares CLR, Wilairatana P, Silva LR. Biochemical aspects of the inflammatory process: A narrative review. Biomed Pharmacother. 2023;168:115764.
47. Abdulkhaleq L, Assi M, Abdullah R. The crucial roles of inflammatory mediators in inflammation: A review. Vet World. 2018;11:1-10.
48. Baral M, Chakraborty S, Chakraborty P. Evaluation of anthelmintic and anti-inflammatory activity of Amaranthus. Int J Curr Pharm Res. 2010;2(4):2–5.
49. Olajide OA, Ogunleye BR, Erinle TO. Anti-inflammatory properties of *Amaranthus spinosus* leaf extract. Pharm Biol. 2004;42(7):521–525.
50. Starlin T, Prabha PS, Thayakumar BKA. Screening and GC-MS profiling of ethanolic extract of *Tylophora pauciflora*. Biomed Inform. 2019;15(6):425–429.
51. Gopalakrishnan K, Udayakumar R. GC-MS analysis of phytocompounds of leaf and stem of *Marsilea quadrifolia* (L). Int J Biochem Res Rev. 2014;4(6):517–526.
52. Aparna V, Dileep KV, Mandal PK. Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. Chem Biol Drug Des. 2012;80(3):434-439.
53. Banjare J, Salunke M, Indapurkar K. Estimation of serum malondialdehyde as a marker of lipid peroxidation in medical students undergoing examination-induced psychological stress. J Sci Soc. 2017;44:137-139.

HOW TO CITE THIS ARTICLE

Yusuff AA, Ogunmoyole T, Johnson OD. Protective effects of *Amaranthus spinosus* leaf extract against CCl_4-induced hepatorenal injury in rats: Insights from GC-MS analysis. J Phytopharmacol 2025; 14(1):14-22. doi: 10.31254/phyto.2025.14103