



Modulation of Oxidative Stress and Inflammatory Cytokines as Therapeutic Mechanisms of *Ocimum americanum* L Extract in Carbon Tetrachloride and Acetaminophen-Induced Toxicity in Rats

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Abstract

Liver diseases have now become a global canker due to increasing drug abuse and several viral infections. The current medicines on the market are woefully inadequate and limited in the application against these diseases. Fortunately, medicinal plants continue to serve as a potential source of drug discovery that could be explored to improve the situation. The present study, therefore, evaluated the hepatoprotective activities of the aqueous extract of various parts (leaves, flower and stem) of *Ocimum americanum* L on carbon tetrachloride (CCl₄)- and acetaminophen-induced toxicity in rats. The protective effect of the plant was assessed using biochemical parameters, histology, levels of liver antioxidants, and expression of some pro-inflammatory cytokines (NF- κ B and IL-1) in the liver. The leaves and stem extracts, orally administered for 7 days at 250 mg/kg, effectively prevented CCl₄-induced elevation of serum biochemical parameters, prooxidants, as well as the expression of NF κ -B and IL-1, which were comparable to Silymarin (standard drug). A comparative histopathological analyses of the liver exhibited virtually normal architecture compared with CCl₄-treated group. The findings showed that the hepatoprotective effect of *Ocimum americanum* was probably due to the inhibition of oxidative stress and downregulation of proinflammatory cytokines by the effective parts of the medicinal plant.

Keywords

Ocimum americanum, hepatoprotective, acetaminophen, carbon tetrachloride, inflammation, medicinal plants

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Introduction

The liver is one of the most important organs that supports almost every other organ and function in the body.¹ The main function of the liver is to filter blood from the digestive tract before distribution to the rest of the body. Chemical agents also pass through the liver before entry into blood.²

An estimated 350 million human lives are lost worldwide (Africa and other continents) annually due to liver diseases.³ The World Health Organization estimates over 2 million deaths per year attributable to liver injuries induced by drug therapies to diseases contributing to about 8% to 16% prevalence. Among such population, 1 million people die as a result of acute and chronic liver diseases.⁴ In Ghana, data from Korle Bu Teaching Hospital, Accra, indicated 2.8% to 5.4%

prevalence of liver disease annually.⁵ According to other data by the World Health Organization, about 5477 deaths in Ghana were due to liver diseases, accounting for 2.92% of all deaths. Globally, Ghana has a prevalence of 26 to 33 deaths per 100 000 due to liver-related conditions.⁶

Drugs for treating liver diseases such as interferon, colchicines, penicillamine, and corticosteroids are found to contain

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numerous side effects.⁷ There is, therefore, the need to develop some effective therapeutic agents from plant sources to treat liver diseases and having infinitesimal or virtually no side effects.

Ocimum americanum L. (family Lamiaceae) is an edible plant (vegetable) used as a food flavoring ingredient and as a spice, especially in preparing chicken soup in Ghanaian local settings. It is widely cultivated in Ghana and locally called *akobesa*. Studies have shown that *O americanum* could cure digestive tract disease with related antimicrobial properties.⁸ The current study, therefore, focused on the pharmacological properties of *O americanum* as a hepatoprotective agent in carbon tetrachloride (CCl₄)- and acetaminophen-induced toxicity in rats.

Methods

Chemicals

Ethanol, methanol, Na₂CO₃, aluminum chloride, potassium acetate, and carbon tetrachloride were of reagent grade and obtained from BDH (New Delhi, India). Reagents for the determination of biochemical indices were obtained from Elitech (Paris, France). All other reagents were of analytical grade.

Plant Collection, Preparation of Extracts, and Phytochemical Screening

Ocimum americanum was collected from Trabuom in the Atwima Kwanwomani District (Plus Code J66Q+G3 Kumasi), Ashanti Region of Ghana, in the morning between 7:00 and 9:00 AM. The plant was authenticated by Dr George Sam in the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology (KNUST), and a voucher specimen (KNUST/HM1/2018/L035) deposit at the Department's herbarium. The various plant parts including leaves, flowers, and whole stem were used for the study. They were shade-dried for 3 weeks, milled coarsely, and stored in zip-locked bags before extraction.

An aqueous extract of the different plant parts was prepared by decoction using freshly boiled distilled water. The sample (50 g) was suspended in 500 mL of water and extracted twice for 24 hours. Filtration of the extract was done using cotton wool and freeze-dried to obtain the *O. americanum* extracts (OAE) of leaves, flowers, and stem. Qualitative determination of glycosides, tannins, flavonoids, alkaloids, saponins, coumarins, triterpenoids, and sterols were also performed on the extracts using standard procedures described by Harborne⁹ and Trease and Evans.¹⁰

Induction of Hepatotoxicity

Hepatotoxicity was induced in experimental animals using carbon tetrachloride (CCl₄) and acetaminophen (Paracetamol, Para). The animals were treated with 1 mL CCl₄/kg body weight (bw) intraperitoneally (ip) in olive oil (1:1 vol/vol) on the second and third days of treatment.¹¹ The other animals were also treated with 500 mg Para/kg bw (Paracetamol IV Pfizer Solution) ip from days 1 to 7.

Animals

Male Wistar albino rats purchased from the animal facility of University of Ghana School of Medical Sciences, Accra-Ghana, were used

Table 1. Grouping of Animals for the Experiment.

Group number	Group name	Treatment
I	Normal	Animals orally received 1 mL/kg bw distilled water daily for 7 days
II	CCl ₄ only	Animals orally received 1 mL/kg bw distilled water daily for 7 days and 1 mL/kg bw CCl ₄ dissolved in olive oil (1:1 v/v) ip on second and third days
III	Para only	Animals received 1 mL/kg bw distilled water orally and 500 mg/kg bw Para by ip daily for 7 days
IV	Silymarin + CCl ₄	Animals orally received 100 mg/kg bw Silymarin dissolved in distilled water daily for 7 days and 1 mL/kg bw CCl ₄
V	Silymarin + Para	Animals orally received 100 mg/kg bw Silymarin dissolved in distilled water and 500 mg/kg bw Para (IV)
VI	Stem only	Animals orally received 250 mg/kg bw stem extract dissolved in distilled water daily for 7 days
VII	Stem + CCl ₄	Animals received 250 mg/kg bw stem extract for 7 days and 1 mL/kg bw CCl ₄
VIII	Stem + Para	Animals received 250 mg/kg bw stem extract for 7 days and 500 mg/kg bw Para (IV)
IX	Flower only	Animals orally received 250 mg/kg bw flower extract for 7 days
X	Flower + CCl ₄	Animals received 250 mg/kg bw flower extract for 7 days and 1 mL/kg bw CCl ₄
XI	Flower + Para	Animals received 250 mg/kg bw flower extract for 7 days and 500 mg/kg bw Para (IV)
XII	Leaves only	Animals orally received 250 mg/kg bw leaves extract for 7 days
XIII	Leaves + CCl ₄	Animals received 250 mg/kg bw leaves extract for 7 days and 1 mL/kg bw CCl ₄
XIV	Leaves + Para	Animals received 250 mg/kg bw leaves extract for 7 days and 500 mg/kg bw Para (IV) by ip daily for 7 days

for this experiment. The animals were transferred to the animal holding facility of the Department of Biochemistry and Biotechnology, KNUST, and they were kept in aluminum cages bedded with wood shavings. The animals were allowed to acclimatize to laboratory conditions for 2 weeks. Optimum laboratory conditions of between 24 and 26 °C and 40% to 70% relative humidity were maintained throughout the study. Animals were allowed to eat and drink freely during this period and throughout the study. The animals were identified by marking them exclusively on their tails with permanent markers. The animal study was conducted based on guidelines as stipulated by the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA, New Delhi, India) and the Guide for Care and Use of Laboratory Animals.¹²

Groupings and Treatment

Table 1 shows the different groups and the associated treatments. All animals were treated for 7 days with an overnight fast prior to first treatment. The single dose of 250 mg/kg bw was selected based on the

safety profile of the leaves that support such dose as the most tolerable.

Evaluation of Body Weight

The body weight of each animal was measured prior to the commencement of the treatment and on the last day of treatment. Percent change in body weight was calculated as follows:

$$\text{Percent change in weight} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Evaluation of Hematological Parameters

All treatments were performed for 7 days. Animals were fasted overnight and sacrificed on the eighth day by cervical dislocation. Incisions were quickly made in the neck area of sacrificed animals and blood samples collected into EDTA tubes for the hematological assessment using Sysmex Haematology System. The parameters including hemoglobin (HGB), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, lymphocytes, neutrophils, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), plateletcrit, platelet distribution width (PDW), and platelet larger cell ratio (P-LCR) were determined.

Evaluation of Some Biochemical Parameters

Blood samples (about 3 mL each) were placed in gel-activated tubes, allowed to stand for 30 minutes, and centrifuged at 5000 rpm for 15 minutes in a refrigerated centrifuge (Eppendorf Centrifuge 5804R). Sera were aspirated into 3 mL Eppendorf tubes and stored at -20°C prior to analyses. The change in the function of the liver was assessed using the levels of total proteins (TP), albumin (Alb), globulin (Glo), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ -glutamyltransferase (γ GT), and bilirubin (total bilirubin [TBil] and direct bilirubin [DBil]) using Selectra E (Vital Scientific) and reagents from ELITECH. The levels of indirect bilirubin (IBil) was estimated as the difference between TBil and DBil.

The percentage protection of treatment was calculated using the formula below. This was based on principal indicators of liver protection: relative liver weight, ALT, AST, ALP, γ GT, and TBil.

$$\text{Percent protection} = \frac{\text{Toxin value} - \text{Test value}}{\text{Toxin value} - \text{Normal value}} \times 100$$

Histopathological Analysis

Livers of sacrificed animals were excised, washed in cold buffered saline, and blotted dried. They were weighed to obtain the absolute liver weight (ALW). The relative liver weight (RLW) was calculated as follows:

$$\text{RLW} = \frac{\text{ALW}}{\text{Body weight at sacrifice}} \times 100$$

The liver sections were fixed in 10% buffered formalin, embedded in paraffin, and then 5 μm sections were cut from each block. The paraffin-embedded liver sections were stained with hematoxylin-eosin for histopathological examination. Each sample from the various

groups was examined in a blinded manner with light microscopy (Olympus Manual System Microscope BX43) and observed at 100 \times and 400 \times magnification. The changes in the liver architecture were observed and the extent of damage indicated by a pathologist.

Immunohistological Analysis

The immunohistochemical staining was performed using the streptavidin-biotin peroxidase complex kit (M IHC Select Detection System, HRP/DAB, Merck, Germany; Lot: 2775482) with a slight modification of the procedure as previously described by Jarikre and Emikpe¹³ and also used previously.¹⁴ Primary antibodies used were monoclonal mouse nuclear factor- κ B (NF- κ B) and interleukin 1 (IL-1) at a dilution of 1:100 in phosphate-buffered saline. Tissue sections from the CCl₄ and Para only treated rats served as negative controls. The slides were mounted with coverslips and DPX for examination.

The photomicrographs were taken with the aid of a digital camera (Amscope MU900) attached to the microscope. The images were quantified for staining intensity using the reciprocal intensity of the stained markers (NF- κ B, IL1) on the open-source Fiji (Image J) software. The optical density of the staining intensities was calculated using the following formula:

$$\text{OD} = \log_{10} \frac{\text{Maximum reciprocal intensity}}{\text{Mean reciprocal intensity}}$$

Antioxidant Analyses

Preparation of Liver Homogenate. Parts of liver tissues (1 g each) were stored in PBS. They were homogenized separately in 10 mL of 100 mM KH₂PO₄ buffer containing 1 mM EDTA, pH 7.4, and centrifuged at 12 000 rpm for 30 minutes at 4 $^{\circ}\text{C}$. The supernatant was collected and stored as the liver post mitochondrial fraction (PMF).

Assay of Superoxide Dismutase Activity. Superoxide dismutase (SOD) activity was determined using the method of Oyagbemi et al.¹⁵ Briefly, 100 mg of epinephrine was dissolved in 100 mL distilled water and acidified with 0.5 mL concentrated hydrochloric acid to prevent auto-oxidation of epinephrine for up to 4 weeks. Then 0.01 mL of hepatic PMF was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2), followed by the addition of 0.3 mL of 0.3 mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds; 1 unit of SOD activity being the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome in 1 minutes.

Assay of Glutathione Peroxidase. The glutathione peroxidase (GPx) activity was measured as previously described.¹⁶ The reaction mixtures contained 0.5 mL of potassium phosphate buffer (pH 7.4), 0.1 mL of sodium azide, 0.2 mL of GSH solution, 0.1 mL of H₂O₂, 0.5 mL of PMF, and 0.6 mL of distilled water. The mixture was incubated in a water bath at 37 $^{\circ}\text{C}$ for 5 minutes, and 0.5 mL of trichloroacetic acid (TCA) was added and centrifuged at 6000 rpm for 5 minutes; 1 mL of the supernatant was added to 2 mL of dipotassium hydrogen phosphate (K₂HPO₄) and 1 mL of Ellman's reagent. The absorbance was then read at 412 nm.

Assay of Reduced Glutathione. Reduced glutathione (GSH) content was estimated by the method of Jollow et al.¹⁷ Briefly, 0.5 mL of 4% sulfosalicylic acid (a precipitating agent) was added to 0.5 mL of PMF and centrifuged at 4000 rpm for 5 minutes. To 0.5 mL of the resulting

Table 2. Phytochemical Constituents of Aqueous Extract of *Ocimum americanum*.

Phytochemical	Leaves	Flower	Stem
Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Glycosides	+	+	+
Alkaloids	+	+	+
Coumarins	+	–	–
Triterpenoids	–	–	+
Sterols	–	–	+

Abbreviations: +, present; –, absent.

supernatant was added 4.5 mL of Ellman's reagent (0.04 g of DTNB in 100 mL of 0.1 M phosphate buffer, pH 7.4). The absorbance was then read at 412 nm.

Assay of Thiobarbituric Acid Reactive Substances. Thiobarbituric acid reactive substances (TBARS) were estimated as malondialdehyde (MDA) in the PMF.¹⁸ To 1.6 mL of Tris-KCl, 0.5 mL of 30% TCA, 0.4 mL of sample, and 0.5 mL of 0.75% thiobarbituric acid (TBA) prepared in 0.2 M HCl were added. The reaction mixture was incubated on water bath at 80 °C for 45 minutes, cooled on ice and centrifuged at 6000 rpm for 15 minutes. The absorbance was measured at 532 nm. Lipid peroxidation in units/mg protein was calculated with a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Statistical Analysis

The results were expressed as mean \pm standard error of mean and the difference in means calculated using multivariate analyses of variance, followed by Tukey's post hoc test. The data were also analyzed using GraphPad Prism 7 (GraphPad Software Inc). All analyses were conducted assuming a significance value of 5%.

Results

Phytochemical Constituents

Phytochemical analyses of *Ocimum americanum* extracts (leaves, flowers, and stem) showed the presence of tannins, flavonoids, saponins, glycosides, alkaloids, and coumarins but the absence of triterpenoids and steroids in the leaves (Table 2).

Effect of Treatment on Body and Liver Weights of Animals

The different treatments had a varied effect on body weight (Table 3). All treatments showed a positive effect on weight gain, except leaves extract co-administered with CCl₄. Stem extracts of *O. americanum* showed the highest increases though not significantly different from controls (normal, CCl₄ only and Para only). Administration of CCl₄ caused a significant increase in the relative liver weights signaling a toxic effect on the liver (Figure 1). The OAE treatment was able to reverse the increases caused by CCl₄ to near normal. No significant differences were observed with Para treatments.

Table 3. Effect of Different Treatments on the Body Weight of Animals.*

Treatment	Percent change in body weight (%)
Normal	17.39 \pm 4.37
CCl ₄ only	9.71 \pm 5.25 ^a
Para only	12.37 \pm 2.09
Silymarin + CCl ₄	6.32 \pm 1.60 ^a
Silymarin + Para	4.32 \pm 3.18 ^{ac}
Stem only	14.93 \pm 9.88
Stem + CCl ₄	16.74 \pm 9.34 ^b
Stem + Para	27.53 \pm 5.96 ^c
Flower only	6.00 \pm 1.81 ^a
Flower + CCl ₄	4.97 \pm 1.24 ^a
Flower + Para	4.00 \pm 1.99 ^{ab}
Leaves only	3.81 \pm 2.59 ^a
Leaves + CCl ₄	-1.53 \pm 1.34 ^{ab}
Leaves + Para	7.79 \pm 4.55 ^a

*Statistical significance: a ($P < .05-.001$) from Normal; b ($P < .05-.001$) from CCl₄ only; c ($P < .05-.001$) from Para only.

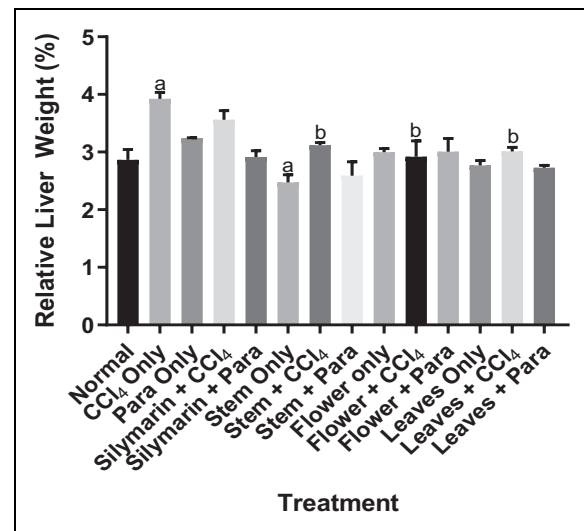


Figure 1. Relative liver weight of normal and treated animals. Statistical significance: a ($P < .05-.01$) from Normal; b ($P < .05-.0001$) from hepatotoxin only.

Effects of Different Treatments on Biochemical Parameters

CCl₄ administration resulted in significant increases ($P < .05-.001$) in the levels of ALT, AST, γ GT, TBil, and IBil, and a decrease in serum protein levels of the animals (Table 4). However, silymarin, stem, flower, and leaves extract treatments resulted in decreases in these parameters to near-normal levels. Para (acetaminophen) only treatment did not result in any significant changes in liver function parameters.

Effects of Different Treatments on Hematological Parameters

The different treatments had no significant changes in the levels of WBC, RBC, HGD, HCT, P-LCR, and PCT of the

Table 4. Effects of Different Treatments on Biochemical Parameter.*

Parameters	Normal	CCl ₄ only	Para only	Silymarin + CCl ₄	Silymarin + Para	Stem only	Stem + CCl ₄	Stem + Para	Flower only	Flower + CCl ₄	Flower + Para	Leaves only	Leaves + CCl ₄	Leaves + Para
Alb (g/L)	39.37 ± 7.53	40.07 ± 0.87	39.67 ± 1.63	35.70 ± 3.18	45.43 ± 1.18	35.73 ± 3.55	38.77 ± 1.31	35.60 ± 3.33	31.90 ± 1.95	36.87 ± 2.85	38.23 ± 3.45	41.07 ± 2.24	39.77 ± 1.15	40.27 ± 0.18
TP (g/L)	103.97 ± 10.95	94.13 ± 2.38	94.47 ± 2.05	96.30 ± 6.21	104.77 ± 2.80	110.73 ± 14.55	93.10 ± 4.10	93.53 ± 4.13	86.57 ± 3.39	96.67 ± 1.33	95.73 ± 4.99	91.53 ± 6.13	97.57 ± 2.97	97.07 ± 0.29
Glo (g/L)	64.60 ± 6.71	54.07 ± 1.54	54.80 ± 3.40	60.60 ± 3.76	59.33 ± 2.02	75.00 ± 11.78	54.33 ± 2.95	57.93 ± 5.02	54.67 ± 3.15	59.80 ± 4.18	57.50 ± 1.56	50.47 ± 5.04	57.77 ± 1.82	56.87 ± 0.47
ALT (U/L)	73.53 ± 8.39	636.60 ± 47.73 ^a	109.43 ± 11.15	326.90 ± 46.89 ^{ab}	111.53 ± 13.62	96.57 ± 12.77 ^b	128.83 ± 18.79 ^b	131.73 ± 9.61	84.23 ± 1.56 ^b	358.37 ± 28.36 ^{ab}	113.63 ± 9.84	81.83 ± 4.05 ^b	323.33 ± 7.10 ^{ab}	115.10 ± 5.17
AST (U/L)	300.67 ± 25.59	976.57 ± 73.86 ^a	383.67 ± 2.43	723.53 ± 43.40 ^{ab}	289.50 ± 30.77 ^c	283.73 ± 36.17 ^{bc}	159.80 ± 33.36 ^{ab}	291.87 ± 9.95 ^c	290.67 ± 17.59 ^{bc}	348.87 ± 12.50 ^b	298.60 ± 27.05 ^c	309.83 ± 20.52 ^{bc}	447.30 ± 16.54 ^{ab}	331.67 ± 0.37 ^c
AST/ALT	4.17 ± 0.49	1.57 ± 0.13	3.57 ± 0.35	2.33 ± 0.39	2.63 ± 0.23	3.13 ± 0.71	1.33 ± 0.48	2.23 ± 0.13	3.47 ± 0.19	1.00 ± 0.10	2.63 ± 0.07	3.80 ± 0.06	1.40 ± 0.06	2.90 ± 0.12
ALP (U/L)	237.10 ± 12.29	268.20 ± 15.08	286.93 ± 80.36	306.80 ± 31.48	261.27 ± 10.11	267.17 ± 48.95	304.83 ± 66.91	218.83 ± 18.85	205.67 ± 25.48 ^c	291.93 ± 50.29	227.20 ± 17.96	174.93 ± 4.99 ^{bc}	216.57 ± 7.46	151.27 ± 2.26 ^{ac}
γ-GT (U/L)	3.23 ± 0.43	10.20 ± 1.48	10.07 ± 0.94	2.73 ± 0.07	2.50 ± 0.51	4.40 ± 0.32	3.37 ± 0.18	4.57 ± 0.09	5.33 ± 0.18	5.17 ± 0.09	1.37 ± 0.48	2.30 ± 0.45	11.67 ± 0.61	0.73 ± 0.23
T-bil (μmol/L)	3.27 ± 0.66	6.41 ± 0.14 ^a	2.40 ± 0.31	2.80 ± 0.11	2.35 ± 0.15	3.76 ± 0.72	2.18 ± 0.51	2.04 ± 0.49	3.69 ± 0.38	2.50 ± 0.37	2.38 ± 0.44	3.29 ± 0.55	2.62 ± 0.19	1.71 ± 0.03
D-Bil (μmol/L)	1.37 ± 0.20	1.60 ± 0.37	1.08 ± 0.17	1.22 ± 0.20	0.89 ± 0.03	1.53 ± 0.07	0.87 ± 0.07	1.33 ± 0.29	1.73 ± 0.06	1.25 ± 0.29	0.89 ± 0.14	1.50 ± 0.13	1.25 ± 0.34	0.93 ± 0.02
IBil (μmol/L)	1.90 ± 0.51	4.81 ± 0.49 ^a	1.31 ± 0.40	1.57 ± 0.10 ^b	1.45 ± 0.13	2.23 ± 0.79	1.31 ± 0.54 ^b	0.71 ± 0.36	1.96 ± 0.33 ^b	1.24 ± 0.08 ^b	1.48 ± 0.39	1.79 ± 0.46 ^b	1.37 ± 0.49 ^b	0.78 ± 0.02

Abbreviations: γGT, γ-glutamyltransferase; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBil, direct bilirubin; Glo, globulin; TBil, total bilirubin; TP, total proteins.

*Statistical significance: a ($P < .05-.001$) from normal; b ($P < .05-.001$) from CCl₄ only; c ($P < .05-.001$) from Para only.

Table 5. Effect of Different Treatments on Haematological Parameters.

	WBC	RBC	HGB	HCT	PLT	LYM%	NEUT%	LYM#	NEUT#	P-LCR	PCT
Normal	11.37 ± 1.33	8.05 ± 0.26	14.07 ± 0.19	48.27 ± 1.35	859.67 ± 150.56	81.3 ± 0.97	18.7 ± 0.97	9.23 ± 0.98	2.13 ± 0.35	6.3 ± 0.32	0.6 ± 0.1
CCl ₄ Only	6.93 ± 0.58	9.35 ± 0.37	16.6 ± 0.51	57.8 ± 1.4	679 ± 184.91 ^a	52.73 ± 2.3	47.27 ± 2.3	3.67 ± 0.33	3.27 ± 0.32	10.97 ± 1.17	0.54 ± 0.14
Para Only	10.5 ± 0.35	8.81 ± 0.38	15.2 ± 0.56	53.03 ± 1.87	834 ± 70.12	78.83 ± 3.02	21.17 ± 3.02	8.3 ± 0.52	2.2 ± 0.25	6.33 ± 0.41	0.6 ± 0.06
Silymarin + CCl ₄	8.9 ± 1.96	8.85 ± 0.89	16.23 ± 1.21	56 ± 4.83	681 ± 130.02 ^a	68.87 ± 3.04	31.13 ± 3.04	6.23 ± 1.64	2.67 ± 0.32	10.07 ± 0.79	0.53 ± 0.11
Silymarin + Para	9.03 ± 0.07	7.52 ± 0.2	14.37 ± 0.33	47.67 ± 0.46	795 ± 34.95	79.1 ± 3.04	20.9 ± 3.04	7.13 ± 0.28	1.9 ± 0.26	6.9 ± 0.5	0.57 ± 0.03
Flower Only	7.8 ± 0.36	7.36 ± 0.46	13.23 ± 0.72	44.97 ± 2.81	812 ± 100.68	75 ± 5.17	25 ± 5.17	5.87 ± 0.63	1.93 ± 0.35	5.17 ± 0.99	0.55 ± 0.05
Flower + CCl ₄	6.87 ± 1.76	7.12 ± 0.73	13.3 ± 1.05	44.37 ± 3.91	645 ± 86.43 ^a	55.97 ± 2.75	44.03 ± 2.75	3.87 ± 1.07	3 ± 0.71	6.33 ± 0.6	0.45 ± 0.07
Flower + Para	10.97 ± 0.84	7.93 ± 0.2	14.6 ± 0.55	48.93 ± 0.94	770.67 ± 108.98	77.27 ± 2.35	22.73 ± 2.35	8.53 ± 0.87	2.43 ± 0.03	7.6 ± 0.4	0.56 ± 0.08
Leaves Only	8.83 ± 0.96	7.85 ± 0.33	14.5 ± 0.51	48.37 ± 2.6	695 ± 77.86 ^a	79.9 ± 0.56	20.1 ± 0.56	7.07 ± 0.81	1.77 ± 0.15	8.37 ± 1.3	0.51 ± 0.06
Leaves + CCl ₄	9.83 ± 1.17	7.85 ± 0.15	14.43 ± 0.37	49.73 ± 0.67	931.67 ± 109.65 ^b	73.17 ± 2.45	26.83 ± 2.45	7.23 ± 1.03	2.6 ± 0.25	7.8 ± 1.32	0.69 ± 0.1
Leaves + Para	9.27 ± 1.52	8.02 ± 0.41	15.03 ± 0.67	49.77 ± 1.84	804.33 ± 121.55	79.97 ± 1.27	20.03 ± 1.27	7.4 ± 1.1	1.87 ± 0.43	7.23 ± 0.74	0.59 ± 0.09
Stem Only	10.7 ± 0.96	7.64 ± 0.22	14.47 ± 0.44	48.4 ± 1.27	1003 ± 108.01 ^a	71.17 ± 3.47	28.83 ± 3.47	7.6 ± 0.67	3.1 ± 0.55	6.33 ± 0.39	0.72 ± 0.08
Stem + CCl ₄	5.4 ± 0.68	10 ± 1.64	18.37 ± 2.77	62.63 ± 10.62	546.67 ± 112.0 ^{ab}	68.63 ± 2.09	31.37 ± 2.09	3.7 ± 0.5	1.7 ± 0.21	8.57 ± 0.97	0.23 ± 0.08
Stem + Para	11.95 ± 1.05	7.63 ± 0.11	14.35 ± 0.35	47.7 ± 0.80	1046.5 ± 9.50 ^{bc}	75.25 ± 6.05	24.75 ± 6.05	9.05 ± 1.55	2.90 ± 0.50	7.35 ± 0.35	0.77 ± 0.01

Statistical Significance: ^a($p < 0.05 - 0.001$) from normal; ^b($p < 0.05 - 0.001$) from CCl₄ only; ^c($p < 0.05 - 0.001$) from Para only.

Abbreviation: HCT, hematocrit; HGB, hemoglobin; LYM#, number of lymphocytes; LYM%, percent lymphocytes; NEUT#, number of neutrophils; NEUT%, percent neutrophils; PCT, plateletcrit; P-LCR, platelet larger cell ratio; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

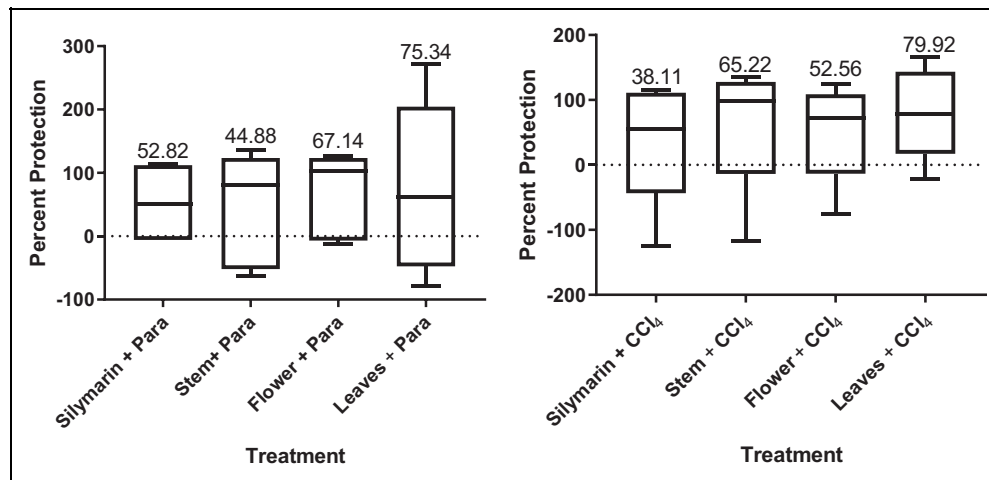


Figure 2. Percent liver protection of various treatments against Para and CCl₄ toxicity.

Table 6. Effect of Different Treatments on Oxidative and Antioxidant Profiles of Liver.

	Total Protein (g/dL)	MDA ($\mu\text{mol}/\text{mg}$ protein)	GSH ($\mu\text{mol}/\text{g}$ protein)	GPx ($\mu\text{mol}/\text{min}/\text{mg}$ protein)	SOD (units/mg protein)
Normal	13.65 \pm 1.53	0.57 \pm 0.09	88.68 \pm 11.39	38.21 \pm 0.97	6.03 \pm 0.69
CCl ₄ only	20.69 \pm 2.51	2.62 \pm 0.2 ^a	74.37 \pm 12.27	42.75 \pm 1.63	4.02 \pm 0.49
Para only	18.44 \pm 1.82	0.82 \pm 0.27	89.62 \pm 11.40	43.65 \pm 2.18	4.28 \pm 0.39
Sily + CCl ₄	15.36 \pm 1.66	0.72 \pm 0.36	102.94 \pm 15.67	42.98 \pm 1.94	5.09 \pm 0.54
Sily + Para	24.92 \pm 2.67	0.31 \pm 0.02	73.36 \pm 9.14	39.63 \pm 0.95	3.52 \pm 0.47
Stem only	6.73 \pm 0.49	0.59 \pm 0.04	257.32 \pm 15.84 ^a	34.47 \pm 1.88	11.22 \pm 0.71 ^a
Stem + CCl ₄	7.24 \pm 0.85	0.59 \pm 0.04	245.07 \pm 16.43 ^{ab}	34.83 \pm 2.11	10.79 \pm 0.74 ^{ab}
Stem + Para	7.73 \pm 0.82	0.74 \pm 0.08	226.47 \pm 18.73 ^{ac}	37.24 \pm 1.95	10.28 \pm 0.90 ^c
Flower only	11.68 \pm 0.81	0.78 \pm 0.15	141.16 \pm 8.54 ^a	35.84 \pm 0.80	6.91 \pm 0.41
Flower + CCl ₄	15.03 \pm 0.84	0.93 \pm 0.2	111.57 \pm 5.20 ^b	43.81 \pm 2.81	5.36 \pm 0.29
Flower + Para	14.92 \pm 0.47	0.91 \pm 0.11	96.83 \pm 9.68	50.71 \pm 3.03	5.30 \pm 0.19
Leaves only	6.87 \pm 0.70	0.70 \pm 0.07	264.94 \pm 22.61 ^a	38.54 \pm 2.16	11.56 \pm 0.97 ^a
Leaves + CCl ₄	7.91 \pm 1.16	0.56 \pm 0.07	242.99 \pm 24.60 ^{ab}	36.13 \pm 1.75	10.53 \pm 1.07 ^{ab}
Leaves + Para	7.03 \pm 0.41	0.62 \pm 0.03	256.60 \pm 15.01 ^{ac}	35.63 \pm 1.77	10.95 \pm 0.63 ^{ac}

Statistical Significance: ^a($P < 0.05 - 0.001$) from normal; ^b($P < 0.05 - 0.001$) from CCl₄ only; ^c($p < 0.05 - 0.001$) from Para only.

animals (Table 5). However, varied changes were observed in the levels of lymphocytes and platelets.

Effect of Different Treatments on Percent Protection

The percent protection of the silymarin, stem, flower, and leaf extracts on CCl₄ and Para-induced hepatotoxicity were recorded and calculated based on the principal indicators of relative liver weight, ALT, AST, ALP, γ GT, and TBil (Figure 2). The leaf extract of *O americanum* showed the highest protection against both CCl₄ and Para.

Effect of Different Treatments on Oxidative and Antioxidant Profiles of Liver

Extracts of *O americanum* resulted in significant increases in the levels of reduced glutathione (GSH) and superoxide dismutase (SOD) with the leaves and stem showing the best activities (Table 6).

Effect of Different Treatments on Liver Histology

CCl₄ and Para treatment resulted in significant changes in liver structure with centrilobular hepatocellular vacuolar degeneration and multiple foci of necrosis and inflammation (Figure 3B and C). Administration of flower extract of *O americanum* did not have any positive effect on the liver structure as shown by the existence of moderate centrilobular atrophy and inflammation in flower only and with hepatotoxicant (Figure 3F-G). However, treatment with leaves only had no observable effect on the liver as well as co-treatment with Para. Co-treatment of leaves with CCl₄ showed some severe areas of coagulation, necrosis, and inflammation (Figure 3I-K). Treatment with Stem extracts alone or with hepatotoxicant had no observable effect.

Effect of Different Treatments on Liver Inflammatory Marker

Figures 4 and 5 show the effect of treatment on the liver expression of NF- κ B and IL-1 in rat hepatocytes of normal and treated

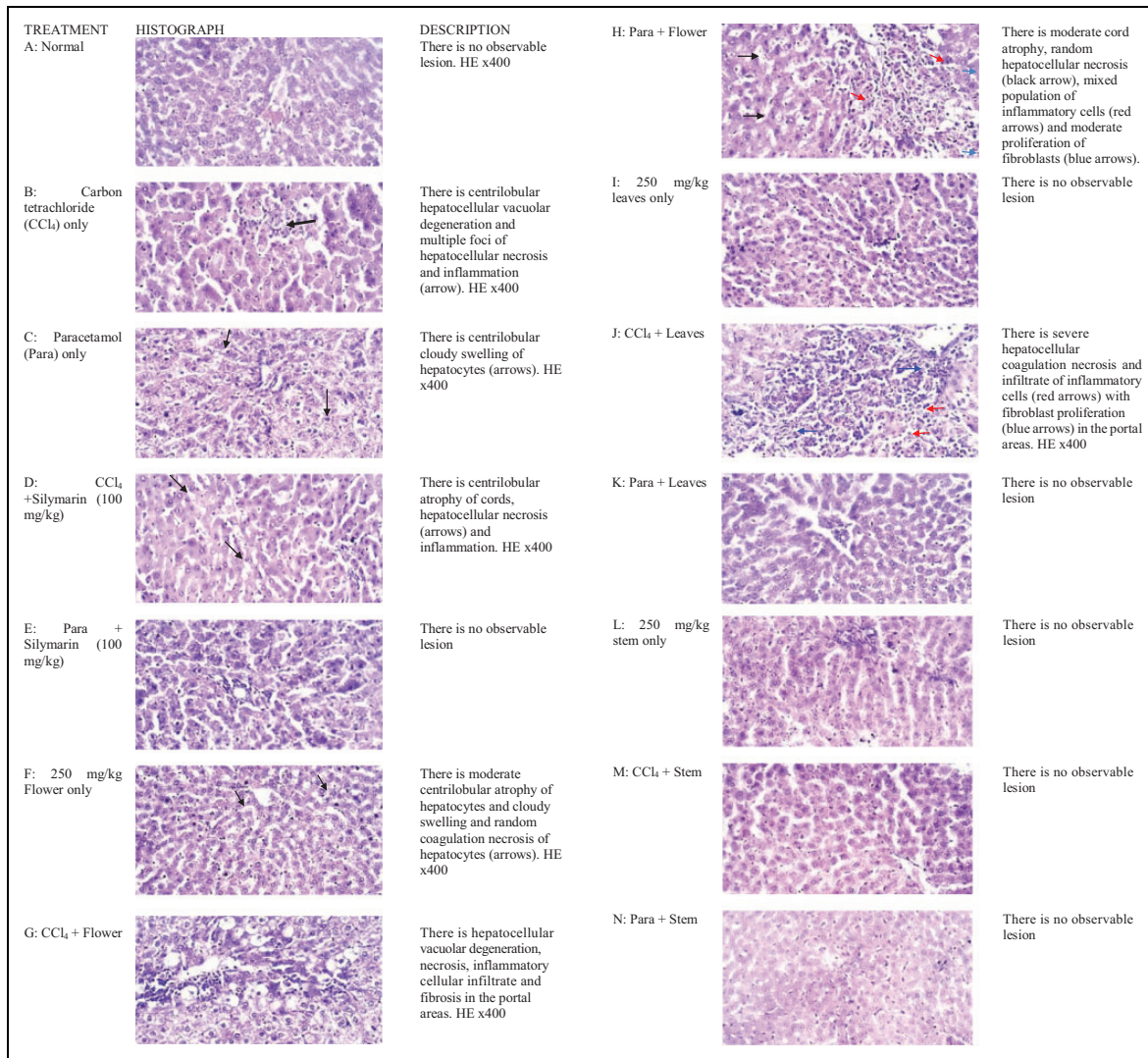


Figure 3. Effect of various treatments on liver microarchitecture.

animals. Figure 6 shows the percentage expression of NF- κ B (A) and IL-1 (B). CCl₄ only showed high expression of NF- κ B (A) and IL-1 (B); extract of leaves and flower with CCl₄ was unable to inhibit the expression of NF- κ B and IL-1. Stem extract, however, inhibited the expression to near-normal levels.

Discussion

The current study was aimed at assessing the hepatoprotective potential of aqueous extracts of *O. americanum* parts on CCl₄ and acetaminophen (Para) induced liver toxicity in rats. Most cases of orthodox and conventional medical treatments affect the body weight of an organism. The study showed the stem extracts having the highest weight gain though both leaves and flower showed significant positive effects on the weight of the animals. The result indicated that *O. americanum* does not affect the dietary pattern of the animals making them gain weight as a result of high appetite for food. The negative effects

of group administered with the leaves alongside the CCl₄ might be attributed to the toxicity of CCl₄ and changes in physiological process which could either alter the appetite and diet leading to poor nutrition and reduced weight.

Except for the normal group, the relative liver weight of all CCl₄ and Para treated groups indicated liver toxicity. The observed increase in relative liver weights following the administration of CCl₄ was confirmed as a result of some physiological changes observed in the liver during organ harvesting. Presence of lipid molecules and pale macroscopic appearance of the liver indicated possible hepatotoxicity.¹⁹ Liver weight gain can also be attributed to the adaptive mechanism of the hepatocytes in counteracting the toxic effect of the CCl₄ by hypertrophy.¹¹ This might also be due to the infiltration of the liver by inflammatory cells subsequently resulting in liver necrosis.²⁰ The OAE treatment of the various plant parts (flowers, leaves, and stem) was able to significantly ($P < .05-.001$) reverse the liver hypertrophy caused by CCl₄ and Para virtually to normal. All extract treatments alone had

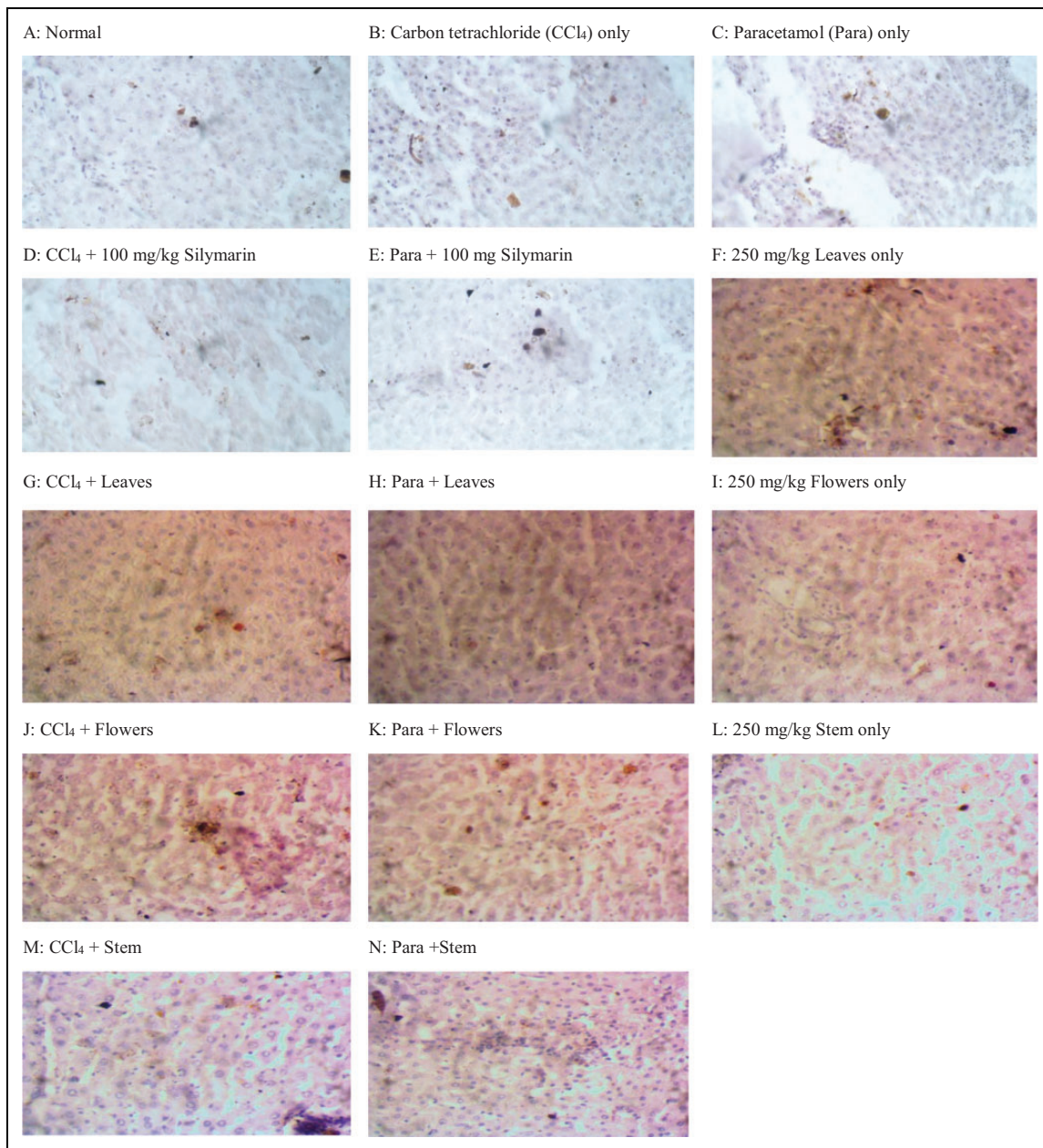


Figure 4. Effect of various treatments on the expression of NF- κ β in liver tissues.

no negative physiological effect on the liver. The organs, especially the liver of all OAE, looked healthy and reddish-brown with no lipid molecules, hence was able to give maximum protection to the liver.

Liver damage is identified and diagnosed by measuring the activities of liver function biomarker enzymes including AST, ALT, ALP, γ GT, and bilirubin, which are released into the blood from damaged cells. Low levels of these liver biomarkers in most cases do not have any clinical significance, hence indicating a healthy liver whereas elevated levels serve as relevant indicators of liver toxicity. ALT is a more selectively liver parenchymal enzyme than AST; greater levels of γ GT also support in diagnosing liver

disease.²¹ In the present study, CCl₄ only group resulted in significant increases in these parameters contrasted with the normal group. The result indicated and confirmed the high hepatotoxic effect of CCl₄ as reported in other studies.^{20,22} This situation is attributable to trichloromethyl radical, a degradative product of CCl₄, which covalently binds with sulfhydryl groups of several membrane molecules like GSH leading to their depletion, hence causing lipid peroxidation. The lipid peroxidation starts a cascade of reactions leading to liver necrosis and damage to membranes with subsequent leakage of enzymes. Damage to the hepatocytes is followed by the release of pro-inflammatory chemokines and cytokines.²³

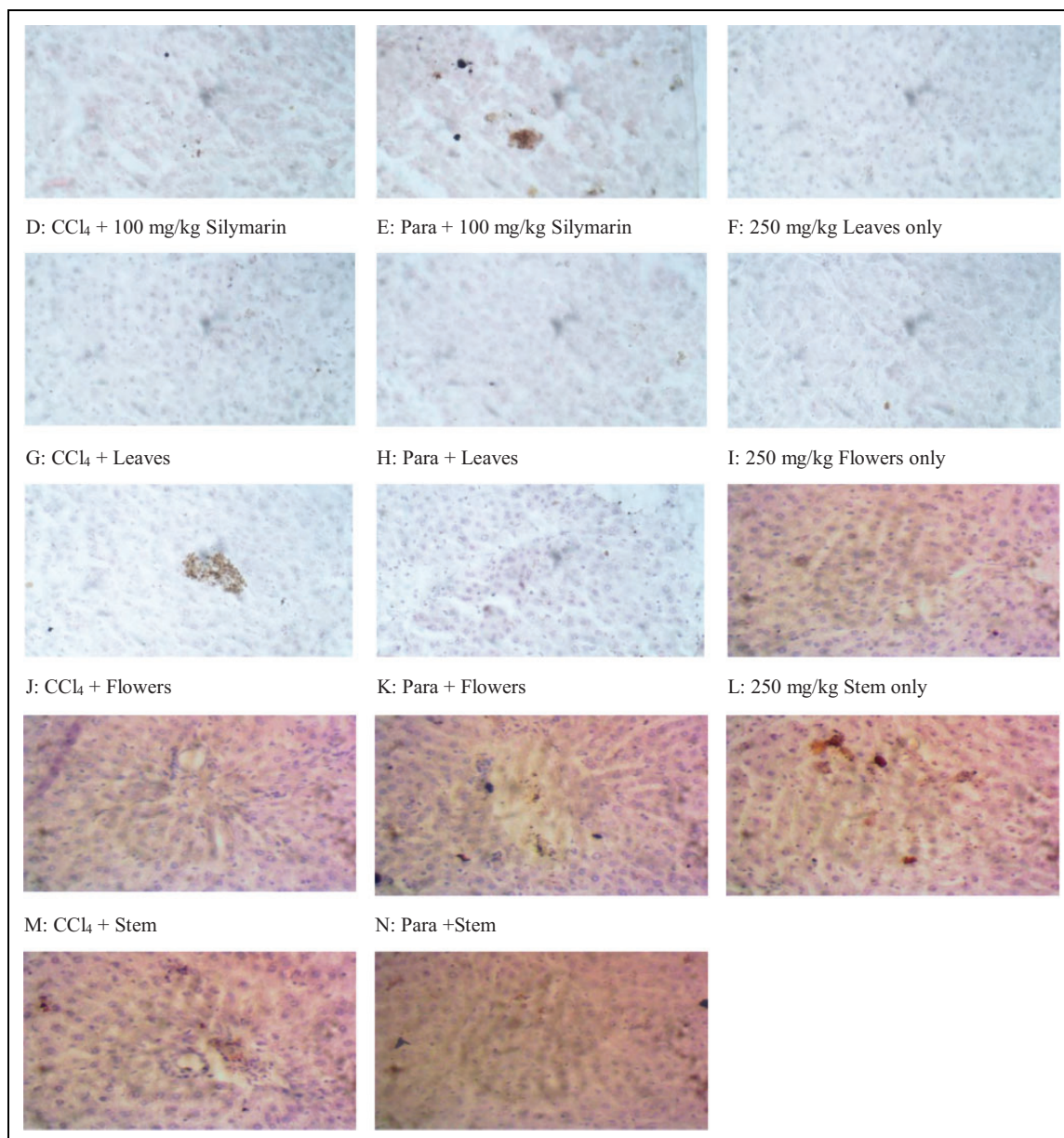


Figure 5. Effect of various treatments on the expression of IL-1 in liver tissues.

An abnormal increment in the levels of bilirubin in blood plasma shows hepatobiliary disease and severe aggravation of hepatocellular function. Increase in the concentration of unconjugated (indirect) bilirubin in the blood may result from a defect in the capacity of the liver to conjugate bilirubin for discharge.²⁴ The OAE caused significant ($P < .05-.001$) decrease in unconjugated (indirect) bilirubin levels. The result suggested that the extract may activate the constitutive androstane receptor, which is a key controller of bilirubin clearance in the liver.¹¹ In light of raised bilirubin levels, the constitutive androstane receptor activates expression of multiple components of the bilirubin clearance pathway bringing about increased clearance due to general glycosides²⁵ and could be used as an anti-jaundice agent. The presence of glycosides and

tannins in the OA extract did contribute to its ability to reduce bilirubin through the process of glucuronidation and improved immune response. The positive effect of Silymarin against CCl_4 confirms its activity.²⁶ Acetaminophen-induced toxicity had no significant increase in all liver enzyme (AST, ALT, ALP) and bilirubin in the current study as compared to CCl_4 , but slightly elevated levels of these enzymes in Para group as compared to the normal group which indicated some liver damage.

Plasma proteins, for the most part, are produced by the liver; the principal exception being immunoglobulins. Severe liver damage decreases the production of various proteins resulting in reduced serum levels of total protein, albumin, and globulins. Decreased protein production may render other abnormal

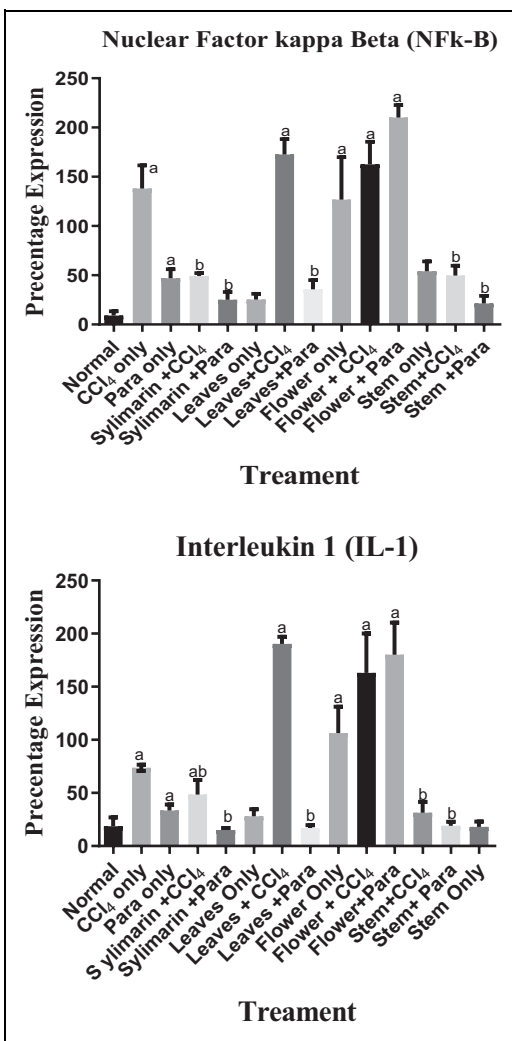


Figure 6. Percentage expression of (A) nuclear factor- $\kappa\beta$ (NF- $\kappa\beta$) and (B) interleukin 1 (IL-1) of normal and treated animals. Statistical significance; a ($P < .05-.01$) from Normal; b ($P < .05-.0001$) from hepatotoxin only.

test values, for instance, depletion of coagulation factors (all are globulins), which may result in prolonged prothrombin or activated partial thromboplastin times. The results showed no significant ($P < .05-.001$) effect in total protein, albumin, and globulin among all groups. Therefore, indicating no severe damage to the liver concerning protein production.

Studies have also reported that the decreasing levels of antioxidant molecules (like GSH) and antioxidant enzymes like glutathione, SOD, glutathione peroxidase, and catalase, which cause a biochemical cascade of event that leads to lipid peroxidation signify hepatic damage.²⁷ Oxygen radical-initiated lipid peroxidation may add to the impaired cellular function and necrosis associated with liver tissues. In the present study, CCl_4 only group caused significant increases in the hepatic MDA levels, a product of lipid peroxidation, indicating very high levels of reactive hydroxyl and peroxy radicals. Furthermore, the results demonstrated that all plant parts of OA

treatment prevented the increase in MDA, probably as part of scavenging the very reactive hydroxyl and peroxy radicals. Oxidative stress-induced tissue damage can be mediated by promoting the balance toward a lower oxidative status. Preventive antioxidant molecules such as SOD, GSH, and GPx are associated directly with the elimination of reactive oxygen species. Glutathione is an important parameter in intracellular mechanisms of protection against various harmful stimuli, such as oxidative stress.²⁸ In the present study, there was a significant ($P < .05-.001$) decrease in GSH in all control (CCl_4 and Para only) groups, supporting the concept that depletion of tissue GSH is one of the major attributes to lipid peroxidation and subsequent tissue damage. The results indicated that all parts of OA extract showed the ability to eliminate ROS. The reason is that *O. americanum* functioned as a free radical scavenger, hence increasing the available free GSH, which detoxifies the reactive intermediary oxygen products of lipid peroxidation induced by CCl_4 and acetaminophen.

Superoxide dismutase represents the first line of defense that assists in clearing singlet oxygen and splitting superoxide radicals to H_2O_2 ,²⁹ which is done by hepatic catalase that scavenges the H_2O_2 produced, while GPx acts on lipid peroxides to prevent the formation of lipid peroxidation products. The results on SOD indicated that the stem and leaves extract produced a significant increase in SOD levels. The decreased levels of SOD in exclusively CCl_4 and Para only treated groups further confirmed the toxic potential of the compounds on the liver resulting in the accumulation of superoxide radicals and the production of oxidative stress.³⁰ The OA extracts served as a free radical scavenger and therefore increased the available free GSH and SOD, which detoxifies the intermediary oxygen products of lipid peroxidation.

The protective effect of OAE extracts was further supported by histological observation of liver microstructure. The histogram of flower extract-treated groups confirmed its low percentage protection. Stem-treated groups indicated that they prevented cellular organelle degeneration as it was able to revert the damage. OAE leaves extract gave maximum protection to hepatocytes as well as hepatic enzymes as the biochemistry results indicated a reverse of severe changes observed in groups treated with CCl_4 only and Para only. Therefore, OAE prevented histopathological changes associated with hepatotoxicity from CCl_4 and Para as Chakraverty et al³¹ and Arthur et al¹¹ also reported on the activity of different plants on such compounds.

Inflammation is another liver damage indicator. Cytokines do regulate the host response to inflammation and immunity. NF- $\kappa\beta$ is a signaling molecule that induces the expression of proinflammatory cytokines such as IL-1 and tumor necrosis factor.³² IL-1 is a proinflammatory cytokine that shows that inflammation has taken place and upregulation shows the severity of inflammation.³³ Platelets have been shown to secrete cytokines to the site of infection or injury in the process of inflammation.³⁴ This study shows that CCl_4 and Para cause liver damage due to significant ($P < .01$) increase in proinflammation cytokine as well as platelets. Leaves and stem extract of

OAE expressed hepatoprotection due to its ability to down-regulate the expression of proinflammatory cytokine (IL-1 and NF- κ B).

It is therefore proposed that the leaf and stem extracts of *O. americanum* offered significant protection on the liver of drug-induced damage by positive modulation of biochemical parameters, improving the production of GSH and SOD, while inhibiting the production of malondialdehyde and down-regulating the expression of NF- κ B and IL-1. These activities further improved liver microarchitecture.

Conclusion

Crude extracts of *O. americanum* have a hepatoprotective effect due to the presence of alkaloid, phenolic compounds, and flavonoids. *O. americanum* could scavenge free radicals generated by hepatotoxins (CCl₄ and Para), hence reducing lipid peroxidation by increasing antioxidant enzyme activity and reducing hepatic enzymes in blood of experimentally induced liver toxicity. This indicates that OAE has therapeutic potential as a hepatoprotective agent.

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Conceptualization and methodology: Afua Kobi Ampem Genfi, Christopher Larbie, Benjamin O. Emikpe, Ademola A. Oyagbemi. Formal analysis, investigation: Afua Kobi Ampem Genfi, Christopher Larbie, Benjamin O. Emikpe, Ademola A. Oyagbemi, Clement O. Adjei, Caleb K. Firemping. Writing of original draft, and review and editing: Afua Kobi Ampem Genfi, Christopher Larbie. All authors approved the final manuscript.


Declaration of Conflicting Interests

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Ethical Approval

There was no formal ethical approval sought for the present study, but the study protocol was reviewed and approved by a veterinarian on the research team. All animal experiments were conducted in accordance with standard protocols.

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