

## ***Hippocratea africana* root extract and fractions attenuated carbon tetrachloride-induced oxidative stress and liver injuries in rats**

**Abstract.** *Hippocratea africana* root is used locally in ethnomedicine to treat various diseases such as diabetes, malaria and poisoning. This work aimed to investigate the antidotal potential of the root extract and fractions against carbon tetrachloride induced liver injury to justify its ethnomedicinal uses and give scientific proof to its claimed antidotal activity in traditional medicine. Therefore, 40 rats were randomly divided into eight (8) groups of 5 rats each and treated as follows: Group 1- (normal control) received oral 10 mL/kg of distilled water, Group 2- served as the organotoxic group, groups 3 - 5 were orally administered with 200, 400 and 600 mg/kg of root extract respectively, groups 6 and 7 were pretreated with 400 mg/kg of DCM and aqueous fractions respectively, and group 8, the positive control group was given 100 mg/kg of silymarin. All treatment lasted 8 days, and carbon tetrachloride (1.5 mL/kg) was given to all treatment groups. Assay of liver function parameters, antioxidative stress markers as well as the histopathological study of the liver were used to assess the hepatoprotective activity of root extract and fractions. Administration of the root extract (200-600 mg/kg) caused significant ( $p < 0.05-0.001$ ) reductions in the levels of liver biomarker enzymes (ALT, AST, and ALP), direct and total bilirubin and elevation of serum level of total protein elevated by  $\text{CCl}_4$  administration. The effects were dose-dependent in most cases. The extract and fraction further caused significant ( $p < 0.05-0.001$ ) and non dose- dependent elevation in the levels of GSH, GPx, CAT, GST, and SOD when compared to the organotoxic group, while MDA level was reduced. The root extract and fractions of *H. africana* did not affect hematological indices of the treated rats but caused significant ( $p < 0.001$ ) dose-dependent increases of WBC and platelets counts at the highest dose (600 mg/kg) relative to the organotoxic group. Histology of the liver sections of extract, fractions, and silymarin-pretreated animals showed insignificant reductions in the pathological features compared to the organotoxic-treated animals except at the lowest dose (200 mg/kg). The chemical pathological improvements observed were not reflected markedly in histopathological observations, suggesting weak hepatoprotective potentials. The results showed that root extract and fractions of *Hippocratea africana* have weak hepatoprotective potentials against  $\text{CCl}_4$ -induced liver toxicities, which may be due to the activities of its phytochemical components and short duration of study.

**.Keywords:** anti-toxicant, antioxidant, *Hippocratea africana*, oxidative stress, liver protective,

### **1.INTRODUCTION**

Drug-associated organ toxicities have continued to be on the increase worldwide. Drugs used for therapeutic purposes such as acetaminophen, gentamicin, rifampicin, carbon-tetrachloride, doxorubicin and antineoplastic drugs, industrial raw material and wastes as well as chemicals and other environmental pollutants like agrochemicals, have variously been linked to organs toxicities [1]. Multiple-organs toxicities resulting from accidental, acute large doses and long-term use of these drugs and chemicals have been linked with hepatotoxicity, nephrotoxicity, cardiotoxicity and testicular/ovarian toxicity. These organs are susceptible when exposed to the effects of these drugs, thereby compromising their functions due to the toxic effects of these drugs on their cells [2]. Although various remedial and management approaches are available, therapeutic strategies aimed at alleviating these toxic potentials of drugs and chemicals are still inadequate and unsatisfactory. Thus, necessitating investigation of agents that can ameliorate these deleterious effects of drugs [3].

Liver disease is responsible for two million deaths yearly, accounting for 4% of all deaths worldwide mostly in men [4], causing about 1.32 million deaths in 2017 [5]. In Nigeria, liver-related diseases have led to increases in hospital admissions in recent times and contributed significantly to annual deaths nationwide [6]. The high cost of management of organ dysfunction in Nigeria and other third-world countries cannot be afforded by many, including the poor and low-income earners, thereby constituting a huge financial burden on families of subjects with such medical conditions [7]. Herbal medicine has become an alternative option in managing and treating organ dysfunctions since it is affordable, readily available, and with less toxic effects. The search for a plant with effective organ protective potential will drastically reduce the financial cost of managing organ dysfunctions and minimize the hazardous effects associated with using available contemporary drugs.

“*Hippocratea africana* (Willd.) Loes. ex Engl. (Celastraceae) syn. *Loeseneriella africana* (Willd.) N.Hallé is a perennial tropical climber that grows widely in tropical African rainforests” [8]. “It is also called the African paddle-pod and ‘*Eba enang enang*’ by the Ibibios of Nigeria. The plant's roots have been widely employed in ethnomedicine by the Ibibios of the southern part of Nigeria to treat many ailments such as fever, convulsion, malaria, ulcers, body pains, infections, diabetes, and diarrhea, among others” [9]. “The plant's root is also used for its antidotal or anti-poison potential to treat liver diseases such as jaundice and hepatitis” [10,11,12]. Previous scientific reports have shown that the root extract possesses antimalarial” [13,14], angioedema and antinociceptive [15], antidiabetic and hypolipidemic [16,17], antidiarrhoeal and antiulcer [18], hepatoprotective [19], antileishmanial, cytotoxicity and cellular antioxidant [20], antibacterial, anticonvulsant and depressant [21], *in vivo* alpha-amylase and alpha-glucosidase inhibitory [22], *in vitro* antioxidant [17,23], kidney protective potentials against doxorubicin-induced kidney toxicity [24] and genotoxic [25] activities. “GCMS analysis of the root fractions revealed the presence of spiro hexane-1-carboxylic acid, ethyl ester, 3-methoxy-2-methylphenol, 2,3-benzofuran dione, 6-hydroxy-4-(p-hydroxy benzyl),  $\delta$ -3-Carene and  $\alpha$ -terpineol in ethyl acetate fraction” [26], monoterpenes (thujene, limonene, linalool,  $\alpha$ -phellandrene,  $\alpha$ -terpineol, and sabinene) and sesquiterpenes (dehydromevalonic lactone), in the n-hexane fraction of the root extract [19]. Also, two xanthones, 1,3,6,7-tetrahydroxanthone and 1,3,6-trihydroxy-7-methoxyxanthone have been isolated from the root of *H. africana* [23]. We report hepatoprotective and antioxidative stress effects of the root extract and fractions of *H. africana* against carbon tetrachloride-induced liver injury in rats.

## 1. MATERIALS AND METHODS

### 2.1 Plants collection

“In November 2021, fresh roots of *Hippocratea africana* were collected in bushes in the Uruan area, Akwa Ibom State, Nigeria. Prof Magaret Bassey, a taxonomist from the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria, identified and authenticated the plant. Herbarium specimen UUDPHB 30 (i) was deposited at the Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo”. [50]

### 2.2 Preparation of extract and fractions

“The fresh roots of *H. africana* were washed, reduced into smaller pieces, and shade-dried in the laboratory for two weeks. These were later pulverized using an electric grinder. The pulverized root of *H. africana* (1 kg) was soaked in ethanol (50%) for 72 h. The liquid filtrate obtained was concentrated in a rotary evaporator at 40°C. The crude extract was dissolved in 500 mL of distilled water, amounting to 20 g, and partitioned with dichloromethane (DCM, 5 x 500 mL) on an equal volume till no color change was observed to obtain DCM and aqueous fractions. The extract and fractions were stored in a refrigerator at 4°C until used for the experiment”. [50]

### **2.3 Animals**

In this study, male albino Wistar rats (125 - 140g) sourced from the University of Uyo Animal House and sheltered in plastic cages were used. The rats were fed with pelleted standard Feed (Guinea feed) and given unlimited access to water.

### **2.4 Experimental Design**

In this experiment, 40 male rats were shared randomly into 8 different experimental groups of 5 rats each that were treated as follows: Group 1, which served as the control group (normal control), received oral 10 mL/kg of distilled water for eight consecutive days. Group 2, the organotoxic group, had animals administered 10 mL/kg of normal saline orally for 8 days. Groups 3 -5 served as the extract-treated groups and were orally treated with 200, 400, and 600 mg/kg of root extract daily for 8 days. Groups 6 and 7 animals were pretreated with 400 mg/kg of DCM and aqueous fractions, respectively for 8 days. Group 8 animals in the positive control group were orally administered 100 mg/kg of silymarin for 8 days.

On the 8<sup>th</sup> day, liver injuries were induced in the treated animals in groups 2 - 8 by administering carbon tetrachloride (1.5 mL/kg, i.p) dissolved in corn oil mixed at a ratio of 1:3. All the animals were weighed again and sacrificed under light diethyl ether vapor, 24 hours after carbon tetrachloride administration,

### **2.5 Collection of Blood Samples and Organs**

Blood samples were collected into plain centrifuge tubes and EDTA bottles from the sacrificed animals by cardiac puncture. The blood in the centrifuge tubes was centrifuged for 15 minutes at 2,500 rpm at room temperature to separate the serum to avoid hemolysis and used for biochemical assays. The blood samples collected into EDTA bottles were used for hematological analysis. The livers were surgically removed, weighed, and fixed in 10% formaldehyde for histological process.

### **2.6 Hematological Analysis**

The following hematological parameters were determined from the blood samples collected into EDTA bottles: Red blood cell counts (RBC), hemoglobin level (Hb), total and differential White Blood Cell Count (WBC), packed cell volume (PCV), and platelet Count. These parameters were determined at the Haematology Department of the University of Uyo Teaching Hospital using an automated Haematology analyzer .

### **2.7 Biochemical Analysis**

#### **2.7.1 Liver Function Test**

The following liver function indices were determined: aspartate aminotransaminase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total plasma protein and albumin, and Total and direct bilirubin. Randox analytical kits were used for these determinations according to standard procedures of manufacturer's protocols [27] at the Chemical Pathology Department of the University of Uyo Teaching Hospital.

#### **2.7.2 Oxidative Stress Markers**

The antioxidant enzymes assays were performed on the liver homogenates of rats used in this study. These oxidative stress markers were used to assess the extract's antioxidative stress potential.

#### **2.7.3 Preparation of liver Homogenate**

After the rats were sacrificed humanely under light diethyl ether, the rat livers were surgically removed and weighed. They were briefly rinsed in ice-cold 1.15% KCl solution and preserved in a clean sample bottle containing 0.9% NaCl. Liver homogenates were constituted in a ratio of 1 g of wet tissue to 9 ml of 1.25% KCl using motor-driven Teflon-pestle. The different homogenates were centrifuged at 7,000 rpm for 10 min at 4°C. The supernatants collected after that were used for the

assays of superoxide dismutase (SOD) [28], catalase (CAT) [29], glutathione peroxidase (GPx) [30], reduced glutathione (GSH) [31] and malondialdehyde (MDA) content [32]. The assays were performed on all the liver homogenates of rats used in this study.

## 2.8 Histopathological studies

The excised livers from rats in different treatment groups were fixed in 10 % buffered formalin for histological processes. These were then processed and stained with hematoxylin and eosin (H&E) [33], according to standard procedures at the Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo. Morphological changes in the excised organs were observed and recorded. Histologic pictures were also taken as micrographs.

## 2.9 Statistical Analysis and Data Evaluation

The ANOVA (one-way) was used to analyze statistically the data obtained from this work followed by a post-test (Tukey-Kramer multiple comparison test). Differences between means at a 5% significance level, i.e.,  $p \leq 0.05$ , were considered significant.

# 3.RESULTS AND DISCUSSION

## 3.1 Effect of *H. africana* root extract on liver weights of rats with carbon tetrachloride-induced toxicity

Pretreatment of rats with root extract and fractions of *H. africana* did not cause a significant effect ( $p > 0.05$ ) on the liver weights of rats following administration of carbon tetrachloride when compared to normal control as well as the organotoxic group (Table 1).

## 3.2 Effect of treatment with root extract and fractions of *H. africana* on the hematological parameters of rats with carbon tetrachloride-induced hepatotoxicity.

Table 2 depicts the effect of root extract and fractions of *H. africana* pretreatment on hematological indices of rats with carbon tetrachloride-induced toxicities. It was observed that pretreatment of the animals did not cause any significant ( $p > 0.05$ ) alteration in the levels of RBC, hemoglobin concentration, and percentages of PCV, lymphocytes, basophils, monocytes, eosinophils, and neutrophils when compared to normal control following the administration of  $\text{CCl}_4$  (1.5 mL/kg) to rats after 8 days of pretreatment with the root extract and fractions. However, significant ( $p < 0.01$ ) elevations in the WBC count and decreases in platelet counts were recorded following carbon tetrachloride administration compared to normal control. Pretreatment of rats with carbon tetrachloride-induced liver toxicity with the root extract and fractions of *H. africana* caused dose-dependent increases of WBC and platelets counts, which were significant ( $p < 0.001$ ) at the highest dose (600 mg/kg) relative to the organotoxic group. Other blood indices such as RBC counts, hemoglobin concentration, PCV, neutrophils, basophils, lymphocytes, monocytes, and eosinophils percentages were not affected significantly ( $p < 0.05$ ) following extract/fractions pretreatment when compared to the organotoxic group (Table 2).

**Table 1:** Effect of *H. africana* root extract on Liver weights of rats with carbon tetrachloride-induced liver toxicity

<b>PARAMETERS/ TREATMENT</b>	<b>Dose mg/kg</b>	<b>Liver</b>
Normal control	-	7.43±0.29
Carbon tetrachloride	1.5ml	7.46± 0.29
Silymarin+CCl4	100	6.95±0.54
Extract+CCL4	200	5.73±0.22
	400	7.16±0.33
	600	7.88±0.42
Aqueous fraction	400	6.84±0.15
DCM fraction	400	8.40±0.71

Note: Data were expressed as mean ±SEM. n = 5.

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**Table 2:** Effect of *H. africana* root extract and fractions on hematological parameters of rats with carbon tetrachloride-induced toxicity

Treatment	Dose mg/kg	WBC (L)	NEUT. (%)	LYM (%)	MONO (%)	EOSINO (%)	BASO (%)	RBC (L)	HGB (g/dL)	PCV (%)	PLATELETS. (L)
Control	10	10.89± 0.57	38.27±2.92	61.12±4.67	3.02± 1.45	0.12± 0.06	0.70± 0.12	7.05± 0.18	12.87±0.26	40.65±1.04	819.0± 22.71
CCl <sub>4</sub>	1.5	15.03±0.78 <sup>b</sup>	38.07±0.92	58.85±3.04	1.75± 0.23	0.22 ±0.07	0.92± 0.09	7.52± 0.20	12.93±0.46	40.77±1.52	680.0±12.37 <sup>b</sup>
Crude extract	200	10.85±0.74 <sup>c</sup>	29.72±3.90	66.70±3.28	2.15± 0.60	0.27 ±0.11	1.15± 0.09	7.57± 0.27	13.60±0.43	43.72±1.54	595.0±28.20
	400	13.16±0.56	33.82±3.67	63.12±3.61	1.32± 0.26	0.65± 0.35	1.07± 0.11	7.42± 0.27	13.25±0.41	43.22±1.29	586.5±28.46
	600	19.67±0.75 <sup>d</sup>	30.90±4.04	65.45±4.31	1.90± 0.26	0.67± 0.23	1.07± 0.16	7.73± 0.29	13.52±0.53	42.67±1.89	805.0±22.37 <sup>d</sup>
Aqueous Fraction	400	11.82±0.86	33.85±4.66	60.85±2.66	3.95± 1.25	0.90 ± 0.31	0.95± 0.22	6.93± 0.15	12.70±0.29	40.50±1.19	655.0±22.55
DCM fraction	400	9.65±0.70	31.35±0.91	63.80±0.44	4.17± 0.89	0.87 ± 0.23	0.92± 0.08	7.16± 0.62	12.72±0.78	39.82±2.71	703.0± 27.62
Silymarin	100	11.47±1.05 <sup>d</sup>	39.25±2.44	56.40±3.64	3.15± 0.97	0.53± 0.03	0.77± 0.41	7.73± 0.33	13.77±0.44	43.10±1.69	731.5±20.81

Note: Data is expressed as MEAN ± SEM, Significant at <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001 when compared to control; Significant at <sup>d</sup>p<0.05, <sup>e</sup>p<0.01, <sup>f</sup>p<0.001 compared to organotoxic group (n=5).

### **3.3 Effect of root extract and fractions of *H. africana* pretreatment on liver function parameters of rats with CCl<sub>4</sub>-induced liver injury**

“The results in Table 3 show the effect of the root extract and fractions of *H. africana* on liver function indices of rats with CCl<sub>4</sub>-induced liver injury. Administration of CCl<sub>4</sub> to rats in the organotoxic group caused significant ( $p < 0.05-0.001$ ) elevation in the levels of ALT, AST, ALP, total and combined bilirubin when compared to the normal control, while total albumin and protein levels were significantly ( $p > 0.05-0.01$ ) reduced when compared to normal control. However, significant ( $p < 0.05-0.001$ ) non-dose-dependent decreases in ALT, AST, ALP, total, and combined bilirubin levels were observed following pretreatment with the root extract and fractions when compared to the organotoxic group (group 2). However, the decrease in ALP level was only significant ( $p < 0.05$ ) in group treated with the highest dose of the root extract (600 mg/kg). The total protein and albumin levels significantly ( $p < 0.05$ ) decreased following CCl<sub>4</sub> administration and were elevated by pretreatment of the rats with root extract and fractions” (Table 3). [50]

### **3.4 Effect of *H. africana* root extract and fractions on liver oxidative stress markers of rats with carbon tetrachloride-induced toxicity**

Table 4 shows the effect of pretreatment with root extract and fractions of *H. africana* on liver oxidative stress markers in rats with carbon tetrachloride-induced organ injuries. Carbon tetrachloride administration was found to significantly ( $p < 0.05-0.001$ ) reduced the levels of GSH, GPx, CAT, GST, and SOD, but the MDA level was highly increased when compared to normal control. Pretreatment of rats with CCl<sub>4</sub>-induced organs injuries with root extract and fractions of *H. africana* and silymarin for 8 days caused significant ( $p < 0.05-0.001$ ) and non dose- dependent elevation in the levels of GSH, GPx, CAT, GST, and SOD when compared to the organotoxic group. However, pretreatment of the rats with root extract and fractions of *H. africana* caused reductions in the levels of MDA of various treatment groups, which were only significant ( $p < 0.05$ ) in the groups treated with the DCM fraction and silymarin (Table 4).

### **3.5 Effect of root extract and fractions of *H. africana* on histology of rat liver in carbon tetrachloride-induced hepatotoxicity**

Histological sections of livers of rats pretreated with various doses of root extract and fractions of *H. africana* at magnification (x400) stained with the H&E method revealed that group 1 (normal control, A) treated distilled water (10 mL/kg) had a liver section that showed normal arrays of hepatocytes, portal triad, and congested portal vein. No degenerated epithelial lesion was observed (Figure 1A). The liver sections of the organotoxic group (Group 2, B) treated with carbon tetrachloride (CCl<sub>4</sub>) only (1.5 mL/kg) showed disrupted parenchyma with ballooning degenerated hepatocytes, both nuclear and cytoplasmic vacuolation. Mildly diffused lobular inflammatory infiltrate and congested blood vessels were also seen (Figure 1B). Group 3 (C) rats pretreated with 200 mg/kg of *H. africana* root extract for 8 days, followed by CCl<sub>4</sub>, showed a multifocal area of hepatocytes drop out/ vacuolation, ballooning degeneration, and area of inflammatory infiltrate. Normal multiple lobules with normal hepatocytes were present. Liver sections of rats in group 4 (D) pretreated with 400 mg/kg of *H. africana* root extract and CCl<sub>4</sub> showed disrupted parenchyma with ballooning degenerated hepatocytes, vacuolation, and scaring parenchyma. Group 5 (E) liver sections of rats pretreated with 600 mg/kg of *H. africana* root extract and CCl<sub>4</sub> showed disrupted parenchyma with ballooning degenerated hepatocytes, vacuolation, and scaring parenchyma. There are also mildly diffused lobular inflammatory infiltrates and congested blood vessels. The liver section of rats in group 6 (F) pretreated with an aqueous fraction of *H. africana* root (400 mg/kg) and CCl<sub>4</sub> showed disrupted parenchyma with ballooning degenerated hepatocytes, both nuclear and cytoplasmic vacuolation/drop-out necrosis were observed. Liver sections of group 7 (G) rats treated with dichloromethane fraction of *H. africana* root and CCl<sub>4</sub> showed disrupted parenchyma with ballooning degenerated hepatocytes, vacuolation, and scaring parenchyma. The liver sections of rats pretreated

with silymarin (100 mg/kg) and CCl<sub>4</sub> showed disrupted parenchyma with ballooning degenerated hepatocytes and mild inflammatory infiltrate (Figure 1H).

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**Table 3:** Effect of *H. africana* root extract and fractions on liver function parameters of rats with carbon tetrachloride-induced toxicity

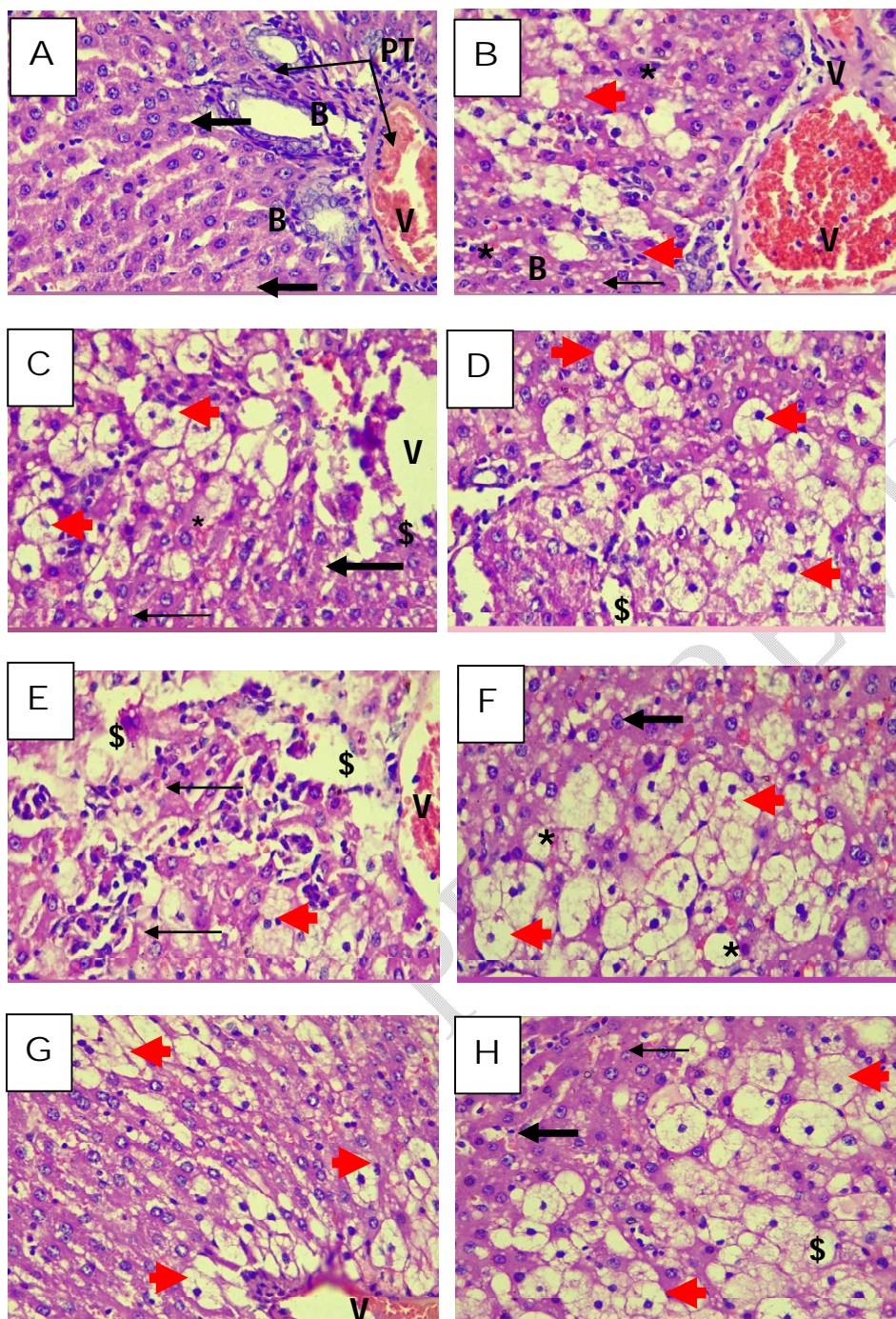
Treatment	Dose mg/kg	Total protein (g/dL)	Albumin (g/dL)	Total Bilirubin ( $\mu\text{mol/L}$ )	ALT (U/L)	ALP (U/L)	AST (U/L)	Combined Bilirubin ( $\mu\text{mol/L}$ )
Control	10	52.0 $\pm$ 1.47	42.50 $\pm$ 2.10	3.35 $\pm$ 0.18	8.37 $\pm$ 1.02	34.75 $\pm$ 1.93	14.75 $\pm$ 1.37	1.77 $\pm$ 0.08
CCl <sub>4</sub>	2000	39.75 $\pm$ 2.56 <sup>a</sup>	30.25 $\pm$ 1.03 <sup>b</sup>	8.02 $\pm$ 0.50 <sup>c</sup>	26.25 $\pm$ 1.65 <sup>c</sup>	55.25 $\pm$ 1.79 <sup>c</sup>	40.50 $\pm$ 2.59 <sup>c</sup>	5.67 $\pm$ 0.42 <sup>c</sup>
Crude extract	200	53.25 $\pm$ 1.37 <sup>f</sup>	35.75 $\pm$ 1.10	5.25 $\pm$ 0.21 <sup>b,f</sup>	21.25 $\pm$ 1.49 <sup>c</sup>	49.0 $\pm$ 1.87 <sup>c</sup>	25.25 $\pm$ 0.85 <sup>b,f</sup>	3.17 $\pm$ 0.12 <sup>c,e</sup>
	400	51.75 $\pm$ 1.49 <sup>f</sup>	34.75 $\pm$ 1.79 <sup>d</sup>	3.87 $\pm$ 0.14 <sup>f</sup>	13.15 $\pm$ 1.55 <sup>f</sup>	47.25 $\pm$ 0.85 <sup>b</sup>	19.25 $\pm$ 0.85 <sup>f</sup>	2.05 $\pm$ 0.10 <sup>e</sup>
	600	57.50 $\pm$ 1.04 <sup>e</sup>	37.0 $\pm$ 1.47	5.20 $\pm$ 0.40 <sup>b,f</sup>	18.0 $\pm$ 2.73 <sup>a,e</sup>	38.50 $\pm$ 1.70 <sup>e</sup>	24.50 $\pm$ 1.70 <sup>c,e</sup>	2.95 $\pm$ 0.31 <sup>c,d</sup>
Aqueous Fraction	400	59.50 $\pm$ 1.04 <sup>a,d</sup>	37.0 $\pm$ 1.47	4.75 $\pm$ 0.22 <sup>f</sup>	14.62 $\pm$ 1.02 <sup>f</sup>	50.50 $\pm$ 1.84 <sup>c</sup>	22.50 $\pm$ 1.04 <sup>a,d</sup>	2.47 $\pm$ 0.11 <sup>f</sup>
DCM fraction	400	52.25 $\pm$ 1.10 <sup>f</sup>	35.0 $\pm$ 1.47 <sup>d</sup>	4.70 $\pm$ 0.32 <sup>f</sup>	16.52 $\pm$ 1.00 <sup>b,d</sup>	50.75 $\pm$ 2.09 <sup>c</sup>	22.50 $\pm$ .84 <sup>a,f</sup>	2.65 $\pm$ 0.21 <sup>e</sup>
Silymarin	100	59.0 $\pm$ 1.68 <sup>d</sup>	38.5 $\pm$ 1.32	4.57 $\pm$ 0.31 <sup>f</sup>	12.65 $\pm$ 0.56 <sup>f</sup>	42.0 $\pm$ 3.24	22.0 $\pm$ 1.68 <sup>f</sup>	2.65 $\pm$ 0.25 <sup>e</sup>

Note: Data is expressed as MEAN  $\pm$  SEM, Significant at <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001 when compared to control; Significant at <sup>d</sup>p<0.05, <sup>e</sup>p<0.01, <sup>f</sup>p<0.001 compared to the organotoxic group. (n=5)

**Table 4:** Effect of *H. africana* root extract and fractions on liver oxidative stress markers of rats with carbon tetrachloride-induced toxicity

Treatment	Dose mg/kg	SOD (U/ml)	CAT (U/g of protein)	GPx ( $\mu\text{g/ml}$ )	GSH ( $\mu\text{g/ml}$ )	GST ( $\mu\text{g/ml}$ )	MDA ( $\mu\text{Mol/ml}$ )
Control	10	0.44 $\pm$ 0.03	5.31 $\pm$ 0.37	0.072 $\pm$ 0.001	1.52 $\pm$ 0.11	0.43 $\pm$ 0.06	0.34 $\pm$ 0.05
CCl <sub>4</sub>	1.5 ml	0.23 $\pm$ 0.01 <sup>b</sup>	1.65 $\pm$ 0.17 <sup>c</sup>	0.033 $\pm$ 0.001 <sup>c</sup>	0.53 $\pm$ 0.02 <sup>c</sup>	0.31 $\pm$ 0.04 <sup>a</sup>	0.58 $\pm$ 0.01 <sup>a</sup>
Crude extract	200	0.29 $\pm$ 0.02	2.92 $\pm$ 0.26 <sup>b,d</sup>	0.024 $\pm$ 0.001 <sup>c</sup>	0.79 $\pm$ 0.04	0.45 $\pm$ 0.01 <sup>d</sup>	0.52 $\pm$ 0.03
	400	0.37 $\pm$ 0.03	3.44 $\pm$ 0.38 <sup>d</sup>	0.041 $\pm$ 0.001 <sup>a</sup>	0.98 $\pm$ 0.06 <sup>d</sup>	0.35 $\pm$ 0.06	0.42 $\pm$ 0.04
	600	0.39 $\pm$ 0.04 <sup>d</sup>	4.51 $\pm$ 1.20 <sup>e</sup>	0.035 $\pm$ 0.001	1.09 $\pm$ 0.06 <sup>d</sup>	0.42 $\pm$ 0.03	0.43 $\pm$ 0.05
Aqueous Fraction	400	0.36 $\pm$ 0.02	2.44 $\pm$ 0.04 <sup>b</sup>	0.046 $\pm$ 0.006 <sup>d</sup>	0.98 $\pm$ 0.09 <sup>d</sup>	0.32 $\pm$ 0.04	0.44 $\pm$ 0.03
DCM fraction	400	0.38 $\pm$ 0.05 <sup>d</sup>	2.68 $\pm$ 0.24	0.050 $\pm$ 0.001	1.11 $\pm$ 0.02 <sup>e</sup>	0.46 $\pm$ 0.02 <sup>d</sup>	0.35 $\pm$ 0.06 <sup>d</sup>
Silymarin	100	0.50 $\pm$ 0.02 <sup>f</sup>	3.06 $\pm$ 0.24 <sup>d</sup>	0.073 $\pm$ 0.008 <sup>f</sup>	1.71 $\pm$ 0.18 <sup>f</sup>	0.53 $\pm$ 0.03 <sup>d</sup>	0.22 $\pm$ 0.02 <sup>f</sup>

Note: Data is expressed as MEAN  $\pm$  SEM, Significant at <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001 when compared to control; Significant at <sup>d</sup>p<0.05, <sup>e</sup>p<0.01, <sup>f</sup>p<0.001 compared to the organotoxic group. (n=5).



**Figure 1:** Liver histological section of rat treated with distilled water 10 mL/kg(A), Carbon tetrachloride 1.5 mL/kg (B), *H. africana* extract 200 mg/kg (C), 400 mg/kg, (D), 600 mg/kg (E), Aqueous fraction (F), DCM fraction (G), Silymarin 100 mg/kg (H) showing portal triad (PT), portal vein (V)ballooning degenerated hepatocytes (red arrowhead), both nuclear and cytoplasmic vacuolation (asterisk), mildly diffused lobular inflammatory infiltrate (Thin arrow), scarring parenchyma (\$) H&E stain, x400 magnification.

“Carbon tetrachloride ( $\text{CCl}_4$ ) is a widely and reliable model for experimental liver damage induction” [34]. “It is documented that carbon tetrachloride ( $\text{CCl}_4$ ) induces hepatic damage through lipid peroxidation, diminished activities of antioxidant enzymes, and generation of free radicals” [35]. “The  $\text{CCl}_4$  induces hepatotoxicity is well established by metabolic activation” [36] and alteration of certain physiological processes, thereby selectively causing injuries to liver cells with minimal effect on normal metabolic function [37]. “The cytochrome  $\text{P}_{450}$  system biotransforms  $\text{CCl}_4$  to produce

trichloromethyl free radical ( $\text{CCl}_3$ ) in the endoplasmic reticulum of the liver, which, when combined with proteins and cellular lipids in the presence of oxygen, form trichloromethyl peroxy radical, which may attack lipids on the endoplasmic reticulum membrane more rapidly than trichloromethyl free radical" [38]. "Thus, trichloromethyl peroxy free radical induces serious lipid peroxidation. The destruction of  $\text{Ca}^{2+}$  homeostasis results in cell death" [38]. "Lipid peroxidation and altered levels of some endogenous scavengers are indirect *in vivo* reliable indices for oxidative stress" [37]. "Moreover,  $\text{CCl}_4$ , which is metabolized by a mixed-function oxidase system in the liver endoplasmic reticulum to the highly reactive trichloromethyl radical (a reactive metabolite), induces auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and resulting in both morphological and functional cell membrane distortion" [38]. "This distortion of the hepatocyte membrane further causes membrane leakages of the hepatocyte cytosolic contents, which are manifested by significant elevation of acute hepatocellular damage of the serum marker enzymes ALT, ALP, and AST as markers for hepatobiliary damage as evident in this study" [39]. "However, of these marker enzymes, ALT is the most reliable. While, AST is known to be abundant in the skeletal muscle, kidneys, cardiac muscle, and testes, and ALP is abundant in the growing bone. Thus, any disease state affecting these extrahepatic tissues can significantly elevate the serum levels of these enzymes" [40]. "In this study,  $\text{CCl}_4$  significantly increased WBC and reduced platelet values compared to the control group, while other blood parameters such as RBC counts, hemoglobin concentration, PCV, neutrophils, basophils, lymphocytes, monocytes, and eosinophils were unaffected. The elevated WBC can be due to the stimulation of the immune defense system" [41]. "Similarly, literature has shown that an increased concentration of antigens in the body results in high values of WBC" [42]. This study observed that the administration of ethanol root extract and fractions of *H. africana* caused significant increases in WBC and platelet counts of the pretreated rats compared to the organotoxic group. Thus, it reflects the presence of cellular injuries and maybe the plant constituents' physiological activities to counter the delirious effect of  $\text{CCl}_4$  by stimulating immunogenic reactions leading to increases in the levels of WBC and platelets.

" $\text{CCl}_4$  has been known to produce hepatic damage by generation of highly reactive trichloromethyl ( $\text{CCl}_3^*$ ) and trichloromethylperoxy radical when metabolized by cytochrome P450" [43,44]. "As shown in the results,  $\text{CCl}_4$  doses induced acute hepatic damage as evidenced by significant elevation of the serum levels of the liver enzymes ALT, AST, and ALP and a significant decrease in the circulatory level of total protein, which conforms with earlier reports of the deleterious biochemical effects of  $\text{CCl}_4$  on hepatic injury" [45]. "Extract/fractions pretreatment significantly attenuated the acute elevation of these enzymes by  $\text{CCl}_4$ , suggesting the extract/fraction ability to counteract the effect of  $\text{CCl}_4$  and its reactive metabolites. Moreover,  $\text{CCl}_4$  administration was associated with significantly decreasing total protein and albumin serum levels. However, treatment with ethanol root extract and fractions of *H. africana* protected the liver from the deleterious effect of  $\text{CCl}_4$  by ameliorating the decreases in the circulatory levels of total protein and albumin, thus stabilizing the endoplasmic reticulum. This is an indication that the extract/fractions preserved hepatic protein synthetic potential. Moreover,  $\text{CCl}_4$  activity has been reported to cause the degeneration of hepatocytes and blockade of the bile ducts, which resulted in a significant increase in serum levels of total bilirubin, direct bilirubin, and ALP" [46]. "Pretreatment with *H. africana* root extract and fractions reduced the elevated serum levels of total bilirubin, direct bilirubin, and ALP. Therefore, a reduction in the levels of ALT and AST towards the normal value indicates the regeneration process from hepatocellular damage. Reductions in ALP, total bilirubin, and direct bilirubin suggest stabilizing the biliary function. An increase in the serum level of the total protein suggests the regeneration of the endoplasmic reticulum, leading to protein synthesis. This may be due to the activities of phytochemical compounds in the extract and fractions under study that are acting to counteract the effect of reactive metabolites of  $\text{CCl}_4$ " [45].

"The histological findings also supported the hepatoprotective activity of the root extract against

CCl<sub>4</sub>-induced liver injury, though not significantly. Disrupted parenchyma with ballooning degenerated hepatocytes, both nuclear and cytoplasmic vacuolation, mildly diffused lobular inflammatory infiltrate, and congested blood vessels, which were severely prominent in the CCl<sub>4</sub> only-treated group, were mildly reduced in the extract/fractions -pretreated groups reflecting a considerable hepatoprotective activity as evidenced in the chemical pathology portraying significant cellular healing. This could have resulted from the short duration of study and administration which with increased treatment time may be improved and reflected on the morphology of the liver architecture. CCl<sub>4</sub>-induced hepatotoxicity produced in rats, which results in hepatic injury, induces the generation of toxic radicals, which can be diminished by the effect of a potent antioxidant in adequate amounts” [44]. “Endogenous antioxidant enzymes play a great role in mopping up the free radicals to form hydrogen peroxide and safer molecules, hence counteracting the toxic effects caused by these radicals. SOD and CAT are important enzymes in the enzymatic antioxidant defense system” [47]. “The root extract and fractions of *H. africana* have been found in this study to elevate the levels of endogenous enzymatic and non-enzymatic antioxidants (SOD, CAT, GPx, GSH) and also reduce the level of MDA in the livers of rats with CCl<sub>4</sub>-induced toxicity. The reduction in MDA portrays a reduction in peroxidation and, hence, free radicals activities. This suggests the involvement of antioxidant/antioxidative stress activity as the mechanism of action for this study's observed hepatoprotective potential of the root extract and fractions. The root extract had previously been reported to increase hepatic antioxidant enzymes such as SOD and CAT” [19]. “This implies that root extract may act by reducing reactive free radicals due to the activities of its antioxidant phytochemical constituents, thereby reducing oxidative damage to the tissues and improving hepatic antioxidant enzyme activity. The results of this study corroborate previously reported hepatoprotective potential of the root extract and fractions of *H. africana* against paracetamol and doxorubicin induced liver injury” [19, 48,49].

The results suggest that the root extract and fractions of *H. africana* were able to exert a hepatoprotective effect by counteracting the effect of toxic electrophile metabolites and trichloromethyl free radicals from CCl<sub>4</sub> by elevating the activities of endogenous antioxidants and thus able to prevent hepatopathy and lipid peroxidation. Reported phytochemical constituents of the plant under study such as  $\delta$ -3-Carene, thujene, limonene, linalool,  $\alpha$ -phellandrene,  $\alpha$ -terpineol, and sabinene) dehydromevalonic lactone, xanthenes, 1,3,6,7-tetrahydroxyxanthone and 1,3,6-trihydroxy-7-methoxyxanthone [19,23,26] with potent antioxidant activities could have protected the pretreated rats from injury by CCl<sub>4</sub> metabolites by reducing oxidative stress. This effect could have been due to the free radicals scavenging potentials of the root extract/fractions and the antioxidative stress activities of their phytoconstituents [19,23,26], which can be attributed to the activities of its phytochemical constituents such as monoterpenes, sesquiterpenes and xanthenes earlier reported to be present in this root extract [19, 23,26].

However, considerable chemical pathological improvements without any significant histopathological changes, following the administration of *Hippocratea africana* root extract and fractions to rats with CCl<sub>4</sub>-induced liver injury, were observed in this study. These may have resulted from several factors such as dosage, duration of study and treatment, as well as the route of administration. Thus, limiting the effectiveness of the root extract and fractions in this study contrary to its previously reported potentials against doxorubicin and paracetamol-induced liver injuries [19,24,48,49].

#### 4. CONCLUSION

The results of this study suggest that the root extract and fractions of *H. africana* possess liver protective and antioxidative stress potentials against injurious substances through the antioxidant activities of its phytochemical constituents.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that no generative AI Technologies such as large language models (CHATGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## ETHICAL APPROVAL

The College approved the Health Sciences Animal Ethics Committee study, University of Uyo.

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