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Hippocratea africana root extract and fractions ameliorated carbon tetrachloride-induced oxidative stress and kidney injuries in rats

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Abstract. *Noah K, Edem UA, Iyanyi UL, Ajaghaku DL, Okokon JE. 2024.* Hippocratea africana *root extract and fractions ameliorated carbon tetrachloride-induced oxidative stress and kidney injuries in rats. Asian J Nat Prod Biochem 22: 51-58.* A common cause of renal failure with potential side effects is drug-induced nephrotoxicity. Renal damage in experiments is frequently induced chemically using carbon tetrachloride (CCl_4), a well-known environmental contaminant with nephrotoxic effects. Intoxicated animals to CCl4 experience experimental oxidative stress in various physiological conditions that promote peroxidative degeneration in different tissues by binding to lipids, proteins, and DNA. The root of *Hippocratea africana* (Willd.) Loes. was investigated to confirm its nephron-protective potential in CCl_4-induced kidney injury in rats. The root extract at the concentrations range of 200-600 mg/kg and its fractions, dichloromethane (DCM) and aqueous at the concentration of 400 mg/kg, was evaluated concerning the kidney damage in the rats induced with carbon tetrachloride (1.5 mL/kg). The anti-oxidative stress and reno-protective potentials of root extract and fractions in rats were determined by observing histological alterations, parameters of kidney function, and oxidative stress markers. The administration of root extract and fractions caused a significant ($p < 0.05$-0.001) increase in oxidative stress markers (SOD, CAT, GPx, SOD, and GSH) in the kidney, while the MDA level was decreased. The root extract/fractions also caused a significant ($p<0.05$-0.001) reduction in serum levels of creatinine, urea, and electrolytes in the rats significantly ($p<0.05$). The kidney histology of the rats treated with *H. africana* extract showed fewer abnormal characteristics than the organotoxic group. The findings demonstrated that the *H. africana* root extract and fractions could preserve the nephron and prevent oxidative stress. They also prevented CCl_4-induced renal damage in rats, possibly due to the phytochemical content with antioxidant properties.

Keywords: Antioxidant, anti-toxicant, *Hippocratea africana*, kidney protective, oxidative stress

INTRODUCTION

Carbon tetrachloride (CCl_4) has been used to study the damage synthetic poisons cause to internal organs in animal models. Upon entering the body by ingestion or skin absorption, CCl_4 diffuses throughout the body. It frequently builds up in the liver, brain, kidney, muscle, fat, and blood (Agency for Toxic Substances and Disease Registry 2000). Animals are intoxicated with CCl_4 in an attempt to create oxidative stress under various physiological conditions. These radicals attach to lipids, proteins, and DNA, causing peroxidative degeneration of many tissues. According to several previous studies, CCl_4 is the most accurate model of the mechanism of Reactive Oxygen Species (ROS) produced in various tissues (Kamisan et al. 2014). After the administration of CCl_4, radicals such as hydroxyl radicals, superoxide anion, hydrogen peroxide, and other radicals are produced, leading to oxidative stress development (Ritter et al. 2004). Long-term exposure to CCl_4 causes necrosis, fibrosis, cirrhosis, inflammatory leukocyte infiltration, and other histological characteristics. It can potentially cause cancer (Qiu et al. 2005).

The kidney is the most significant excretory organ and is essential to preserving the equilibrium of the internal environment. Renal failure, which can be classified as acute or chronic, is a common pathophysiological disorder caused by CCl_4 and can result in death (Shirwaikar et al. 2004). The Reactive Oxygen Species (ROS) are produced due to oxidative stress and are one of the leading causes of acute kidney injury. Proximal tubular toxicity is caused by the direct nephrotoxic effects of toxins such as CCl_4, which include mitochondrial dysfunction, phospholipid damage, increased intracellular calcium concentration, and lysosomal hydrolase inhibition. These effects increase oxidative stress by forming ROS (Hosohata 2016); by encouraging inflammation, ROS either directly or indirectly advances fibrosis. Fibrosis and inflammation may increase the generation of ROS or promote the synthesis of growth factors and cytokines (Siddik 2003). As a result, the accumulation of free radicals within cells can cause lipid peroxidation, and the oxidative degradation of polyunsaturated fats in the membrane causes modifications to the permeability and viscosity of cell membranes (Baud and Ardaillou 1986). Following the in vivo and in vitro studies, CCl_4 raises lipid peroxidation, lowers oxidized glutathione levels in the renal cortex, and

lowers enzyme activity (Khan and Siddique 2012). CCl_4 can alter granular pneumocytes and sub-lethally cause proximal tubular injury in the kidney (Rajesh and Latha 2004).

Moreover, medicinal plants contain various complex chemical components, many of which are well-known for their curative qualities when applied to renal problems. One of the kidney diseases frequently encountered in clinical conditions is acute renal injury (Vijayan 2021). There are fewer effective therapy medications available, which raises the death rate in clinics (Yang et al. 2020; Vijayan 2021). Therefore, the creation of novel therapies or potent medications is desperately needed to treat acute kidney injuries that pose a severe threat to life. *Hippocrata africana* (Willd.) Loes. ex Engl. (Celastraceae) syn. *Loeseneriella africana* (Willd.) N. Hallé is a green forest perennial climber widely distributed in tropical Africa (Hutchinson and Dalziel 1963). It is commonly known as the African paddle-pod and '*Eba enang enang*' by the Ibibios of Nigeria. The Ibibios of Nigeria's Niger Delta region have historically employed the plant root in a variety of ways to treat illnesses like fever, convulsions, malaria, bodily aches, diabetes, and diarrhea (Okokon et al. 2006). Additionally, the plant's root is used for its potential as an antidote or anti-poison to cure liver conditions like jaundice and hepatitis (Etukudo 2000, 2003; Ajibesin et al. 2008). Previous reports showed that the root extract possesses antimalarial (Okokon et al. 2006, 2021), antioedema and antinociceptive (Okokon et al. 2008), antidiabetic and hypolipidemic (Okokon et al. 2021, 2022), antidiarrhoeal and antiulcer (Okokon et al. 2011), hepatoprotective, antileishmanial, cytotoxicity and cellular antioxidant (Okokon et al. 2013), antibacterial, anticonvulsant and depressant (Okokon et al. 2014), in vivo alpha-amylase and alpha-glucosidase inhibitory (Okokon et al. 2021) and in vitro antioxidant (Okokon et al. 2022; Umoh et al. 2021) activities. Earlier studies had reported the presence of spiro hexane-1-carboxylic acid, ethyl ester, 3-methoxy-2-methylphenol,2,3-benzofuran dione,6-hydroxy-4-(p-hydroxy benzyl), δ-3-Carene and α-terpineol in ethyl acetate fraction (Okokon et al. 2017) and the presence of monoterpenes (thujene, limonene, linalool, α-phellandrene, α-terpineol and sabinene) and sesquiterpenes (dehydromevalonic lactone), in the n-hexane fraction of the root extract (Okokon et al. 2013). Also, two xanthones, 1,3,6,7-tetrahydroxyxanthone and 1,3,6-trihydroxy-7-methoxyxanthone, have been isolated from the root of *H. africana* (Umoh et al. 2021). This study aims to determine the anti-oxidative stress and kidney protective properties of *H. africana* root extract and fractions against carbon tetrachloride-induced renal damage in rats.

MATERIALS AND METHODS

Plants collection

Fresh roots of *Hippocratea africana* (Willd.) Loes. roots were collected from bushes in the Uruan area of Akwa Ibom State, Nigeria, in November 2021. A taxonomist from the Department of Botany and Ecological Studies at the University of Uyo in Uyo, Nigeria, identified and verified the plant. The herbarium specimen was deposited in the Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo.

Preparation of extract and fractions

Fresh *H. africana* roots were cleaned, chopped into smaller pieces, and dried in a shaded area for two weeks. Dried roots were ground using an electric grinder. The root powder of *H. africana* (HAE) was immersed in 50% ethanol for 72 hours. The filtrate was concentrated at 40°C in a rotary evaporator. The crude extract (20 g) was dissolved in 500 mL of distilled water and partitioned with an equal volume of dichloromethane (DCM, 5×500 mL) till no colour change was observed to obtain dichloromethane and aqueous fractions. The extract and its fractions were refrigerated at 4°C until used in the following experiment.

Animals

This study used male Wistar rats. The animals were obtained from the University of Uyo Animal House and placed in plastic cages. They were fed standard pelleted Feed (Guinea feed) and given unlimited access to water. The College of Health Sciences Animal Ethics Committee at the University of Uyo approved the study.

Administration of ethanol root extract and fractions of *Hippocratea africana* on carbon-tetrachloride-induced toxicity in rat

In this model, eight (8) groups of five rats were randomly selected from forty (40) rats. Group 1 (the control or normal control group) was given 10 mL/kg of distilled water orally for eight consecutive days. The organotoxic group was represented by Group 2, which was orally given 10 mL/kg of normal saline for eight days. As the extract-treated groups, groups 3 through 5 received oral administration of 200, 400, and 600 mg/kg of root extract daily for eight days, respectively. Animals in groups 6 and 7 were given 400 mg/kg pretreatments of DCM and aqueous fractions for 8 days. Group 8 was the positive control group and received 100 mg/kg of silymarin orally for 8 days. On the eighth (8th) day, animals in groups 2-8 received carbon tetrachloride (1.5 mL/kg, i.p) dissolved in corn oil mixed at a ratio of 1:3. Finally, 24 hours after carbon tetrachloride administration, all animals were weighed again and sacrificed under light diethyl ether vapour.

Collection of blood samples and organs

After 8 days of treatment (24 hours after the last treatment), the rats were weighed again and sacrificed by light diethyl ether vapor.

The blood samples were collected by cardiac puncture into plain centrifuge tubes and used immediately. The blood in the centrifuge tubes was centrifuged immediately at 2,500 rpm for 15 minutes, and to avoid hemolysis, the serum was separated at room temperature and then used for biochemical assays. The kidneys were surgically removed, weighed, and fixed in 10% formaldehyde for histological process.

Biochemical analysis

Kidney function test

The test was conducted in the Chemical Pathology Department of the University of Uyo Teaching Hospital. It used diagnostic kits to determine the biochemical parameters as markers of kidney function, i.e., levels of electrolytes (Na, K, Cl, and HCO_3), creatinine, uric acid, and urea.

Preparation of renal homogenate

After the other kidney was removed, the fat and surrounding connective tissues were separated from the kidney. After longitudinally cutting each kidney, the renal cortex was isolated and maintained at -8°C. The renal cortex was then homogenized in 0.05 M, pH 7.4 cold potassium phosphate buffer. The renal cortical homogenates were centrifugated for 10 minutes at 4°C at 5,000 rpm. The resulting supernatant was used to measure the enzyme activities using colorimetric assay, i.e., glutathione peroxidase (GPx) (Lawrence and Burk 1976), reduced glutathione (GSH) (Ellman 1959), catalase (CAT) (Rasheed et al. 2020), superoxide dismutase (SOD) (Marklund and Marklund 1974), and malondialdehyde (MDA) content (Esterbauer and Cheeseman 1990). The anti-oxidative stress potentials of the extract were evaluated using these oxidative stress indicators.

Histopathological studies

The other removed kidneys were preserved for histopathology studies using 10% buffered formalin. Following standard protocols, they were processed and stained with hematoxylin and eosin (H&E) (Drury and Wallington 1980) at the Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo. Morphological changes were observed, and micrographs were made from histological images.

Statistical analysis

Data collected from this study were analyzed statistically using ANOVA (one–way) followed by a post-test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at a 5% significance level, i.e., $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of *H. africana* root extract on kidney weights of rats induced by carbon tetrachloride

The administration of carbon tetrachloride, root extract, and fractions of *H. africana* did not significantly affect ($p>0.05$) the kidney weights of rats compared to the normal control and the organotoxic group (Table 1).

Effect of root extract and fractions of *H. africana* on kidney function parameters of rats with carbon tetrachloride-induced kidney injury.

The administration of 1.5 mL/kg of carbon tetrachloride to normal rats resulted in a significant ($p<0.05$-0.001) increase in blood urea, creatinine, and electrolytes (K^+, Na^+, Cl^-, and HCO^{-3}) compared to normal control. Rats treated with silymarin and root extract/fractions (200–600 mg/kg) showed elevated levels of serum urea and creatinine; however, electrolytes were significantly ($p<0.05$-0.001) decreased. The effect was not dose-dependent, with the DCM fraction having the highest impact. Furthermore, in comparison to the organotoxic group, the reduction in the level of Cl^- was only significant ($p<0.05$) in the groups treated with root extract (400 mg/kg) and silymarin, respectively (Table 2).

Table 1. Effect of *H. africana* root extract administration on organ weights of rats induced with carbon tetrachloride

Parameters/treatment	Dose (mg/kg)	Kidney
Normal control	-	1.06±0.06
Carbon tetrachloride	1.5mL	1.05±0.02
Silymarin+CCl4	100	1.10±0.09
Extract+CCL4	200	1.11±0.04
	400	1.01±0.08
	600	1.07±0.02
Aqueous fraction	400	1.00±0.06
DCM fraction	400	1.17±0.06

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

Table 2. Effect of *H. africana* root extract and fractions on the parameters of kidney function of rats induced with carbon tetrachloride

Treatment	Dose (mg/kg)	Urea (mMol/L)	Creatinine (µmol/L)	Chloride (Mmol/L)	Potassium (mMol/L)	Sodium (mMol/L)	Bicarbonate (mMol/L)
Control (distilled water)	10 mL/kg	3.57± 0.17	77.75±3.42	39.25±1.70	3.57± 0.20	114.5± 1.65	20.02± 0.31
CCl4	1.5 mL/kg	7.87± 0.39^{c}	161.50±6.41^{c}	54.0±3.02^{c}	6.35± 0.50^{c}	168.25±6.86^{c}	35.30± 0.10^{a}
Crude extract	200	6.97±0.23^{f}	140.5± 4.55^{f}	48.0±1.22^{d}	5.40± 0.31^{e}	162.5±5.10^{c}	22.00± 0.28^{d}
	400	5.85±0.15^{b,d}	118.25±2.92^{c,e}	44.50±1.04^{d}	3.30± 0.23^{f}	116.25±4.53^{f}	21.10± 0.20^{d}
	600	5.70±0.67^{b,e}	121.25± 10.0^{b,f}	38.0±1.73^{f}	3.42± 0.24^{f}	111.75±3.35^{f}	20.55± 0.47^{d}
Aqueous Fraction	400	6.90±0.56^{b,f}	138.0±8.59^{f}	34.0±1.29^{f}	4.07± 0.34^{f}	129.5± 7.70^{f}	25.29± 1.24^{d}
DCM fraction	400	6.42±0.14^{f}	128.75±2.92^{a,f}	40.25±1.75^{f}	3.70± 0.24^{f}	122.25±5.79^{f}	21.18± 0.18^{d}
Silymarin	100	5.37±0.14^{d}	108.75±2.68^{c,f}	40.25±1.25^{f}	3.30± 0.14^{f}	113.0± 1.47^{f}	20.42± 0.56^{d}

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

Table 3. Effect of *H. africana* root extract and fractions on oxidative stress markers in CCl_4-induced rat kidney

Treatment	Dose (mg/kg)	SOD (U/ml)	CAT (U/g of protein)	GPx (µg/ml)	GSH (µg/ml)	GST (µg/ml)	MDA (µMol/ml)
Control (distilled water)	10 mL/kg	1.31±0.15	1.85±0.11	0.072±0.008	0.35±0.03	0.45±0.06	0.56±0.02
CCl_4	1.5 mL/kg	0.45±0.19 [b]	0.33±0.01[b]	0.029±0.002[c]	0.16±0.01[b]	0.28±0.01[a]	1.38±0.04 [b]
Crude extract	200	1.09±0.06[f]	2.92± 0.26[f]	0.034±0.002[e]	0.79± 0.04	0.37±0.02[d]	0.50±0.01[f]
	400	0.87±0.01[d]	3.44±0.38[f]	0.041±0.001[f]	1.08± 0.03[f]	0.41±0.02[f]	0.44±0.02[f]
	600	0.99±0.02[d]	4.51± 0.20[f]	0.035±0.002[e]	0.99± 0.02[e]	0.40±0.03[f]	0.41±0.01[f]
Aqueous Fraction	400	0.76±0.02[d]	2.44±0.04[f]	0.046±0.006[f]	0.88± 0.02[e]	0.35±0.02[d]	0.42± 0.02[f]
DCM fraction	400	0.98±0.05[d]	2.68±0.24[f]	0.055±0.003[f]	1.18± 0.02[f]	0.44±0.01[f]	0.38± 0.02[f]
Silymarin	100	1.08±0.37[f]	2.47±0.01 [f]	0.045±0.001[f]	0.61±0.06[d]	0.46±0.01[f]	0.46±0.02 [f]

Note: Data is expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p<0.01, [c]p<0.001 when compared to control; Significant at dp<0.05, ep<0.01, fp<0.001 compared to the organotoxic group. (n=5)

Effect of *H. africana* root extract and fractions on kidney oxidative stress markers of rats induced with carbon tetrachloride

The administration of carbon tetrachloride significantly (p<0.05-0.001) lowers the levels of MDA and increases the levels of GSH, GPx, CAT, GST, and SOD significantly. The organ injuries of CCl_4-induced rats that were treated with root extract and fractions of *H. africana* and silymarin produced a significant (p<0.05-0.001) and non-dose dependent elevation in the levels of GSH, GPx, CAT, GST, and SOD 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 in the groups treated with the DCM fraction and silymarin (Table 3).

Effect of root extract and fractions of *H. africana* on histology of rat kidney in carbon tetrachloride-induced nephrotoxicity

Histological sections of rats' livers in Group 1 (normal control, A) treated with distilled water (10 mL/kg) at magnification (x400) stained with the H&E method showed normal renal tubules and glomeruli with no evidence of pathology. The organotoxic group (Group 2, B) treated with carbon tetrachloride (CCl_4) (1.5 mL/kg) showed normal renal tubules, glomeruli, and congested blood vessels compared to the control group (Figure 1). Group 3 (C) rats treated with 200 mg/kg of *H. africana* root extract and CCl4 showed normal renal tubules and glomeruli with no evidence of pathology (Figure 1). Rats in group 4 (D) treated with *H. africana* root extract (400 mg/kg) and CCl_4 showed normal renal tubules and glomeruli with no evidence of pathology. Group 5 (E) rats treated with *H. africana* root extract (600 mg/kg) and CCl_4 showed normal renal tubules, glomeruli, and congested blood vessels. Kidney sections of rats in group 6 (F) treated with an aqueous fraction (400 mg/kg) of *H. africana* root and CCl4 showed normal renal tubules, glomeruli, and congested blood vessels. Kidney sections of rats in group 7 (G) treated with dichloromethane fraction (400 mg/kg) of *H. africana* root and CCl_4 showed normal renal tubules and glomeruli, with no evidence of pathology. The silymarin-treated rats with carbon tetrachloride-induced toxicity (Group 8, H) had kidney sections that revealed normal renal tubules and glomeruli without any evidence of pathology (Figure 1).

Discussion

Acute kidney injury caused by chemicals and drugs is one of the leading causes of death globally. Acute kidney injury, which includes acute renal illnesses and disorders, is characterized by an abrupt and quick loss of excretory kidney function within a few hours or days (Dai et al. 2023). Carbon tetrachloride (CCl_4) is a substance commonly used in experiments to cause damage to the liver and kidneys (Azri et al. 1992) due to lipid peroxidation, producing free radicals and decreasing the activity of antioxidant enzymes (Brent and Rumack 1993). According to previous studies, exposure to CCl_4 damages the kidneys by increasing the generation of reactive oxygen species (Tirkey et al. 2005; Ganie et al. 2011).

Through metabolism, CCl_4 becomes CCl_3 or trichloromethyl free radical. The trichloromethyl peroxyl radical is formed when the trichloromethyl free radical reacts with proteins and lipids in cells and oxygen. It can potentially damage endoplasmic reticulum lipids more quickly than trichloromethyl free radicals (Recknagel and Glende Jr. 1973). Therefore, lipid peroxidation is caused by the trichloromethyl peroxyl free radical. The highly reactive trichloromethyl radical leads to auto-oxidation of the fatty acids in the phospholipids that make up the cytoplasmic membrane, which alters the morphology and function of the cell membrane, disrupts Ca^{2+} homeostasis and finally results in cell death (Recknagel and Glende Jr. 1973). Changes in the levels of several endogenous scavengers and lipid peroxidation are considered good, indirect markers of oxidative stress in vivo (Babu et al. 2001).

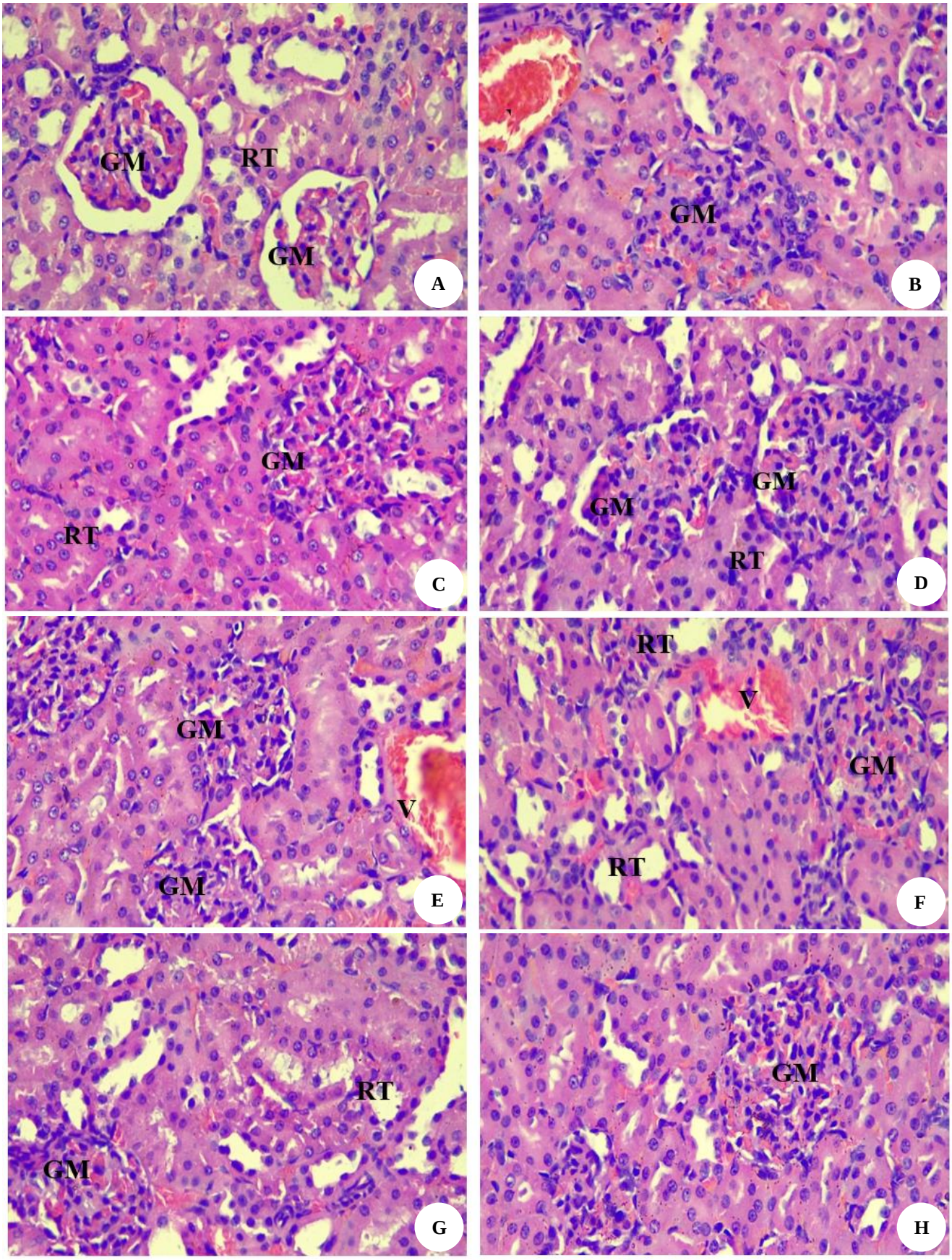

Figure 1. A. Kidney histological sections of rats treated with distilled water 10 mL/kg, B. Carbon tetrachloride 1.5 mL/kg, C. *H. africana* extract 200 mg/kg, D. 400 mg/kg, E. 600 mg/kg, F. Aqueous fraction, G. DCM fraction, H. Silymarin 100 mg/kg. RT: Showing normal renal tubules and GM: Glomeruli, and congested blood vessel with no evidence of pathological lesion

It has been known that several natural compounds have antioxidant activity and can prevent acute kidney injury by reducing the production of free radicals (Tirkey et al. 2005; Jayakumar et al. 2008). The current investigation assessed the anti-oxidative stress and nephroprotective properties of *H. africana* root extract and fractions against CCl_4-induced nephrotoxicity in rats. The administration of CCl_4 (1.5 mL/kg) caused renal function reduction and oxidative stress damage in renal tissues. The animals treated with CCl_4 showed significantly decreased serum concentrations of urea, creatinine, and electrolytes ($p<0.05$-0.001) higher than those of the normal group. The nitrogenous byproducts of blood metabolism, creatinine and urea, are dispersed throughout the body's fluids and are typically eliminated from the blood by the kidney (Safhi 2018). In contrast to the organotoxic group, pretreatment of the rats with the *H. africana* root extract and fractions considerably ($p<0.01$) reduced these parameters non-dose dependently, indicating the nephroprotective efficacy of the root extract and fractions. Oxidative stress was connected to increased urea and creatinine, indicating nephrotoxicity (Lakshmi and Sudhakar 2010).

Numerous investigations have shown that the primary cause of free radical production in various organs, including the liver, kidney, lungs, brain, and blood, is CCl_4 poisoning (Hamed et al. 2012). Additionally, it has been documented that following CCl_4 treatment in rats, the kidney has a higher concentration of CCl_4 than the liver (Sanzgiri and Bruckner 1997), likely due to the kidney's strong affinity for CCl_4 and its substantial cortical cytochrome P450 content. Glutathione (GSH) is a potent antioxidant that significantly prevents cellular damage caused by peroxides and free radicals (Kaur et al. 2003). According to Javed et al. (2011) and Shimeda et al. (2005), CCl_4 showed a significant decrease in GSH because it impairs Hydrogen peroxide (H_2O_2) clearance and encourages the generation of hydroxyl radicals (•OH), which causes oxidative stress. Following treatment with *H. africana* extract/fractions, there was a noticeable and significant ($p<0.05$-0.001) improvement in glutathione concentration and an efficient restoration of lipid peroxidation. The antioxidant enzyme Glutathione Peroxidase (GPx) helps the cell remove excess free radicals and lipid hydroperoxides. Next, a proton is added to GSH to transform it into glutathione disulfide (GSSG), and GR uses NADPH to convert GSSG back to GSH, maintaining the GSH pool in a reduced state. CCl_4 treatment has significantly reduced the concentration of GSH and altered the activity of several essential enzymes, including GPx. A decrease in GSH level may cause the decreased activity of glutathione-metabolizing enzymes in renal tissue. According to an earlier study, CCl_4 altered the activity of these enzymes, which are crucial for scavenging harmful free radicals (Ogeturk et al. 2005). After CCl_4 treatment, the activity of GPx was decreased; however, the kidney tissue treated with *H. africana* extract and fraction showed a considerable increase ($p<0.05$-0.001) in the activity of these enzymes.

The enzymatic antioxidant defense system relies heavily on the enzymes superoxide dismutase (SOD) and catalase (CAT), which work together to scavenge free radicals and transform them into stable molecules like hydrogen peroxide, thereby reducing the cell damage caused by free radicals (Curtis et al. 1972). Superoxide dismutation, which produces H_2O_2, is catalyzed by the metalloenzyme SOD (Freeman and Crapo 1982; McCord 1987). Following CCl_4 treatment, SOD activity was markedly reduced; however, SOD activity was recovered upon administration of the *H. africana* extract/fractions. Catalase reduces H_2O_2 to oxygen and water and protects cells from oxidative stress-related damage. CAT activity was considerably protected from CCl_4 treatment by *H. africana* extract and fraction. It suggests that in addition to significantly ($p<0.05$-0.001) increasing the activity of hepatic antioxidant enzymes, root extract may be lowering reactive free radicals due to the availability of antioxidant compounds, reducing oxidative damage to the tissues. It might be due to the root extract's or fractions' capacity to scavenge free radicals and its anti-oxidative stress activity (Okokon et al. 2013, 2021). It may be connected to the actions of its phytochemical constituents, which include the xanthones, monoterpenes, and sesquiterpenes that are contained in the root extract (Okokon et al. 2013, 2021; Umoh et al. 2021). One crucial indicator of oxidative stress is lipid peroxidation; one byproduct of polyunsaturated fatty acid peroxidation in cells is malondialdehyde (MDA), and increased free radicals lead to overproduction of MDA. After administration of CCl_4, it showed that the amount of MDA in kidney tissue had significantly ($p<0.05$-0.001) increased. Treatment with *H. africana* extract/fractions has significantly reduced MDA levels. It could be due to the antioxidant activity of *H. africana* to scavenge free radicals and prevent lipid peroxidation.

The mechanisms underlying the nephroprotective effects of the root extract and fractions could be caused by antioxidant activity, indicated by the elevation of GSH, GPx, SOD, and CAT, as well as the decrease in MDA levels in the kidney tissues of rats treated with extracts or fractions. These findings confirm the role of oxidative stress in CCl_4-induced nephrotoxicity. These imply that the kidney protective properties of the root extract may be attributed to the phytochemical content to overcome oxidative stress. Rat kidney weights were unaffected by the CCl_4 and extract/fractions treatment compared to the control group. The levels of urea, creatinine, sodium (Na), potassium (K), chlorine (Cl), and bicarbonate were also significantly reduced in the groups treated with root extract and fractions. Nephroprotective potentials may be caused by decreased ($p<0.05$-0.001) kidney function parameters (creatinine, urea, Na, K, Cl, and bicarbonate) after pretreatment with root extract/fractions. These outcomes strengthened the histology results, namely that the kidney tissues in the groups treated with extract and fractions remained comparatively intact compared to those in the organotoxic group. It suggests that the root extract and fractions protected the kidney tissues from the damaging effects of carbon tetrachloride. Histopathological examination of the organotoxic group confirmed the induction of kidney injuries by CCl4, indicated by severely congested blood vessels, as seen in the CCl_4-treated group.

In conclusion, the present study demonstrated that CCl_4 is a potent nephrotoxic substance, which leads to oxidative stress by depleting the activities of antioxidant enzymes, inflammatory cytokine production, and stimulating apoptosis. Treatments with the root extract and fractions of *H. africana* attenuated the CCl_4-induced renal toxicity. Hence, the root extract and fractions of *H. africana* possess nephroprotective and anti-oxidative stress activities against harmful substances due to the activities of its phytochemical constituents.

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