

Nephroprotective Activities of Ethanol Root Extract and Fractions of *Hippocratea africana* Against Doxorubicin-Induced Kidney Toxicity

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Abstract

Hippocratea africana root used locally in the treatment of poisoning was investigated to confirm its antidotal potential in rats. The root extract (200-600 mg/kg) and fractions; dichloromethane (DCM) and aqueous, 400 mg/kg) were evaluated for nephroprotective activity against doxorubicin-induced kidney injury in rats. Kidney function parameters, kidney oxidative stress markers and kidney histology were used to assess the kidney protective effect of the extract. The root extract and fractions (200-600 mg/kg) significantly ($p < 0.05-0.01$) reduced the levels of creatinine, urea and electrolytes that were elevated by doxorubicin. Also, the MDA level elevated by doxorubicin was reduced by the extract and fractions co-administration, while the levels of GSH, GST, SOD, GPx, and CAT that were decreased by doxorubicin were significantly ($p < 0.01$) elevated by the root extract/fractions. Histology of the kidney sections of extract/fractions - treated animals showed reductions in the pathological features compared to the organotoxic-treated animals. The chemical pathological changes were consistent with histopathological observations suggesting marked nephroprotective potential. The anti-toxic effect of this plant may in part be mediated through the chemical constituents of the plant. The plant, *Hippocratea africana* possesses anti-toxicant properties which can be exploited in the treatment of doxorubicin related toxicities.

Keywords: Renoprotective; *Hippocratea africana*; doxorubicin; oxidative stress.

Abbreviations: Dichloromethane (DCM), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Reduced glutathione (GSH), Malondialdehyde (MDA), haematotoxylin and eosin (H&E), Reactive oxygen species (ROS), reduced nicotinamide adenine dinucleotide phosphate (NADPH).

INTRODUCTION

Hippocratea africana (Willd.) Loes. ex Engl. (Celastraceae) known in English as 'African paddle-pod' and 'Eba enang enang' in Ibibio language in Nigeria, is a climber perennial plant distributed widely in tropical Africa (Hutchison and Dalziel, 1973). Traditionally, the plant root has been variously utilized in herbal preparations to treat diseases like malaria and diabetes (Okokon et al., 2006), as well as liver diseases (Ajibesin et al., 2008). Previous reports showed that the root extract possess antimalarial (Okokon et al., 2006; Okokon et al., 2021), antioedema and antinociceptive (Okokon et al., 2008), antidiabetic and hypolipidemic (Okokon et al., 2010; 2022), antiarrhoeal and antiulcer (Okokon et al., 2011), hepatoprotective (Okokon et al., 2013a), antileishmanial, cytotoxicity and cellular antioxidant (Okokon et al., 2013b), antibacterial, anticonvulsant and depressant (Okokon et al., 2014).

Also, earlier studies had reported the presence of δ -3-Carene and α -terpineol (Okokon et al., 2017), isolation of 1,3,7-trihydroxy-6-methoxyxanthone [isoathyriol] and 1,3,6,7-tetrahydroxyxanthone [norathyriol] (Umoh et al., 2021) from ethyl acetate fraction. Monoterpenes and sesquiterpenes have been identified in the n-hexane fraction (Okokon et al., 2013a). We report nephroprotective and antioxidative stress effects of the root extract and fractions of *H. africana* against doxorubicin-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Plants Collection

Fresh roots of *Hippocratea africana* were collected in bushes in Uruan area, Akwa Ibom State, Nigeria in November, 2021. The plant was identified and authenticated by a taxonomist in the Department of

Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo.

Preparation of extract and fractions

Fresh root of *H. africana* were washed, cut into smaller pieces and dried under shade for two weeks. They were powdered using electric grinder. The pulverised root of *H. africana* (HAE) was soaked in ethanol (50%) for 72 hours. The liquid filtrate obtained was concentrated in a rotary evaporator at 40°C. The crude extract (20 g) was dissolved in 500 mL of distilled water and partitioned with equal volume of dichloromethane (DCM, 5 x 500 mL) till no colour change was observed, to obtain DCM and aqueous fractions. The extract and fractions were stored at 4°C in a refrigerator until used for the experiment.

Animals

In this study, male albino Wistar rats were used. The animals were sourced from University of Uyo Animal house and sheltered in plastic cages. The rats were fed with pelleted standard Feed (Guinea feed) and given unlimited access to water. The study was approved by College of Health Sciences Animal Ethics Committee, University of Uyo.

Experimental design

In this study, repeated dose model earlier described by Raskovi et al. (2011) and Olorundare et al., (2020), which lasted for 14 days was used. Groups I rats which served as the untreated control were orally pretreated with 10 mL/kg/day of distilled water. Group 2 rats were given normal saline (10 mL/kg/day) but equally treated on alternate days with 1.66 mg/kg of doxorubicin hydrochloride dissolved in 0.9% normal saline administered on alternate days for 14 days. Groups' 3-5 rats were orally pretreated with 200 mg/kg/day, 400 mg/kg/day, and 600 mg/kg/day of *Hippocratea africana* dissolved in distilled water 2 hours before treatment with 1.66 mg/kg of doxorubicin in 0.9% normal saline administered intraperitoneally on alternate days for 14 days, respectively. Groups 6 and 7 were pretreated with 400 mg/kg of DCM and aqueous fractions respectively. Group 8 rats which served as the positive control group were equally pretreated with 100 mg/kg/day of silymarin two hours before treatment with 1.66 mg/kg of doxorubicin in 0.9% normal saline administered intraperitoneally on alternate days for 14 days.

Collection of blood samples and organs

After 14 days of treatment (24 hours after the last administration) the rats were weighed again and sacrificed under light diethyl ether vapour. Blood samples were collected by cardiac puncture and used

immediately. Blood samples were collected into plain centrifuge tubes. The blood in the centrifuge tubes were centrifuged at 1500 rpm for 15 minutes to separate of serum at room temperature used for biochemical assays. The rats' kidneys were identified, harvested, and weighed.

Kidney function test

The following biochemical parameters such as levels of electrolytes (Na, K, Cl, and HCO₃), creatinine and blood urea were assayed as markers of kidney function using diagnostic kits at the Chemical Pathology Department of University of Uyo Teaching Hospital.

Oxidative Stress Markers

The antioxidant enzymes assays were performed on kidney homogenates of rats that were used in this study. These oxidative stress markers were used to assess antioxidative stress potentials of the extract.

Preparation of Renal Homogenate

In each rat, the kidneys were removed and one kidney was fixed in 10% formaldehyde for histological processes, while the other kidney was dissected free from the surrounding fat and connective tissue and used for assays of oxidative markers. The kidneys were longitudinally sectioned, and renal cortex was separated and kept at -8°C. Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05M, Ph 7.4). The renal cortical homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for the determination of superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Lawrence and Burk, 1976), reduced glutathione (GSH) (Ellman, 1959) and malondialdehyde (MDA) content (Esterbauer and Cheeseman, 1990).

Histopathological studies

The kidneys of the animals that were surgically removed and fixed in 10% formaldehyde were processed and stained with haematoxylin and eosin (H&E) (Drury and Wallington, 1980), according to standard procedures at Department of Chemical Pathology, University of Port Harcourt Teaching Hospital, Port Harcourt. Morphological changes observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrographs.

Statistical Analysis and Data Evaluation

Data obtained from this work were analysed statistically using ANOVA (one -way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of root extract and fractions of *H. africana* on body and organs weights of rats with doxorubicin-induced toxicity

Administration of *H. africana* root extract and fractions to rats with doxorubicin-induced organs toxicities caused considerable improvement of the body weights compared to the organotoxic group. The crude extract caused a pronounced dose-dependent effect (7.14 -8.09%) when compared to the organotoxic group with the dichloromethane fraction treated group exerting the highest effect (9.30%). Silymarin also improved the weight of the treated animals considerably (7.99%). The weights of kidneys of the group treated with doxorubicin only were found to be reduced when compared to those of the normal control group though not statistically significant ($p>0.05$). However, treatment of rats with doxorubicin-induced toxicities with the root extract and fractions of *H. africana* improved the organs weights though insignificantly ($p>0.05$) except in the group treated with aqueous fraction (Table 1).

Evaluation of effect of root extract and fractions of *H. africana* on kidney function parameters of doxorubicin-induced kidney injury in rats.

Table 2 shows the effect of root extract/fractions of *H. africana* on kidney function parameters of rats. Administration of doxorubicin (1.66 mg/kg) to the rats caused significant ($p<0.05-0.001$) elevation of serum urea, creatinine and electrolytes (K^+ , Na^+ and HCO_3^-) except Cl^- when compared to normal control. These increased levels of serum urea, creatinine and electrolytes (K^+ , Na^+ and HCO_3^-) were significantly ($p<0.05 - 0.001$) reduced when compared to organotoxic group following pretreatment of the rats with silymarin and root extract/fractions (200 – 600 mg/kg), with the DCM fraction having the highest effect in most cases. These reductions were non dose-dependent with the lower doses (200 and 400 mg/kg) exerting more significant effects. However, the Cl^- level was not affected significantly ($p>0.05$) with the root extract/fraction treatment (Table 2).

Effect of root extract and fraction on kidney oxidative stress markers of doxorubicin-induced kidney toxicity in rats.

The effect of *H. africana* root extract/fractions on kidney oxidative stress markers of the rats is as shown in Table 3. Administration of doxorubicin (1.66 mg/kg i.p) on alternate days for 14 days caused significant ($p<0.05-$

0.001) decreases of kidney antioxidant enzymes activities (SOD, GPx, GST, CAT) and GSH levels when compared to control. The MDA level was also elevated by doxorubicin treatment significantly ($p<0.001$) when compared to control. However, repeated administration of root extract/fractions of *H. africana* (200- 600 mg/kg) concomitantly with doxorubicin for 14 days caused non dose-dependent elevations of the enzymatic and non-enzymatic endogenous antioxidants in the treated rats groups which were mostly significant ($p<0.05-0.001$) in the higher doses (400 and 600 mg/kg) of the root extract when compared to the organotoxic groups with DCM fraction being the most active. Dose-dependent and significant ($p<0.05-0.001$) decreases in MDA levels of the extract/fractions treated groups were recorded when compared to control with aqueous fraction as the most active fraction. Similar decreases were also observed in the silymarin-treated group when compared to organotoxic control (Table 3).

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

Histological sections of kidneys of rats receiving various treatments at magnification (x400) stained with H&E method revealed that group 1 (A) treated with distilled water (10 mL/kg) showed normal renal tubules and glomeruli. No evidence of pathology was seen. The organotoxic group (Group 2, B) treated with doxorubicin (1.66 mg/kg) showed focal non-specific inflammation, normal renal tubules and glomeruli (GM) and few congested blood vessels were seen. Rats in group 3 (C) treated with 200 mg/kg of *H. africana* root extract and doxorubicin (1.66 mg/kg), group 4 (D) treated with 400 mg/kg of *H. africana* root extract and doxorubicin (1.66 mg/kg), group 6 (F) treated with 400 mg/kg of aqueous fraction of *H. africana* root and doxorubicin (1.66 mg/kg), group 7 (G) treated with 400 mg/kg of dichloromethane fraction of *H. africana* root and doxorubicin (1.66 mg/kg) and group 8 (H) treated with 100 mg/kg of silymarin of *H. africana* root and doxorubicin (1.66 mg/kg) had kidney sections showing normal renal tubules and glomeruli with no evidence of pathology seen. Group 5 (E) rat treated with 600 mg/kg of *H. africana* root extract and doxorubicin (1.66 mg/kg) showed distorted parenchyma with both normal and dilated renal tubules. The dilated tubular epithelium appears flattened. There were also multifocal interstitial inflammatory infiltrates (figures A-H).

Table 1. Effect of *H. africana* root extract on body and kidney weights of rats with doxorubicin-induced toxicity.

Parameters/ Treatment	Dose mg/kg	Kidney	Body weight		
			Before	After	% increase in body weight
Normal control	-	1.24±0.10	135.6±18.34	151.0± 12.33	11.35
Doxorubicin	1.66	0.94±0.10	130.0 ± 9.45	126.3± 10.43	-2.84
Silymarin+DOX	100	1.23±0.17	132.6± 14.55	143.2 ± 3.15	7.99
	200	1.20±0.05	142.8± 10.56	153.0± 5.29	7.14
Extract+DOX	400	1.14±0.02	140.3± 7.36	151.6 ± 6.22	8.05
	600	1.11±0.06	138.4 ± 8.54	149.6 ± 8.48	8.09
Aqueous fraction	400	1.05±0.11	138.4 ± 6.26	144.6± 10.22	4.47
DCM fraction	400	1.17±0.09	134.3± 8.50	146.8± 13.20	9.30

Data were expressed as mean ±SEM. significant at ^dp<0.001 when compared to normal control; ^ap< 0.05, ^bp< 0.01, ^cp< 0.001 when compared to organotoxic control. (n = 6).

Table 2. Effect of *H. africana* root extract and fractions on kidney function parameters of rats with doxorubicin-induced toxicity.

Treatment	Dose mg/kg	Urea (mMol/L)	Creatinine (µmol/L)	Chloride (mMol/L)	Potassium (mMol/L)	Sodium (mMol/L)	Bicarbonate (mMol/L)
Control	10	4.50± 0.30	94.5±5.86	45.75±1.93	3.47± 0.19	111.0± 5.11	22.00± 0.81
Doxorubicin	1.66	10.50± 0.40 ^c	171.6±2.02 ^c	46.66±0.88	6.53± 0.08 ^c	165.0± 1.73 ^c	30.66± 0.66 ^a
	200	7.13±0.31 ^e	143.0± 6.35 ^a	57.0±2.51	4.13± 0.78 ^f	125.0±16.37 ^d	22.66± 1.66 ^d
Crude extract	400	5.50±0.30 ^f	112.3±11.34 ^e	48.33±0.88	4.63±0.28 ^f	138.6± 6.76	25.0± 1.00
	600	6.16±0.97 ^f	125.6± 16.33 ^d	48.66±1.20	3.40±0.05 ^f	107.3±1.45 ^f	24.0± 2.00
Aqueous Fraction	400	4.80±0.50 ^f	108.5±2.72 ^f	49.50±1.70	4.17±0.55 ^b	118.7± 6.27 ^b	22.33± 1.33 ^d
DCM fraction	400	5.00±0.40 ^f	101.0±8.62 ^f	44.0±1.15	3.10±0.15 ^f	104.6± 1.45 ^f	25.66± 2.33
Silymarin	100	3.60±0.05 ^f	75.6±2.33 ^f	44.60±1.45	3.13± 0.17 ^f	105.0± 2.51 ^f	22.33± 2.60 ^d

Data is expressed as mean ± SEM, Significant at ^ap<0.05, ^bp<0.01, ^cp<0.001, when compared to control; Significant at ^dp<0.05, ^ep<0.01, ^fp<0.001 compared to organotoxic group. (n=6).

Table 3. Effect of *H. africana* root extract and fractions on kidney oxidative stress markers of rats with doxorubicin-induced toxicity.

Treatment	Dose mg/kg	SOD (U/ml)	CAT (U/g of protein)	GPx (µg/ml)	GSH (µg/ml)	GST	MDA (µMol/ml)
Control	10	0.61±0.02	1.51±0.06	0.090±0.002	1.18±0.02	0.40±0.01	0.22±0.02
Doxorubicin	1.66	0.17±0.01 ^c	0.46±0.01 ^a	0.040±0.00 ^a	0.31±0.01 ^b	0.22±0.01 ^c	0.63±0.01 ^c
	200	0.30±0.01 ^b	1.04± 0.15 ^c	0.051±0.001	0.85± 0.15	0.25±0.01 ^b	0.50±0.01 ^b
Crude extract	400	0.39±0.01 ^{a,d}	1.11±0.12 ^c	0.067±0.001 ^e	1.16±0.01 ^d	0.28±0.01 ^b	0.46±0.03 ^b
	600	0.51±0.02 ^e	1.26± 0.05 ^{c,e}	0.064±0.004	1.12± 0.08	0.34±0.01 ^e	0.31±0.02 ^f
Aqueous Fraction	400	0.23±0.06 ^c	1.15±0.02 ^f	0.045±0.003 ^a	1.02± 0.07	0.31±0.001 ^d	0.39± 0.01 ^e
DCM fraction	400	0.48±0.04 ^{a,d}	1.36±0.02 ^{c,d}	0.069±0.002 ^c	1.15± 0.14	0.33±0.01 ^e	0.30± 0.01 ^f
Silymarin	100	0.52±0.02 ^f	1.15±0.06 ^c	0.076±0.002 ^e	1.13±0.01 ^d	0.36±0.01 ^f	0.34±0.02 ^{a,d}

Data is expressed as MEAN ± SEM, Significant at ^ap<0.05, ^bp<0.01, ^cp<0.001, when compared to control; Significant at ^dp<0.05, ^ep<0.01, ^fp<0.001 compared to organotoxic group. (n=6).

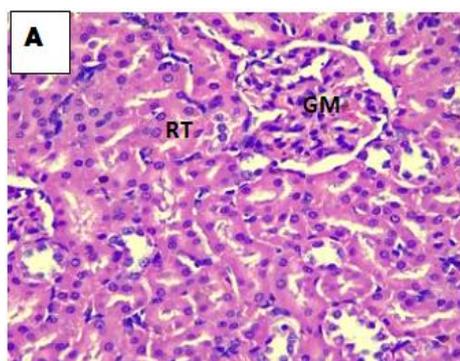


Figure A. Photomicrograph of kidney section of rat treated with distilled water (10mL/kg) showing normal renal tubules (RT) and glomeruli (GM), no evidence of pathology seen. H&E Stain, x400 magnification

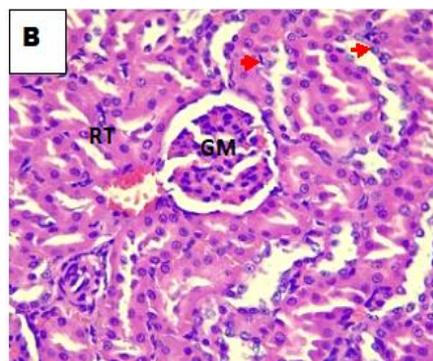


Figure B. Photomicrograph of kidney section of rat treated with doxorubicin (1.66mg/kg) showing focal non-specific inflammation (red arrowhead) with lower magnification, normal renal tubules (RT), and glomeruli (GM). There are few congested blood vessels (V). H&E stain x400

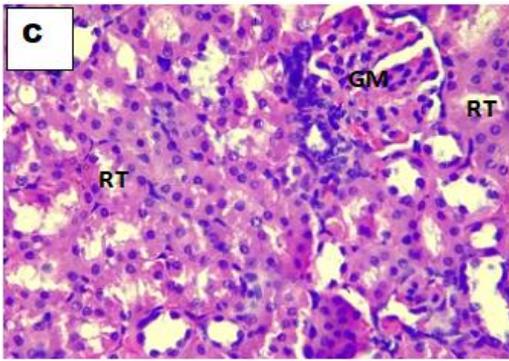


Figure C. Photomicrograph of kidney section of rat treated with 200 mg/kg of *H. africana* root extract and doxorubicin (1.66mg/kg) showing normal renal tubules (RT) and glomeruli (GM), no evidence of pathology seen. H&E Stain, x400 magnification

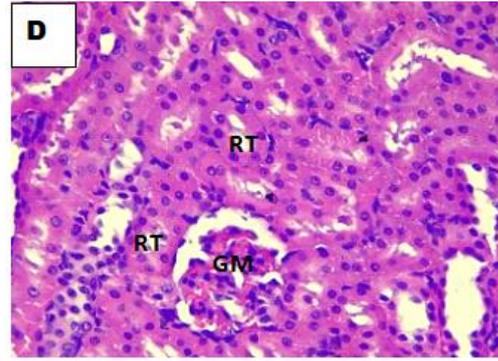


Figure D. Photomicrograph of kidney section of rat treated with 400 mg/kg of *H. africana* root extract and doxorubicin (1.66mg/kg) showing normal parenchymal with renal tubules (RT) and glomeruli (GM). No lesion seen. H&E Stain, x400 magnification

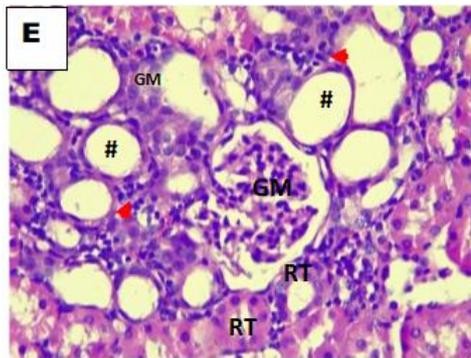


Figure E. Photomicrograph of kidney section of rat treated with 600 mg/kg of *H. africana* root extract and doxorubicin (1.66 mg/kg) showing distorted parenchyma with both normal (RT) and dilated renal tubules (#). The dilated tubular epithelium appears flattened. There are also multifocal interstitial inflammatory infiltrates (red arrowhead). H&E Stain, x400 magnification

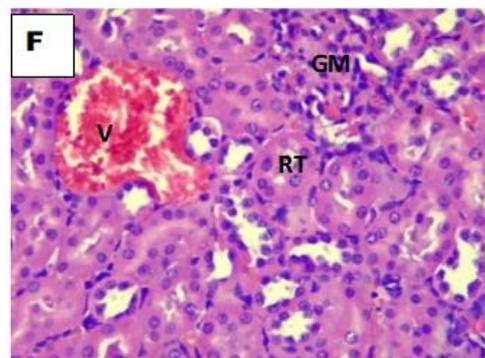


Figure F. Photomicrograph of kidney section of rat treated with 400 mg/kg of aqueous fraction of *H. africana* root and doxorubicin (1.66 mg/kg) showing normal parenchyma with renal tubules (RT), glomeruli (GM) and a focal congested blood vessel (V). No degenerative changes seen. H&E Stain, x400 magnification

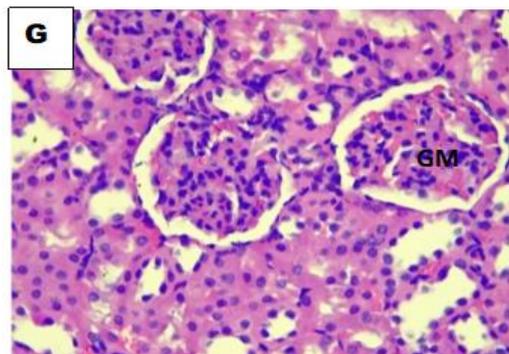


Figure G. Photomicrograph of kidney section of rat treated with 400 mg/kg of dichloromethane fraction of *H. africana* root and doxorubicin (1.66mg/kg) showing normal renal tubules (RT) and glomeruli (GM), no evidence of pathology seen. H&E Stain, x400 magnification

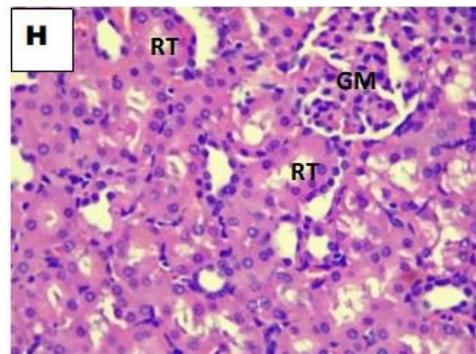


Figure H. Photomicrograph of kidney section of rat treated with 100 mg/kg of silymarin and doxorubicin (1.66mg/kg) showing normal renal tubules (RT) and glomeruli (GM), no evidence of pathology seen. H&E Stain, x400 magnification

Discussion

This work was designed to investigate the effect of root extract and fractions of *Hippocratea africana* on

doxorubicin-induced kidney toxicity in rats in a bid to confirm the folkloric claim of its antidotal activity. Doxorubicin is an anthracycline glycoside antibiotic that

possesses a potent and broad spectrum antitumour activity against a variety of human solid tumours and haematological malignancies (Calabresi and Chamber, 1990). However its use in chemotherapy has been limited largely due to its diverse toxicities, including cardiac, hepatic, hematological and testicular toxicity (Yilmaz et al., 2006). The semiquinone form of doxorubicin is a toxic short-lived metabolite which interacts with molecular oxygen and initiates a cascade of reactions, producing reactive oxygen species (ROS). ROS generation, inflammatory processes and lipid peroxidation have been suggested to be responsible for doxorubicin-induced cardio-, hepato- and nephrotoxicity (Injac et al., 2009; Kalender et al., 2005).

It has been proposed that DOX-semiquinone, an unstable metabolite of DOX, reacts with O_2 , producing H_2O_2 and O_2^- (superoxide). In addition, DOX enhances the activity of extramitochondrial oxidative enzymes such as xanthine oxidase and reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and also interferes with mitochondrial iron export, resulting in formation of ROS (reactive oxygen species) (Bachur et al., 1979).

In this study, doxorubicin administration was found to have caused elevation of serum urea, creatinine and electrolytes (K^+ , Na^+ , Cl^- and HCO_3^-) levels when compared to normal control, which is an indication of a serious injury to the kidney. This finding is consistent with earlier report of Rajasekaran (2019), which similar elevations were reported. It is well documented that kidney injury are indicated by increase in serum level of creatinine and urea (Laskshmi and Sudhakar, 2010) as well as increase serum levels of Na, K, Cl and bicarbonate (James and Mitchel, 2006). However, these increases were reduced significantly by the co-administration of root extract and fractions of *H. africana*.

Doxorubicin is reported to cause nephrotoxicity via oxidative stress as free radicals formed caused tubular atrophy and increased glomerular capillary permeability. Nephrotoxicity by doxorubicin can also result from lipid peroxidation and biological macromolecules damage by iron-dependent oxidative damage (Mohan et al., 2010). Degenerative changes in kidney depend on cumulative dose and duration of treatment as doxorubicin metabolites are partly excreted from the kidney. Another mechanism for renal injury is the conversion of DOX to semiquinone free radical by NADPH-cytochrome P-450 which generates hydroxyl radical and superoxide anion which causes lipid peroxidation (Rashid et al., 2013). The reduction of the levels of urea, creatinine and electrolytes by the root extract and fractions in this study is as a result of their free radical scavenging potentials, thereby protecting the kidney against oxidative stress by free radicals generated by doxorubicin.

The antioxidative burst and antioxidant activities of the root extract and fractions of *H. africana* had

previously been reported (Okokon et al., 2013a; 2022; Umoh et al., 2021). Moreso, the antioxidative stress activities of the root extract and fractions observed in this study further support the antioxidant potentials of the plant. These activities may have contributed to the observed protective effects in this study.

The findings of this study show that administration of doxorubicin (2.5 mg/kg, i.p) on alternate days for 14 days to rats caused significant decreases ($p < 0.05$) in levels of enzymatic and non-enzymatic endogenous antioxidants (GSH, SOD, CAT, GPX and GSH) when compared to control, while the MDA level was elevated. Lipid peroxidation is a marker of oxidative stress and elevations in the amount of MDA, a lipid peroxidation product, have been reported following doxorubicin treatment (Rashid et al., 2013, Rehman et al., 2014, Khames et al., 2019). This trend was observed in this study. Concomitant administration of root extract *H. africana* (200-600 mg/kg) with doxorubicin caused significant ($p < 0.05-0.001$) non dose- dependent elevation in the levels of the antioxidant enzymes (SOD, CAT, GPX) when compared to control. Similarly, GSH level was significantly ($p < 0.001$) elevated following treatment with the extract when compared to control. Also, there were significant ($p < 0.05-0.01$) reductions in the level of MDA of the extract-treated rats. It has been documented that DOX inhibits the activities of endogenous enzymatic and non-enzymatic antioxidants as was the case in this study. So, an imbalance between ROS generation and neutralization leads to oxidative stress and injury to the kidney (Abushouk et al., 2017; Abdel-Daim et al., 2017; Aboushouk et al., 2019). The reduced MDA level caused by the administration of the root extract and fractions may have resulted from reduction in lipid peroxidation and generation of free radicals which might have been scavenged by the phytoconstituents present in the root extract and fractions, revealing the antioxidative stress potentials of the root extract and hence the protective effect on the kidney as was observed.

Histological findings in this study revealed that kidneys of rats treated with doxorubicin (2.5 mg/kg) alone showed pathological signs of injury which were seen as degenerated microvesicles in the tubular lining cells among others. However, co-administration of *H. africana* root extract/fractions and doxorubicin reduced the toxic effects of the doxorubicin as normal glomeruli which were devoid of pathological signs were seen in the kidney sections of the extract-treated rats examined. This further confirms the nephroprotective potential of the root extract which may have been exerted through the antioxidant and antioxidative stress activities of its phytochemical constituents.

CONCLUSIONS

The findings of this study show that the root extract and fractions of *Hippocratea africana* have the potential to

counteract the injurious effect of doxorubicin on the kidney. This activity can be attributed to the antioxidant and antioxidative stress activities of their phytochemical constituents. Thus, the root extract can be used to alleviate and/or prevent doxorubicin-induced renotoxicity.

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Competing Interests: The authors declare that there are no competing interests.

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