Original Research Article

Hippocrateaafricana root extract and fractions ameliorates paracetamol(PCM)-induced oxidative stress and liver injuries in rats.

Abstract

Aim: The antidotal potential of *Hippocrateaafricana* root, which is used locally to cure poisoning, was studied in rats.

Methodology: The hepatoprotective activity of the root extract (200-600 mg/kg), fractions (dichloromethane (DCM) and aqueous, 400 mg/kg) and Silymarin (100 mg/kg), were investigated against paracetamol-induced liver injury in rats.Assay of liver function parameters, markers of antioxidative stressin addition to liver histological analysis were employed to evaluate the hepatoprotective potential of root extract and fractions.

Results: Administration of the root extract (200-600 mg/kg) caused significant (*P*<.05-.001) increases in the blood levels of total protein and decreases in direct and total bilirubin, and the liver biomarker enzymes ALT, AST, and ALP caused by paracetamol administration. Most of the time, the effects were dose-dependent. When compared to the negative control group of animals (organotoxic-treated animals), the histology of the liver sections from animals treated with extract, fractions, and silymarin revealed a decrease in pathological characteristics. These histological observations suggest significant hepatoprotective potentials which were in line with the chemical pathological alterations.

Conclusion: The outcome of the study indicated that root extract and fractions of *Hippocrateaafricana* have antioxidative stress potentials and hepatoprotective action against harmful substances, maybe as a result of its phytochemical constituents' activity.

Keywords: *Hippocrateaafricana*, anti-toxicant, oxidative stress, hepatoprotective, antioxidant

1.0 Introduction

Hippocrateaafricana (Willd.) Loes. ex Engl. (Celastraceae) syn. *Loeseneriellaafricana* (Willd.) N.Halléis a green forest perennial climber widely dispersed throughout tropical Africa [1]It is commonly known as African paddle-pod and 'Ebaenangenang' by the Ibibios of Nigeria. The plant root has been variously used traditionally by the Ibibios of the Niger Delta region of Nigeria to treat illnesses such as fever, convulsion, malaria, body pains, diabetes and diarrhea [2]. For the treatment of liver conditions including hepatitis and jaundice, the plant's root is also used for its antidote or antipoison properties [3-5].It has been reported that the root extract has antimalarial [2,6], antioedema and antinociceptive [7], antidiabetic and hypolipidemic [8,9], antidiarrhoeal and antiulcer [10], hepatoprotective [11], antileishmanial, cytotoxicity and cellular antioxidant [12], antibacterial, anticonvulsant and depressant [13], *in vivo* alpha amylase and alpha glucosidase inhibitory [6], *in vitro* antioxidant[9,14] activities.Previous research has indicated that the ethyl acetate fraction contains spirohexane-1-carboxylic acid, ethyl ester, 3-methoxy-2-methylphenol, 2,3-benzofurandione, 6-hydroxy-4-(p-hydroxybenzyl), ω-3-Carene, and α-terpineol[15]and the n-hexane fraction of the root exract contains monoterpenes (limonene, thujene, linalool, α-phellandrene, α-terpineol, and sabinene) and sesquiterpenes(dehydromevalonic

lactone). Additionally, two xanthones have been isolated from the root of *H. Africana:* 1,3,6,7-tetrahydroxyxanthone and 1,3,6-trihydroxy-7-methoxyxanthone [14]. The hepatoprotective and antioxidative stress properties of *H. africana* root extract and fractions against rats' liver damage caused by paracetamol is the aim of this research.

2.0 Materials and methods

2.1 Collection of plantsand preparation of extract and fractions

The collection of fresh roots of *Hippocratea Africana* in bushes in Uruan area, Akwalbom State, Nigeria was done in November, 2021. A taxonomist from the Department of Botany and Ecological Studies at the University of Uyo in Uyo, Nigeria, carried out the plant identification and authentication.Hebarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo. Fresh *H. africana*roots were cleaned, chopped into smaller pieces, and allowed to dry for two weeks in a shaded area. An electric grinder was utilized to powder them. For 72 hours, the ground-up root of *H. africana* (HAE) was immersed in 50% ethanol.The resultant liquid filtrate was concentrated at 40˚C in a rotary evaporator. To get the DCM and aqueous fractions, the crude extract (20 g) was diluted in 500 mL of distilled

water and divided into equal volumes of dichloromethane (DCM, 5 x 500 mL) until no colour change was noticed. The extract and its fractions were refrigerated at 4˚C until their utilization in the experiment.

2.2 Animals

Male albino Wistar rats were used in this study. The rats were housed in plastic cages after being obtained from the University of Uyo animal house. The rats had unrestricted access to water and were fed pelleted standard feed, also known as Guinea feed. The University of Uyo's College of Health Sciences Animal Ethics Committee approved the study with number UU/FP/AE/22/055.

2.3 Effect of ethanol root extract and fractions of *Hippocrateaafricana*on paracetamol-induced toxicity in rat

In this model, forty (40) rats were randomly divided into eight (8) groups of 5 rats each and treated as follows; Group 1- served as the control group (normal control) and animals in this group received 10 mL/kg of distilled water orally for eight consecutive days. Groups 2- served as the organotoxic group and the animals were administered with 10 mL/kg of normal saline orally for 8 days. Groups 3 - 5 served as the extract treated groups. Animals in groups 3- 5 were respectively and orally administered with 200, 400 and 600 mg/kg of root extract daily for 8 days.For eight days, the animals in groups 6 and 7 received pretreatments with 400 mg/kg of DCM and aqueous fractions, respectively. For eight days, animals in Group 8 (the positive control group) received 100 mg/kg of Silymarin orally.Animals in groups 2 - 8 were given

paracetamol(PCM) (2g/kg, i.p.) dissolved in a mixture of maize oil at a 1:3 ratio on the eighth (8th) day. All of the animals were weighed again and slaughtered under light diethyl ether vapour twenty-four hours after the paracetamol was administered.

2.4 Blood samples and organs collection

At 24 hours, following the final injection, after 8 days of treatment, the rats were weighed once more and sacrificed under a light diethyl ether vapor. After a heart puncture, blood samples were taken and used right away. Blood was drawn into standard centrifuge tubes. In order to separate the serum at room temperature and prevent hemolysis, the blood in the centrifuge tubes was centrifuged right away at 2500 rpm for 15 minutes before being utilized for biochemical tests. For the histology process, the livers were surgically removed, weighed, and preserved in 10% formaldehyde.

2.5 Biochemical analysis

2.5.1 Liver function test

The following parameters were measured: total plasma protein, total and direct bilirubin, aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). At the University of Uyo Teaching Hospital's Chemical Pathology Department, the determinations were made spectrophotometrically using Randox analytical kits in accordance with standard operating procedures and manufacturer's standards [16].

2.5.2 Oxidative stress markers

The study employed liver homogenates of rats, which underwent testing for antioxidant enzymes. The antioxidative stress potentials of the extract were evaluated using these oxidative stress markers: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) reduced gluthathione (GSH) and malondialdehyde (MDA) content.

2.5.3 Preparation of liver homogenate

After the rats were sacrificed humanely under inhaled diethyl ether, the liver of the rats was surgically removed and weighed. They were quickly rinsed in ice cold 1.15% KCl solution and put in a clean sample bottle. These were stored in ice cold 0.9% NaCl. Using a motor-driven Teflon

pestle, homogenates were created using a ratio of 1 g of wet tissue to 9 ml of 1.25% KCl. The homogenates were centrifuged for 10 minutes at 4˚C at 7000 rpm, and the supernatants were utilized for the tests of the concentration of superoxide dismutase (SOD) [17], catalase (CAT) [18], glutathione peroxidase (GPx) [19], reduced gluthathione (GSH) [20] and malondialdehyde (MDA) content [21].

Rat liver homogenates utilized in this investigation were subjected to the assays. The antioxidative stress potentials of the extract were evaluated using these oxidative stress indicators.

2.6 Histopathological studies

The samples underwent conventional protocols at the Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo, where they were processed and stained using haematoxylin and eosin (H&E) [22]. Morphological alterations observed and recorded in the removed organs of the animals sacrificed. Histologic pictures were taken as micrographs.

2.7 Statistical analysis and data evaluation

The data from this study were statistically analyzed using a one-way ANOVA and a post test (Tukey-Kramer multiple comparison test). At the 5% threshold of significance, differences in means were deemed significantie$P \leq .05$.

3.0 Results and Discussion

In this study, adult male wistar rats were used to test the hepatoprotective and antioxidative stress properties of *H. africana* root extract and fractions against oxidative stress and liver injury caused by paracetamol.The parameters employed to evaluate these properties were the effect of the *H. africana*root extract and fractions on liver function tests, oxidative stress markers, and histology. The administration of paracetamol to rats caused significant ($P<.001$) increase in liver weight in comparison to normal control. However, the administration of root extract and fraction of *H. africana* to rats with PCM -induced toxicities caused a significant ($P<.001$) reduction which is not dependent on dosein liver weights of the rats especially in the groups given extract when compared tothe group given paracetamol. No significant effect was recorded on the weights of the rats' kidney for the paracetamol as well as the extract/fractions treatment (Table 1).

Table 1: Effect of the root extract of*H. africana*on organs weights of rats with PCM-induced toxicity

Parameters/treatment	Dose mg/kg	Liver
Normal control	-	7.55±0.17
Paracetamol	2000	6.90± 0.18[c]
Silymarin+PCM	100	6.00±0.13[c,e]
Extract+PCM	200	5.41±0.14[c,f]
	400	6.99±0.12
	600	5.40±0.16[c,f]
Aqueous fraction	400	8.75±0.17[c]
DCM fraction	400	7.97±0.23[c]

Data were expressed as mean ±SD. significant at [c]*P*<.001 when compared to normal control; [d]*P*<.05, [e]*P*<.01, [f]*P*<.001 when compared to organotoxic control. *n* = 5.

When used in excess, the commonly used antipyretic and painkiller paracetamol can have severe side effects that harm the liver. The medication bioactivates to N-acetyl p-benzoquinone imine (NAPQI), a dangerous electrophile that binds to tissue macromolecules covalently and probably oxidizes lipids or protein thiols, the important sulphydryl groups, and modifies calcium homeostasis.[23].The loss of protecting

physiological components like glutathione and tocopherol due to massive reactive species production may damage key biomembrane macromolecules and damage the liver[24].

Rats given 2 g/kg of paracetamol experienced substantial (P <.01-.001) increases in total and combined bilirubin, AST, ALT, ALP, and decreased levels of total protein and albumin when compared to control. When compared to the organotoxic group, pre-treatment with root extract and fractions of H. africana (200–600 mg/kg) resulted in detectably significant (P <.05-.001) decreases in the levels of these enzymes as well as total and combined bilirubin in the extract/fractions-treated groups.The DCM fraction had the highest effect, although these reductions were not dose-dependent. When compared to the paracetamol group, the groups pre-treated with the root extract/fractions had considerably (P <.01-.001) higher levels of total protein and albumin, with the DCM fraction having the greatest effect(Table 2).

The activities of serum ALT, AST, ALP, total and direct bilirubin, and total protein that is initially present in the cytoplasm were estimated in order to evaluate liver function [25]. During hepatopathy, these substances and enzymes seep into the bloodstream, acting as an indicator of liver damage[26].In this study, the paracetamol group had unusually high serum concentrations of ALT, AST, ALP, total and direct bilirubin, as well as a decrease in total protein level. These findings indicate hepatic cell damage and paracetamol-induced liver dysfunction.The reversal of

paracetamol-induced elevated serum enzymes in the groups treated with the extract/fractions may have been facilitated by the extract's ameliorative effect through suppression of intracellular enzyme leakage through its membrane stabilizing activity. The paracetamol group's elevated serum level of ALP is caused by increased synthesis in the context of rising biliary pressure [27] and represents the abnormal change in biliary flow [28]. Consequently, an increase in the pre-treated rats' ALP levels indicates that the extract may be helpful in the treatment and avoidance of diseases such gallstones and cholescystitis.

In the current study, rats given paracetamol showed lower serum levels of albumin and total protein. This could be related to a reduction in the number of hepatocytes in the liver, which would lower the liver's ability to synthesize protein. The degree of hepatopathy was indicated by the lowered amount of total protein in rats given paracetamol. The extract/fractions pre-treated groups showed a significant and dose-dependent reduction in this negative effect on total protein, suggesting that the root extract/fractions enhanced the functional status of the liver cells. Hepatocytes conjugate the hemoglobin metabolic product bilirubin with glucuronic acid to make it more soluble in water. Serum bilirubin levels that rise abnormally are a sign of serious disruption of hepatic function and hepatobiliary illness.Hence, determination of serum bilirubin represents an index for the assessment of hepatic function[29]. Rats given paracetamol had higher blood levels of bilirubin, but the root extract

and fractions pre-treated group had much lower levels. Following extract administration, a drop in serum bilirubin levels demonstrated how well the extract had worked to return the liver to its normal functioning state.

The administration of paracetamol was seen to significantly (P <.01-.001) increase the levels of MDA while greatly decreasing those of GSH, GPx, CAT, GST, and SOD. When compared to the organotoxic group, treatment of rats with organ damage generated by PCM using root extract and fractions of *H. africana*and silymarin resulted in a significant (P <.05-.001) and non-dose dependent rise in the levels of GSH, GPx, CAT, GST, and SOD.However, concomitant treatment 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 highest dose (600 mg/kg) of the extract and silymarin (Table 3).

SOD and CAT are important antioxidant enzymes in the enzymatic antioxidant defense system because they help lessen the detrimental effects of free radicals by scavenging them to form safer molecules like hydrogen peroxide.[30]. The root extract and fractions have been observed in this study to increase hepatic enzymatic and non-enzymatic antioxidants such as SOD, GPx, CAT and GSH and also decrease

MDA level, indicating the involvement of reactive free radicals scavenging activity, therefore enhancing the activity of liver antioxidant enzymes and lowering oxidative damage to the tissues. This finding corroborates earlier report by[11].

This effect could be due to the free radicals scavenging potentials of the root extract/fractions and its antioxidative stress activity [6, 11]. These activities can be linked to the phytochemical constituents of the root extract, such as monoterpenes, sesquiterpenes, and xanthones, which have previously been reported to be present [6,11,14].

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

Treatment	Dose mg/kg	Total protein(g/ dL)	Albumin (g/dL)	Total Bilirubin (µmol/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	Combined Bilirubin (µmol/L)
Control	10	75.0± 2.55	47.50±0.64	3.95±0.18	9.15± 0.18	30.75± 2.42	17.0± 1.08	2.57± 0.22
Paracetamol	2000	56.0± 2.01^{c}	34.0±1.68^{c}	8.37±0.30^{b}	32.25± 1.49^{c}	61.75± 2.75^{c}	41.50± 2.02^{c}	5.85± 0.42^{c}
Crude extract	200	76.50±1.55^{f}	45.50± 1.32^{f}	7.32±0.19^{b,f}	29.75± 1.88^{b}	44.0±1.08^{c,d}	36.50± 0.64^{b}	4.32± 0.23^{a,d}
	400	69.0±1.68^{f}	41.25±1.49^{d}	7.42±0.51^{b,f}	28.0± 1.95^{b}	44.25± 3.56^{c,d}	36.5± 2.59^{b}	3.15± 1.04^{b}
	600	69.75±2.01^{f}	41.50± 1.55^{d}	6.80±0.51^{a,f}	21.5± 2.90^{e}	37.25± 1.75^{f}	33.50±2.50^{b,d}	4.95± 0.49^{a,e}

Aqueous Fraction	400	60.50±1.32[a]	40.5±1.04	4.90±0.28[f]	14.25± 0.90[f]	47.25± 0.85[c,e]	24.25± 0.85[f]	2.57± 0.22[f]
DCM fraction	400	65.0±1.22[d]	41.25±1.49[d]	4.92±0.26[f]	13.95± 0.75[f]	51.75± 3.68[c]	24.25± 1.37[f]	2.35± 0.15[f]
Silymarin	100	66.50±2.32[d]	41.0±2.18	3.52±0.17[f]	11.12± 2.17[e]	40.75± 1.25[f]	13.75± 0.85[f]	1.90± 0.16[f]

Data is expressed as MEAN ± SD, 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]$p<0.001$ compared to organotoxic group. $n = 5$.

Table 3: Effect of *H. africana*root extract and fractions on liver oxidative markers of rats with paracetamol-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	0.35± 0.03	5.85±0.24	0.091±0.002	2.01± 0.08	0.53±0.07	0.41± 0.03
Paracetamol	2000	0.18± 0.01[c]	1.41±0.18[c]	0.036±0.02[b]	0.93± 0.07[c]	0.31±0.03[b]	0.61± 0.02[b]
Crude extract	200	0.20±0.01[b]	2.14± 0.25[f]	0.053±0.04[d]	1.56± 0.16	0.36±0.02[a]	0.59± 0.01[a]
	400	0.29±0.02	1.91±0.30[b]	0.046±0.09[a]	1.02± 0.21[b]	0.33±0.02[a]	0.51±0.03
	600	0.32±0.03	2.63± 0.15[f]	0.068±0.01[d]	1.48± 0.24	0.55±0.03[e]	0.48±0.04[d]
Aqueous Fraction	400	0.18±0.01	2.40±0.16[f]	0.048±0.01[a]	1.12± 0.16[e]	0.24±0.03[c]	0.59± 0.02[a]

DCM fraction	400	0.28±0.02	2.16±0.15[f]	0.040±0.001[b]	1.82± 0.03[f]	0.35±0.01[a]	0.50± 0.02
Silymarin	100	0.43±0.01[f]	3.56±0.32[f]	0.046±0.001[a]	1.04± 0.01[d]	0.40±0.01[d]	0.34± 0.02[e]

Data is expressed as MEAN ± SD, Significant at [a]*P*<.05, [b]*P*<.01, [c]*P*<.001, when compared to control; Significant at [d]*P*<.05, [e]*P*<.01, [f]*P*<.001 compared to organotoxic group. *n*= 5.

Hepatocytes, nuclei, the portal triad, the bile duct, the hepatic artery, and the hepatic vein all had normal cellular profiles and were all contained within normal cellular architecture in Group 1 (normal control, A) treated distilled water (10 mL/kg), according to histological sections of the livers of rats receiving various treatments (Figure 1). After receiving paracetamol (2 g/kg) treatment, the organotoxic group (Group 2, B) showed signs of cellular abnormalities, such as disseminated necrotic region evidence with karyolysis and hemorrhagic parenchyma. In comparison to the control group, very few lobules displayed normal parenchymal with normal hepatocyte arrays (Figure 1). After receiving 200 mg/kg of *H. africana* root extract and PCM, the rats in Group 3 (C) had a partially normal area with normal hepatocyte arrays and moderately hemorrhagic sinusoidal gaps. Focused fatty alterations and activated Kupfer cells were visible in the parenchyma. Confluence necrosis and a multifocal area of hepatocyte drop out were also observed (Figure 1).Group 4 (D) rats were given 400 mg/kg of *H. africana* root extract and PCM, and the rats' livers displayed both arrays of hepatocytes and hydropic hepatocytes (ballooning degeneration), in partially normal areas. There were several sites of confluence necrosis visible in the parenchyma. A liver section revealed multifocal areas of hepatocyte drop out and confluence necrosis, area of inflammatory infiltration, and hyaline degeneration in Group 5 (E) rats treated with 600 mg/kg of *H. africana*root extract and PCM.There were many normal lobules with normal hepatocytes. The liver sections of the rats in group 6 (F) that were given PCM (2000 mg/kg) plus an aqueous fraction of H. africana root displayed moderately congested arteries and normal liver cell arrays. Rats in group 7 (G) with liver sections that were treated with dichloromethane fraction (400 mg/kg) of *H. africana* root and PCM (2000 mg/kg) displayed normal hepatocyte and portal triad arrays with no visible lesions. The liver segment of the silymarin-treated rats with PCM-induced toxicity (Group 8, H) showed normal hepatocyte and blood vessel arrays with no discernible damage (Figure 1).

From a histological perspective, paracetamol was found to have significantly reduced the root extract/fractions pre-treated groups' cellular deterioration and other abnormalities. The histology result is consistent with the liver function parameters' values, suggesting that the extract may have a dose-dependent hepatoprotective impact on liver damage caused by paracetamol.

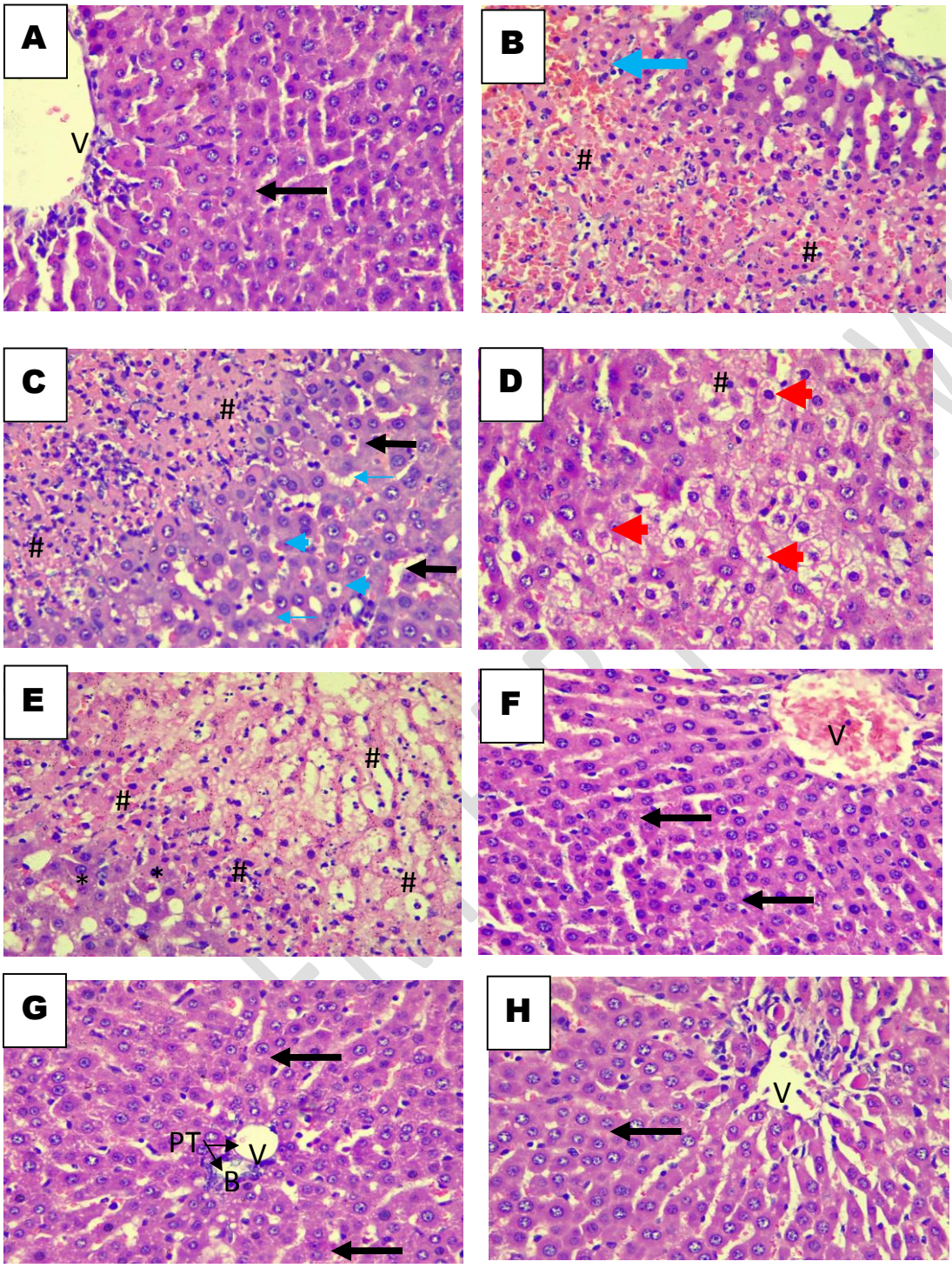

Figure 1: Liver histological section of rat treated with distilled water 10 mL/kg(A), Paracetamol 2g/kg (B), *H. africana* extract 200 mg/kg (C), 400 mg/kg, (D), 600 mg/kg (E), Aqueous fraction (F), DCM fraction (G), Silymarin 100 mg/kg (H) showing normal arrays of liver cells (black arrows) and moderately dilated vessels (V),confluence necrosis (#), area of inflammatory infiltrate and hyaline degeneration (asterisk),activated kupfer cells and focal fatty changes (white arrow) H&E stain, x400 magnification.

4.0 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 substance which is through the activities of its phytochemical constituents.

Ethical approval

All authors hereby declare that 'Principles of laboratory animal care' (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. The study was approved by College of Health Sciences Animal Ethics Committee, University of Uyo with ethical number UU/FP/AE/22/055.

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