

Hepato-Renal Toxicity Following Administration of *Hippocratea africana* Ethanolic Leaf Extract

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Abstract: *Hippocratea africana* (HA) extract has been widely reported for its anti-plasmodial and antioxidant activities. This study was designed to investigate the plants' ethanolic leaf extract on the liver and renal functions, and histology in experimental animal model. Fifteen female adult Wistar rats weighing 150 - 160 g were divided into 3 groups (A - C) of 5 rats each. Group A, the normal control received distilled water 4 ml/ kg body weight, groups B and C received HA extract (524.4 and 786.6 mg/kg body weights respectively); representing 20% and 30% of LD₅₀ which was 2622.2 mg/kg body weight. Extract administration was by orogavage for 28 days, and serum was collected for renal and kidney function tests using standard procedures. Liver and kidney were excised, fixed in 10% formal saline, processed and stained with H&E, and Masson's trichrome stains for light microscopy. Signs of behavioural toxicity or mortality were not observed during the experiment, however, mild to moderate morphological alterations were demonstrated in both the liver and kidney of test animals with the widening of the urinary space, atrophy of the glomerulus, foci hyperplasia, and tubular swelling of the kidney in groups B and C animals, and this was corroborated in the liver, where there was mild to moderate widening of sinusoidal spaces with some presence of fatty changes. The liver function test indicated increasing serum enzymes of which alkaline phosphatase was significantly ($p < 0.05$) increased in groups B and C, whereas renal function test were significantly ($p < 0.05$) increased in these groups particularly of serum creatinine and urea. In conclusion, increasing doses and duration of HA administration may lead to severe hepato-renal complications.

Keywords: *Hippocratea africana*, Liver and Kidney, Histology

INTRODUCTION

Over 80% of the African population rely on the use of herbal medicines, either as decoctions and/or concoctions for the treatment or the management of various diseases which conventional therapies often fail to satisfactorily tackle (Parekh and Jing, 2011; Romm, 2017), thereby increasing the desire all the more to use verifiable medicinal plants which are complementary and better serve as alternatives to synthetic drugs as they are locally sourced, possess bioactive principles that is efficacious, affordable, and low toxicity (WHO, 2017).

Hippocratea africana is a plant of medicinal value from the family Hippocrateaceae, a perennial climber that can be cultivated from seeds, and possesses *in vivo* antiplasmodial activity in mice (Okokon *et al.*, 2006), improves antioxidant levels in hepatocytes (Okokon *et al.*, 2013), promotes erythropoiesis, and reduces atherosclerotic coronary disease markers

(Ndem *et al.*, 2014). The root bark extract of *Hippocratea africana* induces mild toxicity by altering the liver enzymes and histology of Wistar rats (Ndem and Ewere, 2016), but an earlier study had suggested that at doses of 100 to 300 mg/kg body weight of rats, the root extract of *Hippocratea africana* did not cause liver or kidney derangements (Ndem *et al.*, 2013). However, Ndem *et al.*, (2016) demonstrated that root bark extract *Hippocratea africana* at 100 to 300 mg/kg body weight induced homeostatic perturbations in experimental rats, though with no apparent renal distortion of the histoarchitecture, and but concluded that the concentration of creatinine be re-evaluated to confirm possible distortion to the renal function. The investigation of the phytochemical analyses of *Hippocratea africana* revealed the presence of alkaloids, terpenes and flavonoids, which may be responsible for the anti-malarial activity (Phillipson and Wright, 1991). The aim of this present study was

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to investigate the effects of *Hippocratea africana* on the liver and kidney function tests and changes in the histology using the Wistar rat experimental model.

MATERIALS AND METHODS

Collection of plant samples

Fresh leaves of *Hippocratea africana* were harvested from Uruan forest in Uruan Local Government Area of Akwa Ibom State in the rainy season in the month of July. The leaves were identified and authenticated in the Department of Botany, University of Uyo, with specimen voucher number UUPH34a deposited.

Preparation of the extract

The fresh leaves of *Hippocratea africana* were washed gently with tap water to remove debris and pounded using mortar and pestle to reduce it to smaller unit. Then the grounded leaf 1000 g was macerated in 70 % ethanol for 72 hours. The mixture was sieved using a sieve cloth to separate the filtrate from the residue, with the filtrate concentrated in a water bath at 45 °C to obtain a brownish crude extract with a yield of 48.7 g placed in a beaker wrapped with aluminum foil and preserved in a refrigerator at 4 °C until required for the experiment.

Experimental animals

Fifteen adult albino female Wistar rats weighing 150 – 160 g were obtained from the College of Health Sciences Animal House, University of Uyo and used in this study. They were housed in a ventilated room in standard cages under standard laboratory condition and acclimatized for 7 days. Food and water were provided throughout the experimental period. The animals were humanely handled in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (2011). The animals were weighed and allotted into three (3) groups (A - C) of five (5) rats per group, housed in cages with dimensions of 60 cm long x 40 cm wide and 25 cm high) and shavings of soft plywood as beddings which was changed daily. Animals were maintained at temperature of 25 °C ± 3 °C; approximately 12:12 hours light and light cycle daily and were fed with pelletized growers feed from (Grand Cereals Limited, Anambra, Nigeria), and received tap water *ad libitum*. Group A served as the control which was administered distilled water, while B and C were administered medium and high dosage levels of the plant extract based on the weight of the animals. The placebo and extract were administered orally once daily for twenty-eight (28) days using the canula attached to syringe.

Experimental design

Treatment Groups

- A: Normal control (NC) distilled water (4 ml/kg)
- B: Medium dosage HA leaf extract [524.40 mg/kg (20 % of LD₅₀)]
- C: High dosage HA leaf extract [786.61 mg/kg (30 % of LD₅₀)]

Animal sacrifice and collection of samples

At the end of the treatment period, animals were anaesthetized using chloroform and dissected. Blood samples were obtained via cardiac puncture using sterile needles and syringes (2 ml) into labelled sterile plain sample. The serum was obtained by centrifugation of the clotted blood (Desk Centrifuge Ocean Med England Model 80-2) at 4,000 revolutions per minute (rpm) for 25 minutes, and the serum used for the biochemical assays.

Biochemical analyses

The biochemical assays were performed on the serum of ethanolic leaf extract of *Hippocratea africana* administered Wistar rats and control, for the determination of hepatic enzymes concentration; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), spectrophotometrically by the method (Reitman and Frankel, 1957).

Histological studies

The excised liver and kidneys were fixed in 10 % formal saline, thereafter transferred to 70 % ethanol, and then through graded series of ethanol and embedded in paraffin. The paraffin embedded sections were cut at 4 µm using a rotary microtome and stained with haematoxylin and eosin, and Masson's trichrome (Drury and Wallington, 1980; Kiernan, 2008), and then studied under for the light microscope for alternations.

Statistical Analysis

Data were evaluated using mean ± standard error of mean (SEM) and comparison between the test groups and control group was done using student's t- test, means were considered significantly different at (p < 0.05).

RESULTS

The effect of *Hippocratea africana* ethanolic leaf extract on the body weight, liver and kidney functions of Wistar rats is presented in Tables 1, 2 and 3 respectively.

Table 1 Effect of *Hippocratea africana* on Body Weights of Wistar Rats

| Groups (n=5) | Body Weight (Mean ± SEM) | | |
|-----------------|--------------------------|---------------|----------|
| | Initial (g) | Final (g) | % Change |
| A | 159.20 ± 0.04 | 182.20 ± 8.03 | 12.62 |
| B | 150.54 ± 5.00 | 175.60 ± 3.11 | 14.27 |
| C | 153.70 ± 5.69 | 180.20 ± 5.11 | 14.71 |

Table 2 Effect of *Hippocratea africana* on Liver Function Tests in Wistar rats

| Groups (n=5) | AST (U/L) | ALT (U/L) | ALP (U/L) | T-BIL-D (mmol/L) | D-BIL-D (mmol/L) | ALB (g/L) |
|-----------------|--------------|--------------|----------------|---------------------|---------------------|--------------|
| A | 51.74±3.48 | 42.50±3.19 | 174.00±9.51 | 5.78±0.90 | 0.70±0.04 | 29.50±0.67 |
| B | 66.00±9.19 | 46.40± 6.68 | 240.80± 18.30* | 5.72±1.58 | 1.22±0.23 | 30.00±0.71 |
| C | 68.40±8.70 | 49.40±5.78 | 250.20±25.78* | 6.04±0.67 | 1.22±0.37 | 30.20±1.07 |
| P value | 0.486 | 0.675 | 0.030 | 0.977 | 0.289 | 0.832 |

Mean ± standard error of mean (SEM); p < 0.05 compared to control. *- statistically significant compared to control (A)

Table 3 Effect of *Hippocratea africana* on Kidney Function of Wistar Rats

| Groups (n=5) | Creatinine (mmol/L) | Urea (mmol/L) | K (mmol/L) | Na (mmol/L) | Cl ⁻ (mmol/L) | HCO ₃ ⁻ (mmol/L) |
|-----------------|------------------------|------------------|---------------|----------------|-----------------------------|---|
| A | 75.60±2.25 | 13.4±0.55 | 5.44±0.20 | 138.60±1.96 | 101.80±0.49 | 24.40±1.03 |
| B | 82.20±1.92 | 13.10± 0.31 | 5.96±0.15 | 140.20±1.98 | 102.00±1.00 | 27.20±0.49 |
| C | 85.20±3.31* | 14.08±0.53* | 6.32±0.29* | 144.60±0.81 | 105.00±1.02* | 25.00±0.55 |
| P value | 0.05 | 0.03 | 0.04 | 0.06 | 0.01 | 0.58 |

Mean ± standard error of mean (SEM); p < 0.05 compared to control. *- statistically significant compared to control (A)

Histological observations

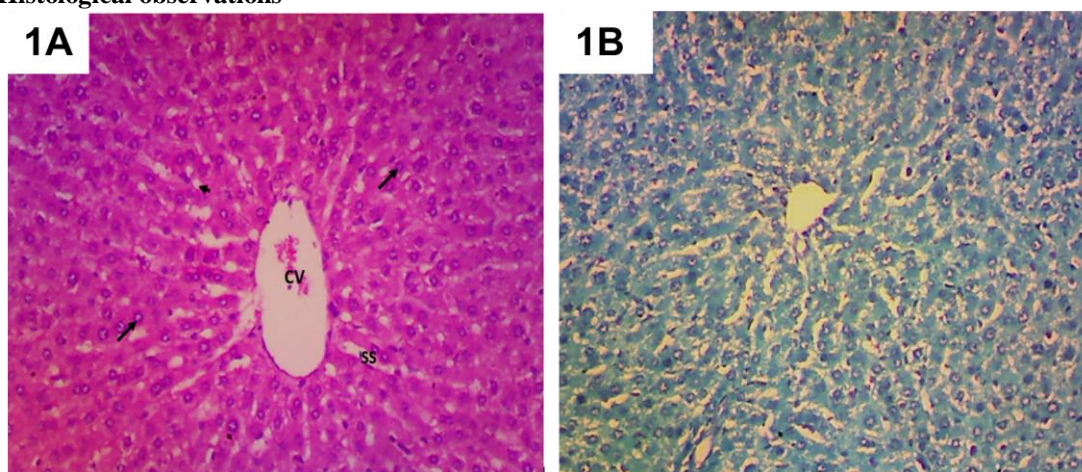


Figure 1 ([A - H&E] and [B - Masson's trichrome]) Photomicrograph of liver section showing normal control (NC) – group A histoarchitecture with array of hepatocytes (arrows), average sized central vein (CV), x100. Inference – appears normal

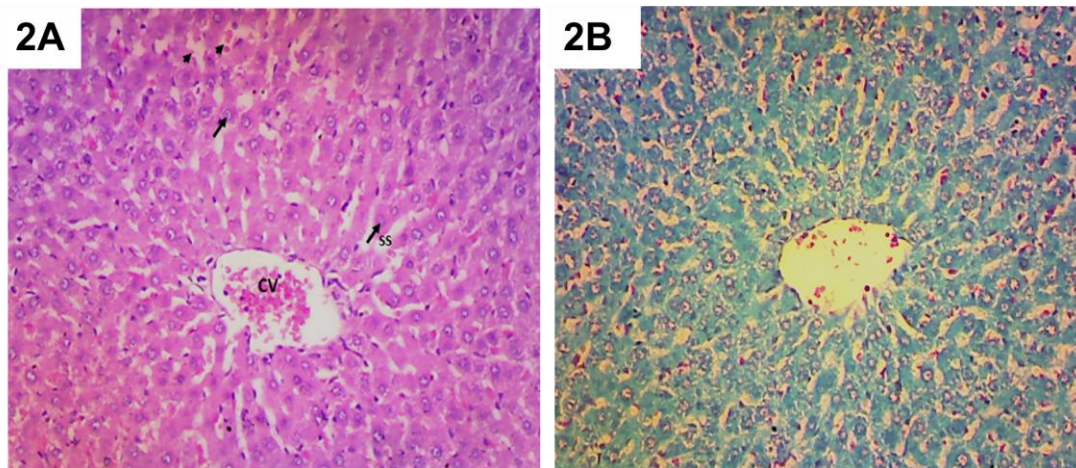


Figure 2 ([A - H&E] and [B - Masson's trichrome]) Photomicrograph of the liver section showing group B with normal array of hepatocytes (arrows), sinusoid (ss) and congested central vein (CV), a focal points of tissue degeneration (arrowhead) with hemorrhagic parenchyma, x100. Inference – mildly affected.

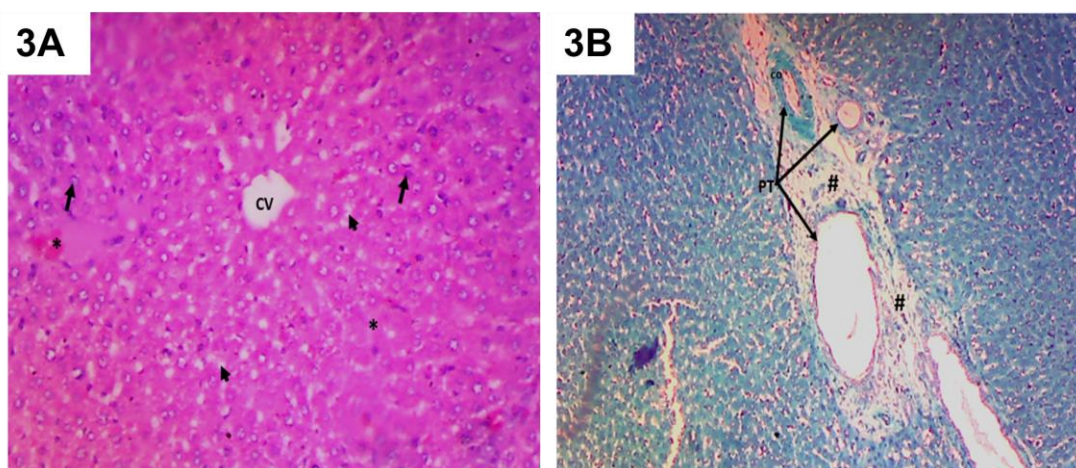


Figure 3 ([A - H&E] and [B - Masson's trichrome]) Photomicrograph showing liver section of group C with normal array of hepatocytes (arrows), average sized central vein (CV) wide areas of fatty changes (arrowheads), x100. The area of portal triad showed evidence of connective tissue proliferation (#), the collagen (co) fiber of the arterial wall was displayed. Inference – moderately affected.

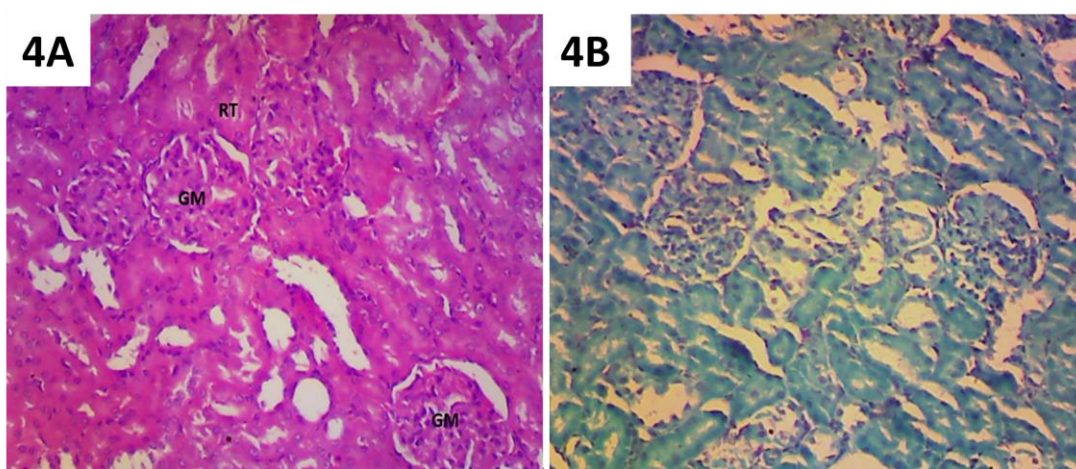


Figure 4 ([A - H&E] and [B - Masson's trichrome]) Photomicrograph of Kidney section x100 of group A animal with renal tissue histology architecture evidence with normal glomeruli (GM), renal tubule (RT). Inference: appears normal

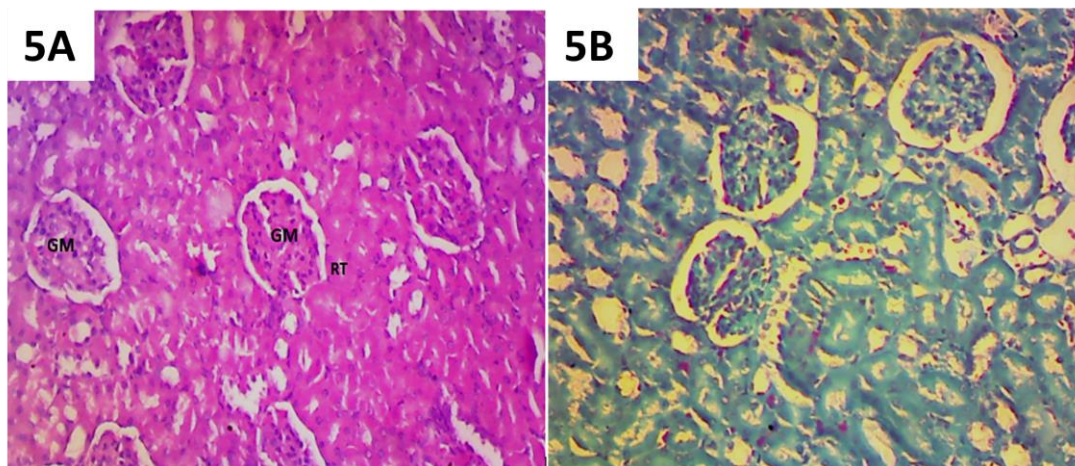


Figure 5 ([A - H&E] and [B - Masson's trichrome]) Photomicrographs of Kidney x100 section of group B animal with renal tissue histology architecture evidence with normal glomeruli (GM), renal tubule (RT), Renal artery (arrow) and a focal inflamed parenchyma (#) and increased urinary space. Inference: mildly affected

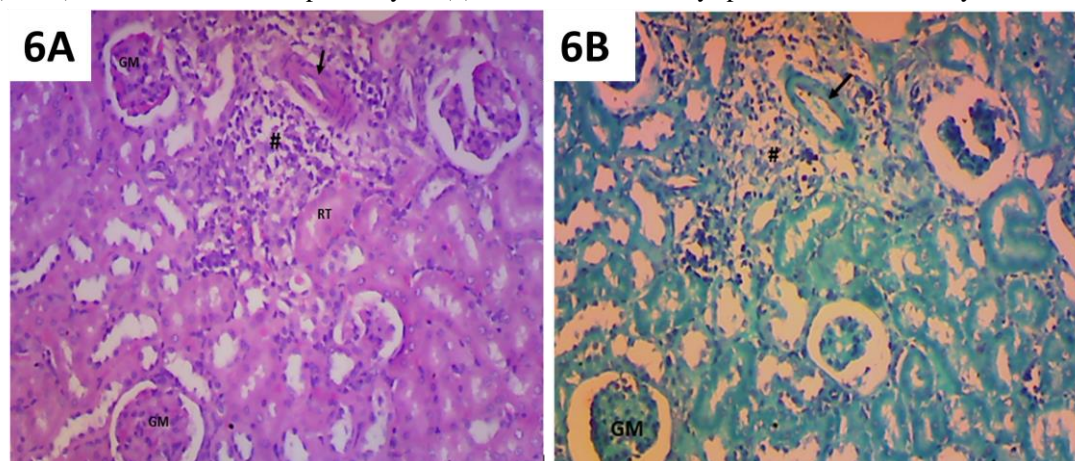


Figure 6 ([A - H&E] and [B - Masson's trichrome]): Photomicrographs of kidney section x100 of group C animal with normal glomeruli (GM), renal tubule (RT), Renal artery (arrow) and a focal inflamed parenchyma (#) Inference: moderately affected

DISCUSSION

This study was designed to investigate a 28 day sub-chronic effect of ethanolic leaf extract of *Hippocratea africana* on possible alteration of liver and kidney histoarchitecture and biochemistry in adult female Wistar rats. The surging levels of plasma ALT, AST and ALP are useful clinical markers for hepatotoxicity, and are estimated to be proportional to the degree of hepatic damage (Jaeschke et al., 2002; Shalan et al., 2005). Low concentrations of ALT, AST and ALP in the blood is to be expected and is considered normal, but an underlying liver disease is the most common factor for a higher than normal level of hepatic enzymes. In this study, ALT showed slight surges in groups B and C when compared with the control group, though not significant, a similar trend was observed for AST concentration. Ndem and Ewere (2016) demonstrated that slight increases in ALT and AST were detected after *H. africana* root bark administration at 100 and

200 mg/kg, indicating mild hepatocellular damages, and that the male Wistar rats expressed elevated liver enzymes than their female counterparts. However, ALP was significantly elevated, and mild elevations of ALP may signal liver disease, which may plausibly be due to overproduction or/and leakage in blood (Thapa and Walia, 2007). These differentials taken together provide insight to the underlying changes in the serum liver enzymes.

Total and direct bilirubin was slightly increased in treated the groups compared with control. Bilirubin acts to excrete anions (Limdi and Hyde, 2003), and bilirubin its levels have prognostic relevance in alcoholic hepatitis, primary biliary cirrhosis, and acute liver failure, but a disproportionate rise in conjugated bilirubin has limited diagnostic value (Feldmann et al., 2002).

Decrease in albumin may be due to decrease protein synthesis which results from deficits in regulations or trauma to the hepatocytes, as in the severity of liver disease, serum albumin reduces indicating diminished albumin synthesis (Limdi and Hyde, 2003). Data from this study showed that albumin was slightly decreased in the treated groups compared to control, but decreases in serum albumin is not specific for liver disease and though their decreased levels may be associated with protein malnutrition, and nephrotic syndrome (Daniel and Marshall, 1999). However, serum albumin levels may be normal in diseases like drug related hepatotoxicity, acute viral hepatitis and obstructive jaundice (Thapa and Walia, 2007).

Potassium was significantly increased in the treated groups compared to the control, and hyperkalemia implies perturbations in the resting potential of muscles and nerve but it's been argued that concentration between 5.5 to 6.0 mmol/L does not cause fatality (Acker *et al.*, 1998), as observed in this study in which all treated rats survived the entire duration of the experiment. Potassium values less than 3.0 mmol/L is implicated in arrhythmia, tachycardia and cardiac arrest, but these values are associated with bicarbonate which measures the buffering capacity of plasma (Tierney *et al.*, 2001).

The sodium ion showed a non-significant change with increases in groups B and C when compared with normal control. Sodium excretion is associated and directly related with chloride ion because most sodium ions excreted is coupled with chloride ion at excretion (Guyton and Hall, 2011), and it is vital to the induction of electrical signals needed for cellular communication in the musculature and nervous systems, as significant decrease or increase may pose cellular dysfunction and fatality (Syzdek *et al.*, 2007). Low concentration of sodium is indicative of dehydration and shock (Hamouti *et al.*, 2011), which was not detected in this study.

Likewise serum urea and creatinine levels showed increasing trends; with urea being significantly increased in the test groups compared to control, and these markers are linked with renal function integrity, and frequently involved in renal injury (Crook, 2006). These biochemical findings are in agreement with the renal histoarchitecture wherein severity of damage is based upon distortions in the morphologic components such as the glomeruli, tubules, interstitium and blood vessels (Alpers, 2005).

Routine histology of the liver in test groups showed evidence of hepatocyte and sinusoidal space distortions, mild to moderate fatty liver and portal triad inflammation compared to the control group which is comparable with the liver function

assessments (figures 2 and 3). Morphological features of inflammation include tissue destruction, caused by inflammatory cells or the underlying traumatic agent (Kumar *et al.*, 2008). A self healing mechanism becomes activated via replacement of damaged tissue with connective tissue via the proliferation of small blood vessels during fibrosis (Majno, 1998). The expression of collagen is a sensitive observable index in liver derangement presented as fibrosis, as it accounts for about 50 % total protein in fibrotic liver (Wang *et al.*, 2013). Our data demonstrates that the connective tissues were well expressed in the HA-administered groups compared to the control.

The renal histoarchitecture in the treated groups (figures 5 and 6) presented prominent glomerular atrophy and widening of urinary spaces at increasing doses of the *H. africana* leaf extract compared to control. It has been demonstrated that most glomerular and tubules distortions are caused by toxic or infectious agents (Alpers, 2005). The kidney serves to concentrate urine, excrete metabolic waste products and foreign chemicals, regulates water and electrolyte balances, body fluid osmolarity, acid base balance, arterial pressure, secretion, metabolism and excretion of hormones, glucogen storage and gluconeogenesis (Alpers, 2005; Guyton and Hall, 2011).

In conclusion this sub-chronic study provides evidence that the administration of leaf ethanolic extract of *Hippocratea africana* at medium and high doses of the LD₅₀ possess nephrotoxic and hepatotoxic potential to the liver and kidney of Wistar rats with mild to moderate histoarchitectural distortions. Therefore regardless of the ethnopharmacological importance of extract of *Hippocratea africana*, medium or high dose concentration and prolonged usage is discouraged.

CONFLICT OF INTEREST

None declared

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