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Synergistic Hepatocurative Effects of Hydro-ethanol Leaf Extracts of *Kigelia africana* and *Guiera senegalensis* against Carbon tetrachloride-Induced Liver Disease in Wister Rats

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ABSTRACT

Several medicinal plants and their phytochemical constituents including the leaves of *Kigelia africana* (KA) and *Guiera senengalensis* (GA) are used traditionally without toxicological assessments. This study aimed to evaluate hepatocurative synergistic effects of leaf extracts of KA and GS against liver injury induced by carbon tetrachloride (CCl_4) in albino rats. Forty albino rats of same sex were used for evaluation of sub-chronic toxicity and liver damage. Sixteen rats were divided into four groups, 4 rats each in group I, II, III and IV. Twenty-four albino rats were used for evaluation of liver damage and divided into 4 groups, six rats each in group a, b, c and d. The study has shown that leaf extracts of KA and GS possessed hepatocurative effect at the dose of 500 and 1000mg/kg in 8 days of administration. Histopathological study showed intense vacuolation, hepatic necrosis and haemorrhage in CCl_4 pre-treated group. The study shows that combined leaf extracts of KA and GS have synergistic hepatocurative effects against CCl_4-induced liver injury.

KEYWORDS: *Kigelia africana*, *Guiera senegalensis*, hydro-ethanol extract, liver injury, carbon tetrachloride, hepatocurative.

INTRODUCTION

The popularity of plant medicine in Nigeria can be attributed partly to the abundance of plant species with medicinal properties in the country. Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria [1]. The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs [2]. *Kigelia* is based on an African name and *africana* means

from Africa. The genus *Kigelia* has one species and occurs mainly in Africa [3]. The genus name comes from the Mozambican Bantu name, kigeli-keia, while the common names, sausage tree and cucumber tree, refer to the long, sausage-like fruit [4]. Its name in Afrikaans Worsboom also means Sausage tree, and its Arabic name means "the father of kit bags" [5].

Guiera senegalensis is one of the medicinal plants used for the treatment and control of diseases which belongs to the combretaceae family and also distributed in the savannah region of west and central Africa, Senegal, Nigeria, Chad, Gambia, Mali, Guinea, Guinea-Bissau, Niger, Burkina Faso, Mauritania and Ghana [6, 7]. G. senegalensis is called *Sabara* by Hausa and other tribes in northern Nigeria. The plant contains abundant phenolic and flavonoidal compounds and is often used in the region to treat diarrhea and fever as well as increase milk production in lactating women [8, 9], medicine for the remedy of gastrointestinal pain and disorder, dysentery, diarrhea, respiratory infections, fever, rheumatism and also act as anti-malarial agent [10]. The leaves are widely administered for pulmonary and respiratory complaints, for coughs, as a febrifuge, colic and diarrhea, syphilis, against dysentery, rheumatism, hypertension, eczema, beriberi, leprosy, gastroenteritis, beriberi, impotence, epilepsy, dieresis, expurgation, bronchitis, tuberculosis, fever, colds and asthma [11, 12].

Liver diseases, which are still a global health problem, may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in liver fibrosis). Since ancient times, mankind has made use of plants in the treatment of various ailments because their toxicity factors appear to have lower side effects [13].

The aim was to investigate the synergistic hepatocurative effects of KA and GS against CCl_4- induced liver disease experimental rats. The objectives were to determine the sub-chronic toxicity, liver function indices for some days interval and histopathological assessments of the liver.

MATERIALS AND METHODS

Analytical grade reagents and chemicals were used.

Experimental Wister Rats

The experimental animals used were 40 adult wister rats weighing between 90-130 g of single sex used during the study. The rats were purchased from the Animal houses, Pharmacology

Department, Ahmadu Bello University Zaria, Kaduna State, Nigeria. The rats were fed with water and food ad libitum. They were kept under laboratory conditions and acclimatized for 7 days before the research commenced at the Animal house, Federal University, Dutsin-Ma, Nigeria.

Preparation of Leaf Extract

Two hundred grams (200 g) of the powdered leaf of KA and GS were dissolved in 1000 ml of 50% ethanol at room temperature for 48 h and stirred at intervals of 30 min. The extract was filtered using a clean, sterile, white muslin cloth and was re-filtered using a whatman filter paper No.1. The filtrate was evaporated using rotary evaporator at a temperature of 45 ^{0}C. The hydro-ethanol extract wash re-constituted by dissolving the dried evaporated samples of the extract in distilled water for the toxicological studies. All the residues obtained were also reconstituted in sterilized distilled water and screened.

Experimental Design

The rats were divided into 4 groups, 6 rats each in group a, b, c and d. The rats in Group A were not induced liver injury, they served as positive control, whereas rats in groups B, C, and D, were induced liver damage using 120 mg/kg CCl_4, following the methodology of Alhassan *et al* [14]. Rats in Group B were not administered leaf extracts of KA and GS but were administered orally with 10 mg/kg Livolin, and they served as negative control. Rats in Groups C and D were administered with a daily dose of 500 mg and 1000 mg /kg body weight of leaf extract of KA and GS. In each group, 2 rats were removed after 2, 5 and 8 days respectively and sacrificed.

Toxicity Studies

Sub-chronic toxicity studies sixteen (16) albino rats weighing 60-130 g were distributed into four (4) groups. The rats in Groups II, III and IV were orally administered with doses 100, 300 and 500 mg/kg bwt once daily for 28 days respectively. Animals in Group I served as control (0.00 mg/kg) and administered distilled water. After 28 days, the animals were sacrificed and the blood collected was centrifuged to obtain sera for biochemical assays.

Biochemical Analysis

Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) were assayed [15]. The assay of Alkaline Phosphatase (ALP) employed the procedure of Rec [16]. Total Bilirubin

(TBL), Conjugated bilirubin (CBL) and unconjugated bilirubin (UCBL) were estimated by the methods of Jendrassik and Grof [17] and Sherlock [18]. Albumin was assessed using Cheesbrough method [19]. Urea was estimated using method of Wybenga et al. [20]. Electrolytes and creatinine were done by the methods of Uriyo and Singh [21] and Henry [22] respectively. Uric acid was by the method of Collins and Diehl [23] and Morin and Prox [24].

Histopathological Assessment

Liver of the rats were fixed in formalsaline, dehydrated in ethanol (50-100%). The tissues were cleared and embedded in xylene and paraffin respectively. A 4-6μM thick were cut. Staining was done with Hematoxylin and Eosin and photomicroscopic examination conducted according to Roy et al. [25].

Statistical Analysis

The results were presented (mean ± standard error of mean). Data from the groups were subjected to one way Analysis of Variance (ANOVA) followed by Tukey multiple comparison test. $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Results of Sub-chronic Toxicity

Table 1 summarizes the results for serum activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and levels of total bilirubin (TB), conjugated bilirubin, total protein (TP) and albumin (ALB) levels after 28 days administration of the extracts. At dose of 100 mg/kg and 500 mg/kg body weight (bwt), the levels of CB, TP, ALT, AST, TB and ALP significantly ($p<0.05$) increases while ALB significantly ($p<0.05$) decreased compared to the control group.

Table 1. Liver function indices of rats administered with combined hydro-ethanol leaf extracts of *Kigelia africana* and *Guiera senengalensis* after 28 days.

Parameters	NC	CE 100 mg/kg	CE 300 mg/kg	CE 500 mg/kg
TB	2.00±0.00^{a}	2.25±0.15^{a}	2.75±0.45^{a}	2.70±0.29^{a}
C.B	0.20±0.00^{b}	0.40±1.99^{b}	0.30±0.00^{b}	0.45±0.15^{b}
ALT	5.50±1.50^{a}	9.00±3.00^{a}	5.50±1.50^{a}	13.00±6.00^{a}
ALP	8.00±0.00 ^{a}	8.50±0.50^{a}	8.50±0.50^{a}	10.50±1.50^{a}
T.P	6.65±0.55^{a}	6.95±0.35^{a}	7.30±0.70^{a}	8.30±0.00^{a}
ALB	3.60±0.20^{ab}	3.20±0.10^{a}	3.75±0.15^{b}	3.00±0.20^{a}

Value are mean ± SEM (n= 4) Values with different superscript in the same column represent significant different (P<0.05). KEY: C.B = Conjugated bilirubin, T.P = Total protein, T.B = Total bilirubin, CE = Combine extract, NC = Normal control, ALT= Alanine amino transferase, ALP= Alkaline phosphatase ALB= Albumin.

Table 2 summarizes the levels of kidney parameters which increased significantly at the dose of 100 mg/kg. At the dose of 300mg/kg bwt, the levels of creatine decreased significantly, Cl^- normalized and K^+, urea, Na^+, and HCO_3^- increased significantly ($p<0.05$), at the dose of 500mg/kg, the levels of creatine, K^+, and Cl^- decreased significantly, urea, Na^+, and HCO_3^- increased significantly ($p<0.05$) compared to control.

Table 2: Renal function indices of rats administered combined hydro-ethanol leaf extracts of *Kigelia africana* and *Guiera Senengalensis* after 28 days.

	CREATININE	UREA	K^+	Na^+	HCO_3^-	Cl^-
N.C	88.00±5.66^{a}	7.65±0.78^{a}	5.60±0.57^{a}	147.50±4.95^{a}	14.00±6.41^{a}	103.00±1.41^{a}
C.E 100	89.00±4.24^{a}	9.00±0.99^{a}	26.10±2.14^{b}	152.00±4.24^{a}	27.00±4.24^{b}	102.50±1.41^{a}
C.E 300	39.60±4.99^{b}	8.50±2.23^{a}	6.10±0.14^{a}	151.00±2.83^{a}	25.50±4.95^{b}	103.00±1.41^{a}
C.E 500	83.00±7.07^{a}	8.25±0.92^{a}	5.45±0.92^{a}	148.50±0.71^{a}	26.50±3.54^{b}	101.00±1.41^{a}

Value are mean ± SEM (n=4) Values with different superscript in the same column represent significant different (P<0.05). KEY: C.E; Combine extract, N.C; Normal control

Results of Liver Function Test

From the results of Table 3, Rats injected with 120 mg/kg CCl_4 had significantly higher ($p<0.05$) serum ALT, ALP, ALB, TB, CB, and TP, with significantly lowered AST than the control group.

Table 3: Effects of *Kigelia africana* and *Guiera Senengalensis* leaf extracts on the activities of serum liver biomarkers of rats after 2 days post induction.

Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (µmol/L)	CB (µmol/L)	TP (g/L)	ALB (g/L)
Normal control	8.14 ± 0.14^a	19.27 ± 0.77^b	51.75 ± 0.32^a	3.06±0.96^a	1.82± 1.42^a	49.53± 9.43^a	25.19 ±5.19^a
120 mg/kg CCl_4	32.00±2.00^b	10.25 ± 0.25^a	110.09±59.9^a	10.15± 0.15^b	4.80± 4.40^a	60.16± 0.16^a	26.53±6.33^b

Values (mean ± standard error of mean) of 2 determinations (n=6). Values in the same column with different superscripts differs significantly ($p < 0.05$). CCl_4: Carbon tetrachloride, AST= Aspartate amino transferase.

The treated group that received dose of 500 mg/kg indicated significant decrease of ALT, AST, TP and significant increase of ALP, TB, CB, and ALB compared to standard drug treated group while at dose of 1000 mg/kg the levels of ALT, AST, ALP, TB, and ALB decreased significantly while CB and TP increased significantly ($p<0.05$) (Table 4).

Table 4: Liver function test after oral administration of *Kigelia africana* and *Guiera Senengalensis* leaf extracts for 5 days.

Parameters	ALT(U/L)	AST(U/L)	ALP(U/L)	TB(µmol/L)	CB(µmol/L)	TP (g/L)	ALB(g/L)
Normal control	8.10±0.71^a	6.58±3.42^a	7.25± 0.05^a	3.00± 1.00^a	0.65± 0.05^a	7.00±0.0^a	4.10±0.10^a
10 mg/kg Livolin	25.00±0.00^b	34.10±6.10^b	108.50± 0.50^c	10.51±2.51^b	2.90± 0.10^b	3.74±0.25^a	19.75±0.25^b
500 mg/kg G.S and K.A	23.07±4.93^b	30.10±0.10^b	119.0±0.00^d	40.0 ± 0.0^c	9.10± 0.10^c	3.30±0.20^a	25.38±5.88^b
1000 mg/kg G.S and K.A	20.87±5.62^b	30.14±1.14^b	9.00± 0.0^b	8.05± 0.50^b	3.00±0.0^b	32.65±29.65^b	4.30± 0.20^a

Values (mean ± standard error of mean) of 4 determinations (n=6). Values in the same column with different superscripts differs significantly ($p < 0.05$).

The treated group (Table 5) that received dose of 500 mg/kg indicated significant increase of all the parameters except TP normalized while at dose of 1000mg/kg there was significant decreased of ALT, ALP and CB, with levels of AST, TB and ALB increased significantly ($p<0.05$) compared to standard drug treated group.

Table 5: Liver function test after oral administration of *Kigelia africana* and *Guiera Senengalensis* leaf extracts for 8 days.

Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (µmol/L)	CB (µmol/L)	TP (g/L)	ALB (g/L)
Normal control	8.12±0.28^{a}	10.0±0.00^{a}	8.25±0.05^{a}	2.00±0.00^{a}	0.80±0.00^{a}	7.00±0.00^{a}	4.0± 0.00^{a}
10 mg/kg Livolin	20.0±0.02^{b}	20.95±0.25^{b}	98.0±0.00^{b}	8.05±0.05^{b}	3.05±0.50^{b}	4.00±0.00^{a}	15.25±0.25^{b}
500 mg/kg GS and KA	25.40±0.30^{b}	30.05±0.05^{c}	115.05±0.00^{c}	9.39±0.39^{b}	7.06±2.96^{c}	4.00±0.00^{a}	25.80±6.10^{b}
1000mg/kg GS and KA	16.85±2.50^{c}	23.32±2.42^{b}	89.95±0.50^{c}	9.79±1.29^{b}	2.80± 0.10^{b}	4.00±0.00^{a}	24.63±5.73^{b}

Values (mean ± standard error of mean) of 4 determinations (n=6). Values in the same column with different superscripts differs significantly ($p < 0.05$).

Figure 1 shows histopathology of liver after 2 days liver injury induction. At x40,100 and 250 respectively, numerous inflammatory cells (arrows) were observed at 400× (arrows at the centre) mild ballon hepatocyte (arrows) outside.

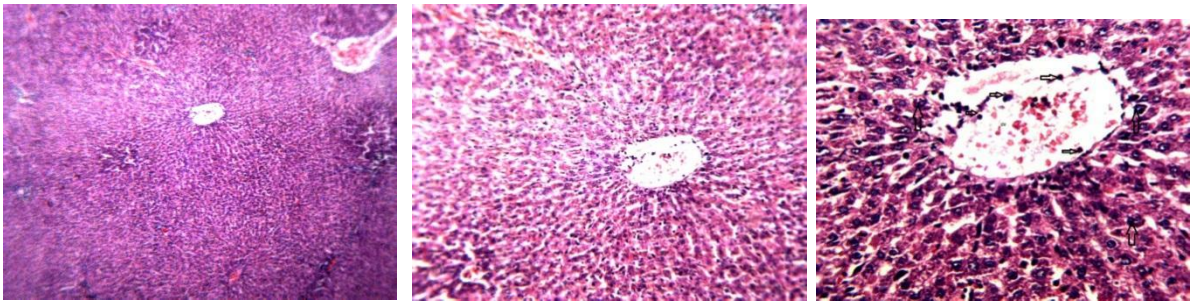

Figure 1:- Histopathology of CCL_4 induced injured liver.

From Figure 2, at x40, 100 and 250 respectively, at the highest magnification an inflamed hepatapocyte was observed (arrowed) while the the triangular arrow shows a clustered of hepatocyte and indicator of inflammatory infiltrate.

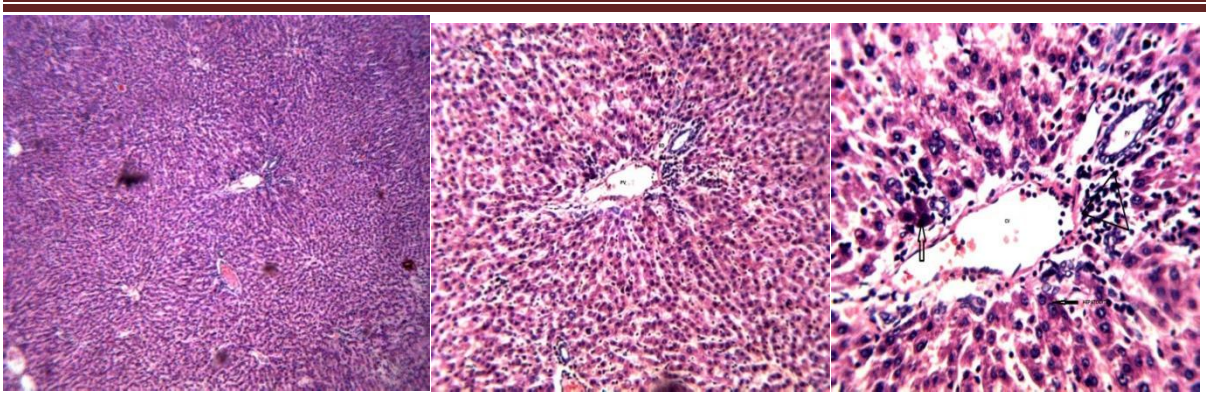

Figure 2:- Histopathology of untreated CCL_4 induced injured liver after 2 days.

Figure 3 shows histopathology of liver after treatment with extract for 5 days. At x40, 100 and 250 respectively, at x400 mild cytoplasmic vacuoles (arror) and severe hemorrhage was present on central vein.

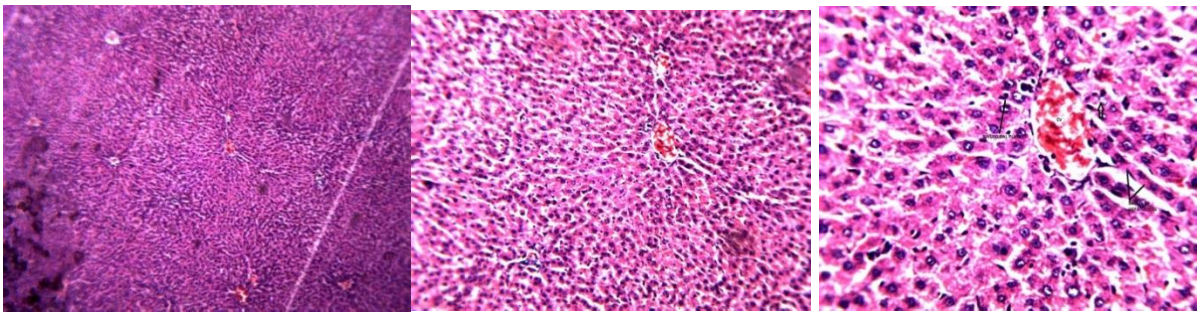

Figure 3:- Histopathology of CCL_4 induced injured liver treated with 500 mg/kg plant extract.

Figure 4 shows histopathology of liver after treatment with extract for 5 days. At x40,100 and 250 respectively, at all magnifications) Normal liver histoarchitectures were seen.

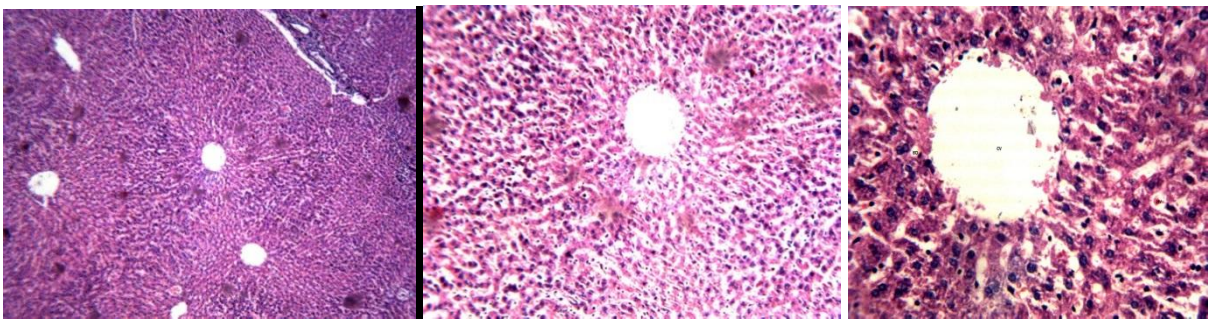

Figure 4:- Histopathology of CCL_4 induced injured liver treated with 500 mg/kg plant extract.

Figure 5 shows histopathology of liver that was not injured. At x40,100 and 250 respectively, revealed the normal architecture of liver section with variable hepatocellular arrangement arror show immunological cells.

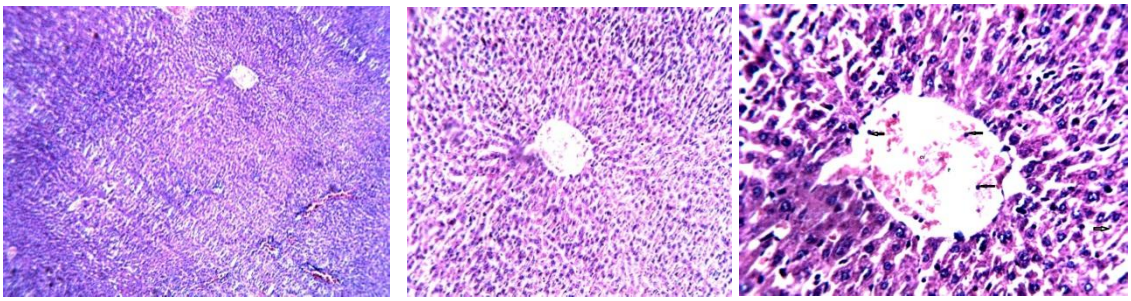

Figure 5:- Histopathology of uninjured liver.

Figure 6 shows histopathology of liver after treatment with extract for 8 days. At x40,100 and 250 respectively, Proliferation of Bile ductule. Bile ductules (arrows) are small, irregularly formed structures that are present at the interface between the portal tract connective tissue and the hepatocytes. They proliferate in response to injury to the biliary system.

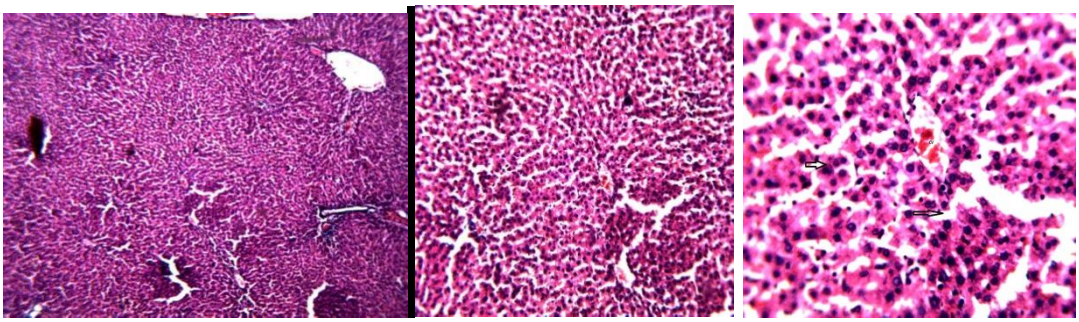

Figure 6:- Histopathology of CCL_4 induced injured liver treated with 1000 mg/kg plant extract.

Figure 7 shows histopathology of liver after treatment with extract for 8 days. At x40,100 and 250 respectively at x400) Several hepatocytes swelling (ballooning degeneration) (arrows) surrounded central vein was observed and severe hemorrhage at the central vein.

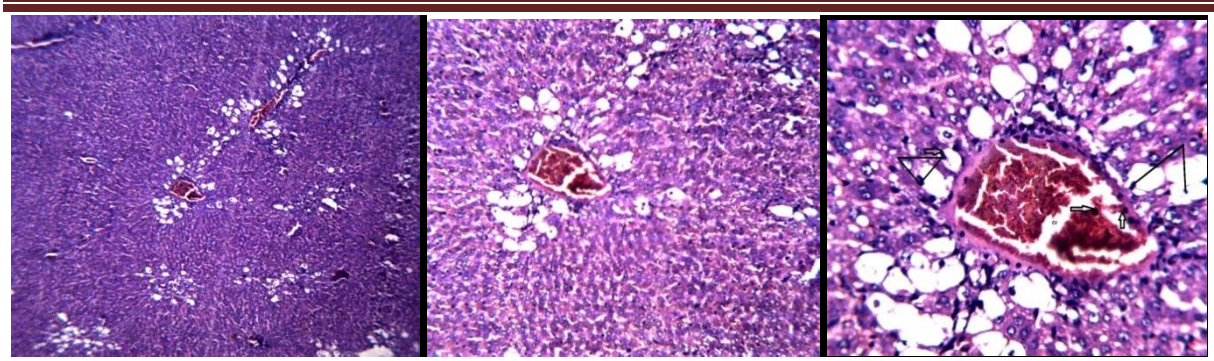

Figure 7:- Histopathology of CCL_4 induced injured liver treated with 1000mg/kg plant extract.

The results show significant difference in liver parameters levels of albino rats (Table 1) treated with combined hydro-ethanol leave extract of *Kigelia africana* and *Guiera senegalensis* and rats treated with normal saline. At dose of 100mg/kg and 500 mg/kg body weight (bwt), the levels of CB, TP, ALT, AST, TB and ALP significantly ($p<0.05$) increased while ALB significantly ($p<0.05$) decreased after 28 days of oral administration. At the dose of 300mg/kg bwt, all the parameters of liver indices significantly increased while ALT normalized when compared to the control group. For Table 2 results, the levels of kidney parameters increased significantly at the dose of 100 mg/kg bwt compared to control group. At the dose of 300 mg/kg bwt, the levels of creatine decreased significantly, Cl^- normalized and K^+, urea, Na^+, and HCO_3^-increased significantly ($p<0.05$) compared to control while at the dose of 500 mg/kg bwt, the levels of creatine, K^+, and Cl^- decreased significantly, urea, Na^+, and HCO_3^-increased significantly ($p<0.05$) compared to control. Thus, the liver and kidneys might have been exposed to certain toxic principle present in the extract at higher concentration.

The effect of the hydro-ethanol leaf extracts after 28 days treatment is presented in Table 1. Total protein, total bilirubin, alkaline phosphatase and the activities of transaminases increased significantly at all doses against the control. From the renal function indices, an increased ($p<0.05$) in urea was observed in groups administered with all dosages (Table 2). The sodium levels increased generally while potassium levels were decreased. From the results of Table 3, rats injected with 120 mg/kg CCl_4 had significantly higher ($p<0.05$) serum ALT, ALP, ALB, TB, CB, and TP, with significantly lowered AST than the control group. This is an indication of possible fibrosis, the initial repair mechanism of liver injury, as indicated by Burtis *et al* [26] that

all cellular damage induces fibrosis as a healing response. After five days treatment (Table 4), at the doses of 500/1000 mg/kg bwt, the levels of ALT, AST decreased significantly while CB increased significantly ($p<0.05$) but at 500 mg/kg treatment, the levels of ALP, TB, ALB increased significantly while TP normalized and then at the doses of 1000 mg/kg ALP, TB, ALB decreased significantly while TP increased significantly compared to standard drug treated group (Group 2). After eight days treatment (Table 5), at the doses of 500 and1000 mg/kg bwt, the levels of AST, TB and ALB increased significantly while TP normalized. At 500 mg/kg treatment, the levels of ALT, ALP and CB increased significantly while at the doses of 1000 mg/kg ALT, ALP and CB decreased significantly ($p<0.05$) compared to standard drug treated group Group 2).

However, the healing effect is more pronounced in the groups that received daily doses of 1000 mg/kg and the negative control group treated with daily doses of 10mg/kg of Livolin for 8 days compared to Group 3 rats which received a daily dose of 500 mg/kg. This may be possibly due to low serum bioavailability of the extract. The results agreed with Said *et al* [27] that rats which received 1000 mg/kg daily doses for 8 days showed significantly lowered ($p<0.05$) serum levels of ALT, AST, ALP, TP and ALB while there was significantly higher ($p<0.05$) levels of TB compared to the negative control.

Carbon tetrachloride is widely used for the experimental induction of liver damage. CCl_4 at a dose of 120 mg/kg body weight (into olive oil 1:1) used in the induction of liver injury in rats showed remarkable elevation of liver enzymes. Histopathological examination showed hepatic necrosis, hemorrhage and intense vacuolation. But administration of the extracts showed its ability to reserve the normal structure of hepatocytes.

Histopathologicial examination of the liver indicated presence of lesions in the hepatocytes of experimental rats administered with the highest dose of the extract (1000 mg/kg body weight). Histopathology examination of rats liver shows significant differences in severity of inflamed hepatocytes. Histoarchitechture between rats liver in treated and untreated groups shows difference number and cluster of inflamed hepatocytes.

The liver is the largest organ within the body, involved in metabolism of drugs. CCl_4 proves highly useful as an experimental model for the study of certain hepatotoxic effects [14]. KA and GS are medicinal plants with lot of properties and activities. These plants possess

various secondary metabolites. It occur widely with lot of common names. They have biological as well as pharmacological activities. The whole plant like stem, fruit, root, bark and leaf, is very much useful as all have a medicinal value.

CONCLUSION

These plants possess various secondary metabolites. They have biological as well as pharmacological activities. Findings from this research work show that combined hydro-ethanol leaf extract of *Kigelia africana and Guiera senegalensis* possess a significant hepatocurative activity. Further work is required to test the plant in the management of liver disease and to isolate the active components of the plant fractions and their mechanisms of action.

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