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Medicinal Potential of *Kigelia africana* Leaf Fractions on Rats Induced CCl_4 Toxicity

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Abstract The study aimed at determining the protective effect of *K. africana* leaf fractions on serobiochemical parameters and histological examination of liver section of rat induced carbon tetrachloride (CCl_4) toxicity. A total of 35 male rats were used for the study. The rats were divided into seven groups, each group having five rats. Group 1 (normal), Group 2 (drug control), Group 3 (CCl_4 control) while Group 4, 5, 6 and 7 were administered with fractions 1, 2, 3 and 4 at 100 mg/kg/day/body weight for 7 days and induced with CCl_4 intraperitonealy. Changes in body weight revealed significantly ($p< 0.05$) decrease in body weight of treated Groups 3, 4, 5, 6 and 7 compared to Group 1 and 2. Administrations of the fractions showed significant ($p< 0.05$) decrease on AST, ALT and ALP in treated Groups 2, 5 and 7 compared to Group 3, 4 and 6. Furthermore, conjugated bilirubin, total bilirubin, albumin and total protein levels significantly ($p< 0.05$) decreased in Groups 2, 5 and 7 compared to Group 3, 4 and 6. Group 4 and 6 also showed significant decrease on biochemical parameters compared to Group 3. Histology of the liver revealed clearly marked hepatic injuries with acute fatty changes, slight and mild necrosis, moderate hepatic injury with moderate fatty changes and mild inflammation in Group 3, 4 and 6. Normal liver architecture was clearly observed in treatment group 2, 5 and 7. The study revealed the hepato-protective effect of *K. africana* fractions against CCl_4 induced liver damage. The study also support claims for the use of the leaf traditionally for the treatment of liver diseases.

Keywords *Kigelia africana*, fractions, serobiochemical parameters, Carbon tetrachloride

1. Introduction

Kigelia africana (*Lam.*) popularly called the sausage tree belongs to the family of *Bignoniaceae.* It is known for its medicinal properties and traditionally used in most African communities. It is a tropical African plant that is widely grown and distributed in South, Central and West Africa. *Kigelia Africana* has so many vernacular names, it is referred to as Rawuya (Hausa, Nigeria), Uturubein (Igbo, Nigeria); Pandoro, Iyan (Yoruba, Nigeria) and Bechi (Nupe, Nigeria) [1] while it is called Mwegea (Swahili, Kenya, Tanzania) and Umfongothi (Zulu, South Africa) [2]. It is known as Balmkheera in Hindi (India) [3].

The leaves and fruits are widely used traditionally in treating ailments such as liver disease, rheumatism, snake bites, evil spirits and venereal diseases like syphilis [4]. The use of the bark, stem, twigs, leaves and fruits of *K. africana* to relieve rheumatism, toothache and headache has also been documented [5].

K. africana extracts have been used pharmaceutically as dietary/herbal supplement and traditionally for treatment of various diseases. It was documented for its strong anti-oxidative effects against hepatotoxicity induced by paracetamol toxicity. The antioxidant activity of the extract was due to the presence of caffeic acid derivative and other compounds present. The plant has some bioactive compounds including flavonoids, tannins, steroids, terpenes, saponins, naphthoquinones, iridoids and caffeic acid in the various plant part fruits, stem, leaves and roots [4]. Studies on the hepato-protective effect of leaf fractions of the plant against carbon tetrachloride (CCl_4) toxicity have not been carried out. It is against this background that the study aimed at investigating the effect of the fractions on biochemical parameters and histopathological examination of the liver section of rat induced CCl_4.

2. Materials and Methods

2.1. Plant Material

Fresh leaf of *K. africana* was collected from Girei Local Government Area, Adamawa State. Girei is located on latitude 9^0 11' 15'' N and longitude 12^0 20' 29''E, on the North bank of River Benue Google earth (2014). The leaves was taxonomically identified and authenticated in the Plant Science Department, Modibbo Adama University of Technology, Yola. The leaves were air dried in the laboratory for 7 days and thereafter made into powder using electric blender. The coarse material was sieved using 0.3 mm Endicott test sieve.

2.2. Extraction and Fractionation

Extraction and fractionation were carried according to [6] [7]. Ethanol was used as the solvent system. The powdered leaves (200 g) were extracted with 500 ml of ethanol (99.9 %) within a period of 24 h and the procedure repeated 3 times using the same powdered extract. The solvent was removed at 45 °C under vacuum. The ethanol extract residue obtained was dissolved in water (500 ml) and exhaustively extracted by consecutive liquid/liquid partition with ethanol (500 ml) with equal concentration of the extract (100 mg/ml) using a separating funnel (1000 ml). The ethanol fractions were evaporated to obtain the fractions. The fractions obtained were tested to evaluate the hepato-protective effect of *k. africana* on albino rats induced CCl_4.

2.3. Experimental Animals

Male albino rats used for the study were obtained from the veterinary research institute VOM, Jos Plateau State, Nigeria with an average weight of 100 – 120 g. The rats were housed in metal cages in the laboratory at temperature between 30 to 37 °C and maintained with free access to standard rat feeds and water. They were exposed to alternate cycle of 12 hours of darkness and light each. Ethical considerations for the use of animals in research were in compliance with the guidelines involving the use of animals for research purpose.

2.4. Experimental Design

In the experiment, a total of 35 albino rats were used. The rats were divided into seven groups as shown in Table 1.

Table 1: Experimental design showing different groups and treatment

Group 1	Normal Control: Fed with normal feed and water
Group 2	Drug Control: Received silymarin (100 mg/kg/day) orally for 7 days and CCl_4 induced intraperitonealy.
Group 3	CCl_4 Control: Orally induced with CCl_4 at dose 2 mL/kg, subcutaneously in the ratio 1:1 dilution with olive oil on 3rd day.
Group 4	Treated with fraction 1 (100 mg/kg/day) and induced CCl_4 on 3rd day.
Group 5	Treated with fraction 2 (100 mg/kg/day) and induced CCl_4 on 3rd day.
Group 6	Treated with fraction 3 (100 mg/kg/day) and induced CCl_4 on 3rd day.
Group 7	Treated with fraction 4 (100 mg/kg/day) and induced CCl_4 on 3rd day.

Rats were sacrificed on the 8th day. Blood samples were collected at slaughter and analyzed. Vital tissues were processed for histopathology.

2.5. Serobiochemical Parameters

Serum samples were analyzed using automated serology analyzer for the activity of Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphate (ALP), Conjugated bilirubin (C.B), Total bilirubin (T.B), Albumin and Total protein (T.P).

2.6. Histopathological Studies

Liver of the sacrificed rats was removed, washed in normal saline and fixed in 10 % formalin. The specimens were then trimmed, washed and dehydrated in ascending grades of alcohol risen it with xylol, embedded in paraffin, section (4-6 μm) and was stain with heamtoxylin and eosin for histopathological examination.

2.7. Statistical Analysis

Data collected were significantly $p< 0.05$ analyzed using SPSS version 21 (USA). The difference between two means was determined using one-way analysis of variance (ANOVA). Values are taken in triplicate and results are reported as mean ± SEM.

3. Results and Discussion

3.1. Changes in Body Weight

Changes in the body weight of albino rats administered orally with daily doses of ethanol fractions are presented in Table 2.

The results showed significant ($p< 0.05$) body weight gain in Group 1 (normal control) compared to other groups. Significant ($p< 0.05$) weight gains were also observed in Group 5 (Fraction 2) and Group 7 (Fraction 4) compared to Group 3 (CCl_4 Control). Weight gain was also observed in Group 4 (Fraction 1) and Group 6 (Fraction 3) compared to Group 3 (CCl_4 Control).

3.2. Biochemical Effect

Findings on the biochemical markers in rats treated with *K. africana* leaf fractions is shown on Table 3 and 4. Significantly ($p< 0.05$) decrease in the activity of AST, ALT and ALP were observed in treatment groups 2 (drug control), group 5 (Fraction 2) and group 7 (Fraction 4) while significant ($p<0.05$) increase were observed in group 3 (CCl_4 Control), groups 4 (Fraction 1) and group 6 (Fraction 3) (table 3). Liver is the most affected organ in the body. It is the site of detoxification and showed fatty vacuolation of the hepatocytes [8]. The findings in this study showed significant elevated level of AST, ALT and ALP in rat group treated with CCl_4, Fraction 4 and 6. The findings support agree with report by [9] that liver injury is characterized as hepatocellular with an elevated level of ALT while AST (mitochondria enzyme) increased level in plasma shows severe tissue injuries. Furthermore, AST, ALT and ALP are also known as markers of hepatic necrosis of liver damage.

Our findings also revealed significant decreased in rats group treated with silymarin (drug), Fraction 5 and 7; hence showed protective effect. Other biochemical parameters used in estimating liver damage include bilirubin, albumin and total protein (table 4). The results also revealed significant increase in bilirubin, albumin and total protein in rats treated with CCl_4, Fraction 4 and 6. Elevated levels of bilrubin, albumin and total protein are pointers to certain diseases such as liver and kidney disease. Effective control of these markers is attributed to an improvement in the hepatic cell's secretory mechanism, thus regarded as hepatic function markers. Increased in biochemical parameters of rats may indicate leakages of cellular enzymes, alteration of membrane permeability and liver cell destruction [10]. Rats group treated with silymarin (drug), Fraction 5 and 7 showed significant decreased. Decreased in the level of liver enzyme in the treatment group may also be attributed to the reduction in lipid peroxidation processes which plays a key role in the activation of antioxidants enzymes by regulating their transcription [11].

Furthermore, decreased level of these biochemical parameters studied revealed the medicinal potential and hepatoprotective effect of silymarin and the fractions thereby preventing leakages of intracellular enzymes and stabilize membrane activity. Silymarin is a drug used for the treatment of toxic liver diseases while the fractions are rich in antioxidants, bioactive compounds and secondary metabolites of plants including phenols, flavonoids, alkaloids, saponins, glycosides and tannins [12].

Table 2: Effect of *K. africana* fractions on body weight of rats induced CCl_4 for 7 days

Treatment Groups	Body weight gain (g) 0 day	Body weight gain (g) 8 days
Group 1 (Normal)	110.08 ± 1.02	14.14 ± 1.08*
Group 2 (Drug control)	107.14 ± 1.19	09.10 ± 1.15*
Group 3 (CCl_4 control)	83.19 ± 2.05	05. 17 ± 2.01^{NS}
Group 4 (FR 1)	86.34 ± 1.29	07.98 ± 1.03*
Group 5 (FR 2)	98.89 ± 1.89	11. 09 ± 1.10*
Group 6 (FR 3)	88.90 ± 2.01	07.18 ± 1.03
Group 7 (FR 4)	95.67 ± 1.09	10.08 ± 1.14*

Values are expressed as Mean ± SEM; (N = 5), *Significant ($p< 0.05$); NS: Not Significant

All treatment groups were administered 100 mg/kg/day of the fractions; FR = Fraction

Table 3: Effect of *K. africana* fractions on enzyme markers of rat induced CCl_4

Groups	AST (iu/L)	ALT (iu/L)	ALP (iu/L)
Group 1 (Normal)	11.33 ± 0.12	10.17 ± 0.15	27.67 ± 0.18
Group 2 (Drug Control)	12.50 ± 0.17^{a}	11.83 ± 0.17^{a}	26.00 ± 0.21
Group 3 (CCl_4 Control)	86.33 ± 0.53	72.83 ± 0.16	94.67 ± 0.42
Group 4 (FR 1)	80.67 ± 0.22$^{\mathbf{c}}$	48.67 ± 0.41^{a}	43.00 ± 0.11^{a}
Group 5 (FR 2)	14.00 ± 0.35$^{\mathbf{ac}}$	13.67 ± 0.32$^{\mathbf{ac}}$	30.00 ± 0.25$^{\mathbf{ac}}$
Group 6 (FR 3)	77.67 ± 0.52^{a}	69.33 ± 0.10	79.00 ± 0.89^{a}
Group 7 (FR 4)	14.67 ± 0.10$^{\mathbf{ac}}$	11.50 ± 0.11$^{\mathbf{ac}}$	25.33 ± 0.22$^{\mathbf{ac}}$

Values are Mean ± SD (N=5),

^{a}Significantly decreased ($p<0.05$) compared to CCl_4 Control

^{b}Significantly decreased ($p<0.05$) compared to Drug Control

^{c}Significantly decreased ($p<0.05$) compared to different fraction

All treatment groups were administered 100 mg/kg/day of the fractions

AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, FR = Fraction

Table 4: Effect of *K. africana* fractions on non-enzyme markers in rat induced CCl_4

Groups	C.B (µmol/L)	T.B (µmol/L)	ALB (g/L)	T.P (g/L)
Group 1 (Normal)	5.87 ± 0.12	13.58 ± 0.10	38.67 ± 0.14	58.33 ± 0.25
Group 2 (Drug control)	6.40 ± 0.12$^{\mathbf{a}}$	11.90 ± 0.24$^{\mathbf{a}}$	39.67 ± 0.33$^{\mathbf{a}}$	60.50 ± 0.19$^{\mathbf{a}}$
Group 3 (CCl_4 control)	58.52 ± 0.48	34.18 ± 0.37	91.17 ± 0.84	144.50 ± 0.20
Group 4 (FR 1)	49.02 ± 0.74$^{\mathbf{a}}$	35.18 ± 0.31	89.50 ± 0.96	126.00 ± 0.79
Group 5 (FR 2)	8.57 ± 0.69$^{\mathbf{ac}}$	12.77 ± 0.38$^{\mathbf{ac}}$	40.17 ± 0.14$^{\mathbf{ac}}$	61.33 ± 0.14$^{\mathbf{ac}}$
Group 6 (FR 3)	51.90 ± 0.10	31.85 ± 0.88	79.50 ± 0.18$^{\mathbf{a}}$	109.17 ± 0.41$^{\mathbf{a}}$
Group 7 (FR 4)	8.88 ± 0.90$^{\mathbf{ac}}$	13.00 ± 0.14$^{\mathbf{ac}}$	37.67 ± 0.63$^{\mathbf{ac}}$	56.67 ± 0.39$^{\mathbf{ac}}$

Values are Mean ± SD (N=5),

^{a}Significantly decreased ($p<0.05$) compared to CCl_4 Control

^{b}Significantly decreased ($p<0.05$) compared to Drug Control

^{c}Significantly decreased ($p<0.05$) compared to different fraction

All treatment groups were administered 100 mg/kg/day of the fractions

T.B = Total bilirubin, C.B = Conjugated bilirubin, T.P = Total protein, ALB = Album, FR = Fraction.

3.3. Histopathological Examination

Pathological changes observed in rats are shown in the various plates below. Plate I showed micrograph of liver section of group 1 (normal control). The micrograph showed normal hepatic lobules displaying normal hepatic plates with moderate sinusoidal spaces. The nuclei of the hepatocytes are oval and vascular, with fine granular acidophilic cytoplasm. The hepatic portal areas are well organized with active blood circulation. The plate showed that the liver surface appears to be normal.

Plate II showed micrograph of liver section of group 2 (drug control). The micrograph showed normal hepatic lobules displaying hepatic plates with moderate sinusoidal spaces. The nuclei of the hepatocytes are ovoid,

vesicular, some prominent nucleoli with fine granular acidophilic cytoplasm. The central veins are congested with signs of heamorrhage and mild necrosis within the surrounding sinusoidal spaces. The cytoplasm of the surrounding hepatocytes is coarse. The hepatic portal areas are well organized with active blood circulation, hence, the result showed a normal architecture with slight hepatic congestion.

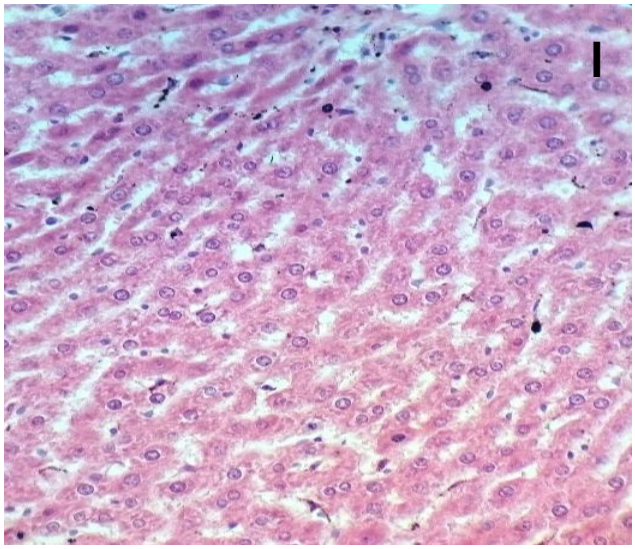
Plate III showed micrograph of liver section of group 3 (CCl_4 control). The micrograph showed clear abnormality of the liver architecture. The section showed hepatic lobules displaying necrosis of the hepatic plates mainly around the portal areas with clear destruction of the blood vessels, the hepatocytes appears damaged with ovoid nuclei, prominent nucleoli and acidophilic cytoplasm. There are moderate presence of fatty changes and increased inflammatory cells. The result showed hepatic injuries with moderate fatty changes inflammation.

Plate IV showed micrograph of liver section of group 4 (Fraction 1). It showed hepatic lobules displaying necrosis of the hepatic plates mainly around the portal areas with marked destruction of the blood vessels. The other hepatocytes appear fairly normal with ovoid nuclei, prominent nucleoli and acidophilic cytoplasm. There are marked presence of fatty change and some inflammatory cells. The result clearly marked hepatic injuries with marked acute fatty changes.

Plates V showed micrograph of liver section of group 5 (Fraction 2). The micrograph showed normal hepatic lobules displaying hepatic plates with moderate sinusoidal spaces. The nuclei of the hepatocytes are ovoid, vesicular, some prominent nucleoli with slight coarse granular acidophilic cytoplasm. The central veins contain blood cells with signs of slight necrosis within the surrounding hepatic plates. The hepatic portal areas are well organized with active blood circulation. The result showed normal liver architecture with slight necrosis.

Plate VI showed micrograph of liver section of group 6 (Fraction 3). The micrograph showed the appearance of a normal liver. The sections also showed hepatic lobules displaying necrosis of hepatic plates mainly around the portal areas with moderate destruction of the blood vessels. The other hepatocytes appear fairly normal with ovoid nuclei, prominent nucleoli and acidophilic cytoplasm. There was moderate presence of fatty changes and increase inflammatory cells. The result indicated a moderate hepatic injury with moderate fatty changes and mild inflammation.

Plate VII showed micrograph of liver section of group 7 (Fraction 4). The micrograph showed normal hepatic plates with wide sinusoidal spaces and slight necrosis. The nuclei of the hepatocytes are ovoid, vesicular and some prominent nucleoli with coarse granular acidophilic cytoplasm. The central vein contains blood cells with moderate number of polymorphs within the sinusoidal spaces. The hepatic portal areas are well organized with active blood circulation. The result indicated a normal liver architecture with mild necrosis.



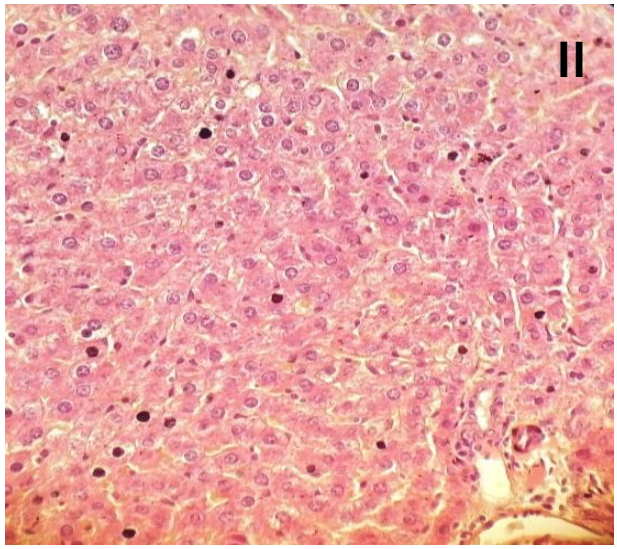


Plate I: *Micrograph of liver section of Group 1(Normal control); (H and E, x 400).* ***Plate II:*** *Micrograph of liver section of rat Group 2 (Drug control); (H and E, x 400).*

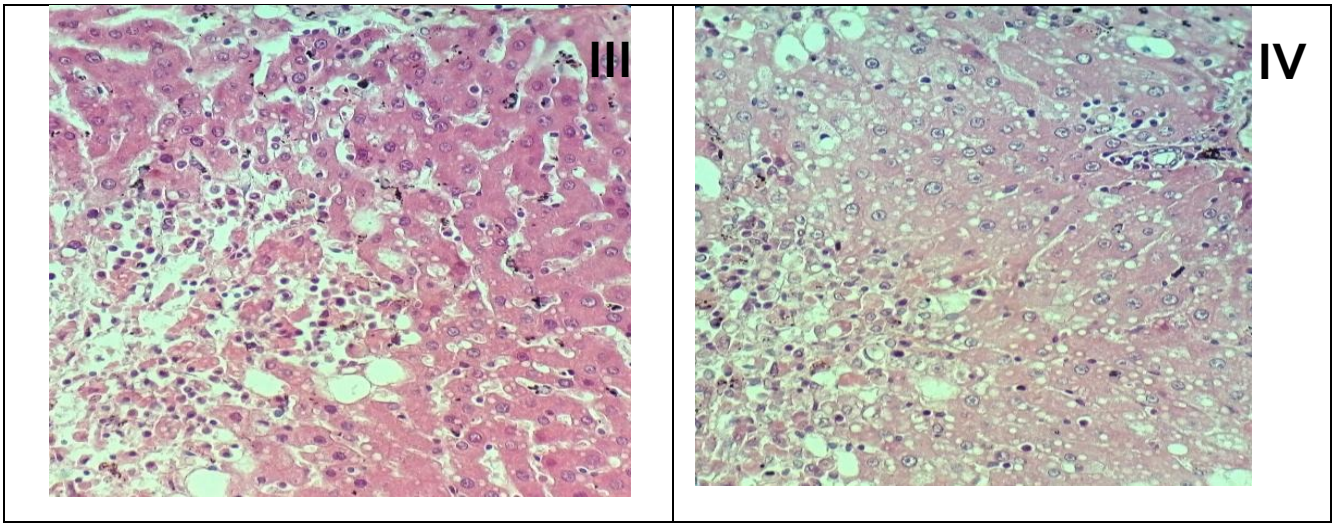


Plate III: *Micrograph of liver section of Group 3 (CCl_4 control); (H and E, x 400).* ***Plate VI:*** *Micrograph of liver section of Group 4 (Fraction 1); (H and E, x 400).*

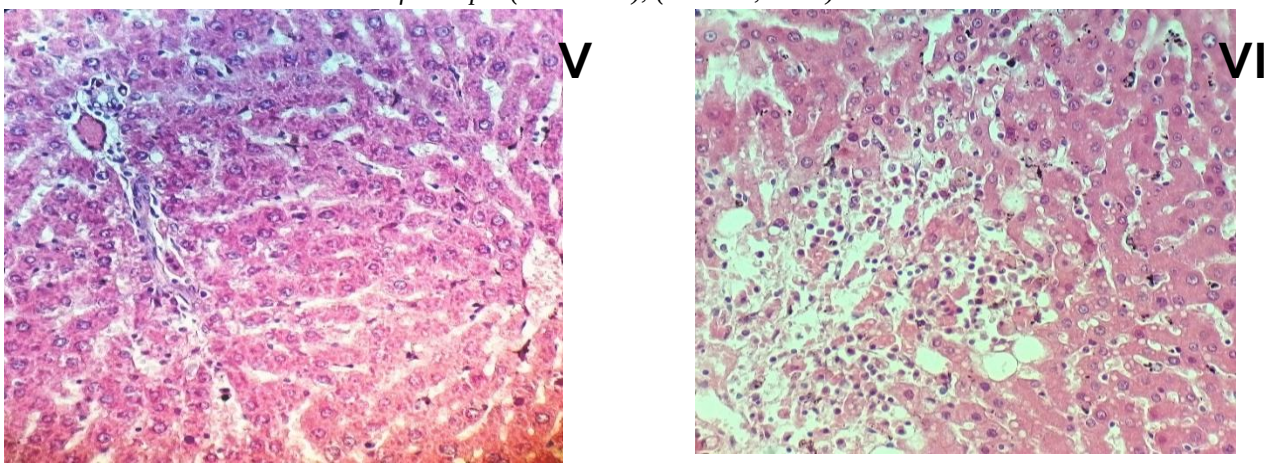


Plate V: *Micrograph of liver section of Group 5 (Fraction 2); (H and E, x 400).* ***Plate VI****: Micrograph of liver section of Group 6 (Fraction 3); (H and E, x 400).*

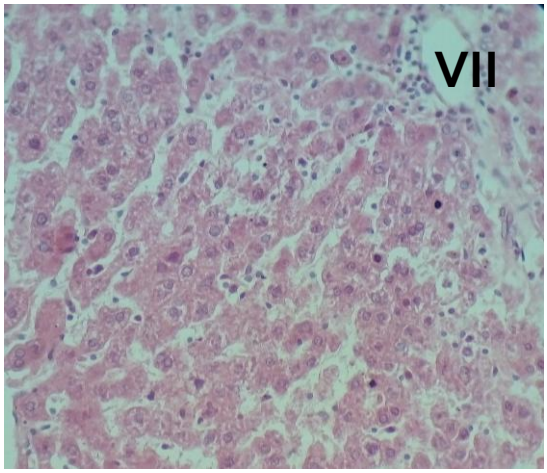


Plate VII*: Micrograph of liver section of Group 7(Fraction 4); (H and E, x 400)*

4. Conclusion

The findings in this study revealed the medicinal potential and protective effect of *K. africana* leaf fractions against liver damage induced carbon tetrachloride (CCl_4). This study support claims for the traditional use of the leaf decoction in the treatment of various ailments such as liver diseases.

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