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Full Length Research Paper

The haematological and biochemical effects of methanol extract of the seeds of *Moringa oleifera* in rats

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The toxic effects of oral administration of the methanol extract of *Moringa oleifera* seed at 0, 400, 800 and 1600 mg/kg was investigated in Wistar rats after 21 days treatment. Treatment with the extract did not significantly (*P > 0.05*) alter the levels of hemoglobin, red blood cell, packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin concentration. In rats, also, the levels of total protein, albumin and globulin in the treated animals were not significantly different from the control. The extract however induced significant decrease (*P<0.05*) in the concentrations of platelets, monocytes, and total white blood cells and significant increase in the levels of alanine and aspartate aminotransferases (ALT and AST), at 1600 mg/kg. *M. oleifera* seed extract also induced portal cellular infiltration, periportal congestion and hydropic degeneration of hepatocytes in the liver as well as cortical congestion and intestitial haemorrhages in the kidney.

Key words: *Moringa oleifera* seed, rats, biochemical, hematological.

INTRODUCTION

Moringa oleifera is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for nutritional, medicinal and industrial uses. Powder from seed kernels of *Moringa* works as a natural coagulant and is used in the rural areas to clarify very turbid water (Gassenschmidt et al., 1995). Immature seeds of *M. oleifera* are used in recipes, the leaves are extensively used as vegetable in many parts of the world and the roots can be made into a condiment similar to horseradish (Prajapati et al., 2003). The therapeutic effects of *M. oleifera* include: antibiotic (Fahey et al., 2002; Haristoy et al*.,* 2005), anticancer (Guevara et al*.,* 1999: Bharali et al*.,* 2003), antiulcerogenic effects (Akhtar and Ahmad, 1995), analgesic (Rao and Ojha, 2003), antiurolithiatic (Bennett et al*.*, 2003) and larvicidal activities (Sharma et al., 2006).

In addition, its beneficial roles in human immunodeficiency/acquired immune deficiency disease (HIV/AIDS) (Burger et al*.,* 2002) and effects on regulation of thyroid hormone status in adult male and female rats have been reported (Pankaj and Anand, 2000). Some earlier studies have found *M. oleifera* seed to be nontoxic and recommended its use as a coagulant in developing countries (Olsen, 1987). Oral test, acute and chronic toxicity tests on rats with both *Moringa stenopetala* and *M. oleifera* seeds (dosages 50 and 500 mg/kg body weight) have been reported to produce no toxic effects, but, rather increased the weights of the rats (Sattaur, 1983; Jahn, 1988). In contrast, Oluduro and Aderiye (2009) reported significant increases in tissue enzymes and marked aggregation of bile canaliculi when 1to10 mg/ml of the seed extract was used. Therefore, there remains considerable concern over the safety of *M. oleifera* seed. This study was designed to investigate the haematological and serum biochemical effects of high dosages of extract of *M. oleifera* seed to ascertain the safety or otherwise of orally ingested dosages of the extract on these parameters.

MATERIALS AND METHODS

Preparation of plant extract

The seeds of *M. oleifera* were air dried and pulverized before the commencement of the methanol extraction. The extraction was

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Table 1. Effects of methanol extracts of *M. oleifera* on haematological parameters of rats.

Parameter	Control	400 mg/kg	800 mg/kg	1600 mg/kg
PCV	37.5±5.3	33.2±6.2 p=0.1128	35.8±2.6 p=0.2483	35.2±3.8 p=0.2039
Hb (g/L)	12.4±1.8	10.9±2.1	11.8±0.9	11.5±1.0
RBC (x10^{12}/L)	12.7±3.5	10.9±4.0	11.7±4.4	9.9±2.0
MCV (fl)	35.2±2.9	31.8±7.2	32.5±7.6	35.7±3.3
MCH (pg)	11.3±1.0	10.2±2.1	10.3±2.7	10.9±1.3
MCHC (%)	36.3±5.4	31.9±3.2	34.2±1.6	32.4±1.8
WBC (x10^{9}/L)	9.68±1.3	7.14±0.1[b]	7.09±0.2[b]	7.05±0.3[b]
Lymphocytes (%)	59.3±1.5	69.8±1.3[a]	69.8±1.6[a]	57.2±0.7[b]
Neutrophils (%)	16.0±5.8	17.1±8.8	10.1±2.2[b]	10.6±3.2*
Monocytes (%)	0.4±0.1	0.7±0.2	0.2±0.1[b]	0.2 ±0.1[b]
Platelet (µL)	10.3±1.9	8.7±2.1	8.7±0.1b	8.5±1.9

p:[a]<:0.001 compared to control; *p*:[b]<0.05.

carried out as described by Njar et al. (1993) and Raji (1995). The pulverized seed weighing 345 g was exhaustively extracted with distillated methanol by means of cold extraction and extract evaporated *invacuo*. The seed extract of *M. oleifera* was concentrated *invacuo* using a rotary evaporator at 40°C. The solvent (distillated methanol) remaining in the extract was finally removed by placing the seed extract in porcelain dishes in temperature-controlled oven to give a residue weighing 15.4 g (a yield of 4.5%). The resulting extract was reconstituted in 15.4 mls of distilled water to give a final concentration of 1000 mg/ml.

Experimental animals

Twenty four healthy white Wistar strain albino rats (160 to 200 g) of both sexes, obtained from the Animal House, Faculty of Veterinary Medicine, University of Ibadan, were used for the study. The rats were fed with rat cubes (Ladokun feeds limited, Ibadan, Nigeria) and water *ad libitum*. Rat was chosen as the experimental animal for the study because toxic substances readily produce demonstrable effects in rats (Farris and Griffith, 1949). Following a one-week period of acclimation to laboratory conditions, the rats were randomly divided into four groups (control group 1, 2, and 3). A varied dosage of the seed extract at 400, 800 and 1600 mg/kg, dissolved in water was administered orally to rats in groups 1, 2 and 3, respectively. The control rats were ingested with distilled water.

Haematology and serum biochemical studies

At the end of the 21 days of treatment, each rat was bled through the orbital sinus into heparinised bottles for haematological studies and blood samples collected in clean non-heparinised bottles were allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis. Packed cell volume (PCV) and haemoglobin concentration were determined by the microhematocrit and cyanmethaemoglobin methods, respectively, as described by Jain (1986). Erythrocyte count was determined by the haematocytometry method as described by Jain (1986). Total white blood cell (WBC) counts were made in a haemocytometer using the WBC diluting fluid and differential leucocytes counts were made by counting the different types of WBC from giemsa stained slides viewed from each of the 30 fields of oil immersion objective of a microscope (Coles, 1989). Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined from the values obtained from red blood cells (RBC) count, haemoglobin concentration and PCV values (Duncan et al., 1994). From the serum, total protein was measured using biuret reaction, while albumin was measured by colorimetric estimation using sigma diagnostic reagent (Sigma Diagnostic, UK.), which contained bromocresol green (BCG). Globulin was obtained from difference total protein and albumn. AST and ALT were determined using a photoelectric colorimeter as described by Duncan et al. (1994), serum urea and creatinine levels were also determined using photoelectric colorimeter as described by Coles (1989).

Histopathology

All the animals from each of the experimental groups 1, 2, 3 and the control were sacrificed 24 h after their respective daily doses. The rats were thereafter quickly dissected to remove the liver and kidney and then transferred into 10% buffered formalin. The organs were dehydrated in ethanol (70 to 100%), cleared in xylene and embedded in paraffin. Tissue sections were examined under a light microscope after staining with haematoxylin and eosin (H and E) (Culling, 1963; Lillie, 1965).

Statistical analysis

All the values were expressed as mean ± S.D. statistical analysis was carried out by using PRISM software package (version 5.0). Statistical significance was assessed by the student t-test and values of probability less than 5% was considered statistically significant.

RESULTS

Effects of extract on haematological parameters of rats

The result of the effects of the methanol extract of *M. oleifera* seed on haematological parameters is presented in Table 1. The extract at the dose of 400, 800 and 1600

Table 2. Effects of methanol extract of *M. oleifera* seed extract on serum biochemical parameters.

Parameter	Control	400 mg/kg	800 mg/kg	1600 mg/kg
Total protein (g/dl)	4.5±0.9	3.8±0.5	4.1±0.4	3.8±0.6
Albumin(g/dl)	1.2±0.1	1.1±0.1	1.0±0.2	1.3±0.1
Globulin (g/dl)	1.9±0.5	2.7±0.4	2.8±0.4	2.6±0.5
AST(U/L)	22.7±1.2	24.3±4.4	25.7±5.6	29.0±7.3[b]
ALT(U/L)	51.4±13.7	57.8±8.3	62.2±9.1	63.2±12.7[b]
Urea (mg/dl)	1.6±0.7	1.3±0.5	1.1±0.2	0.9±0.4[b]
Creatinine (mg/dl)	1.8±0.6	1.7±0.5	1.4±0.6	1.2±0.5[b]

p:[b]<:0.05 compared to control.

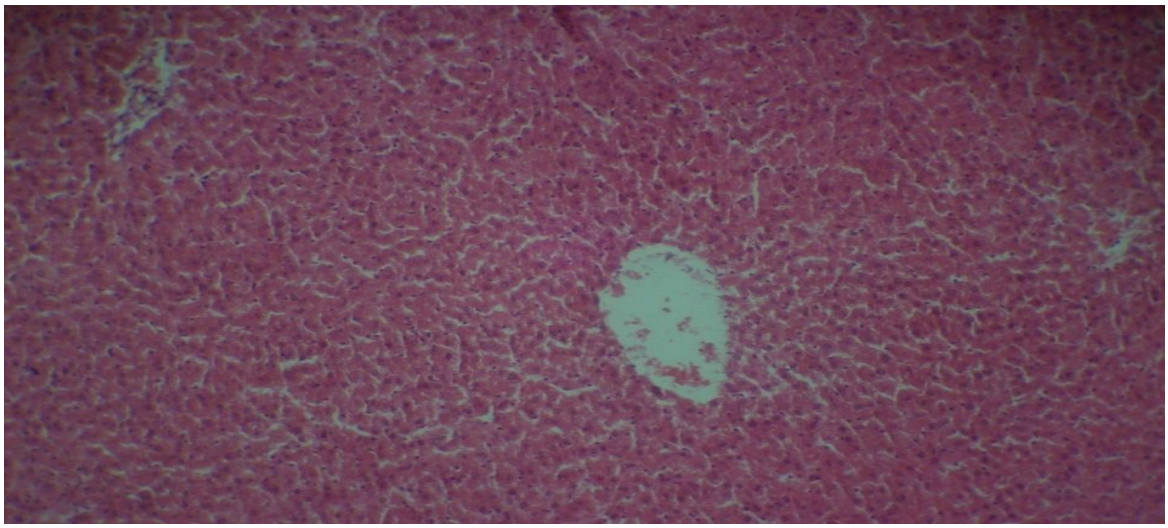

Figure 1. Liver with no visible lesion M x100 H and E.

mg/kg caused significant decrease (*P<0.05*) in total white blood cell and platelet counts. Significant decrease was also observed in the neutrophil and monocyte counts of rats which received 800 and 1600 mg/kg of extract. Decreases found in PCV, haemoglobin (Hb), MCV, MCH and mean corpuscular haemoglobin concentration of the groups administered 400, 800 and 1600 mg/kg were not significantly different from the control group.

Effects of extract on serum biochemical parameters of rats

The result of the effects of *M. oleifera* on serum biochemical parameters is presented in Table 2. There was significant (*P<0.05*) increase in the levels of AST and ALT at the dose of 1.600 mg/kg. There were however no significant (*P>0.05*) changes in the levels of AST and ALT at the doses of 400 and 800 mg/kg. Also, there were significant (*P<0.05*) decreases in levels of urea and creatinine at the doses of 800 and 1.600 mg/kg. The levels of sodium and potassium ions were also significantly (*P<0.05*) decreased at the dose of 1.600 mg/kg.

Histological effects

The result of the histological changes induced by the methanol seed extract of *M. oleifera* is presented in Figures 1 to 7. No visible lesion was observed in the liver (Figure 1) and kidney (Figure 5) of the control group. Mild portal cellular infiltration was observed in the liver (Figure 2) and the kidney showed mild cortical congestion (Figure 6) at the 400 mg/kg. Diffuse sinusoidal congestion and very mild hydropic degeneration was observed in the liver (Figure 3), and the kidney showed mild cortical congestion and interstitial haemorrhages at the cortex (Figure 7) at the 800 mg/kg. Also, severe periportal congestion, portal cellular infiltration and diffuse hydropic degeneration of hepatocytes was seen (Figure 4) at 1.600 mg/kg.

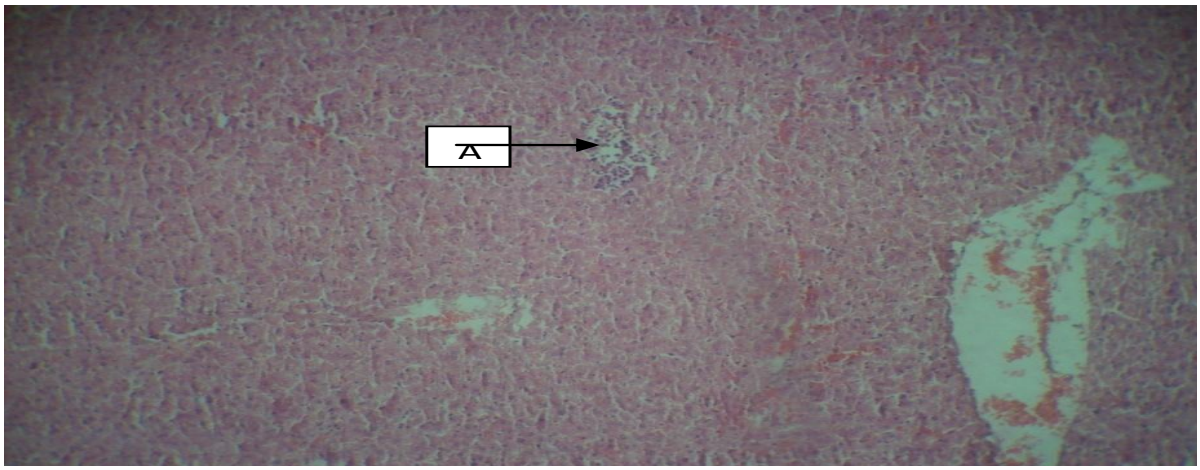

Figure 2. Liver of the 400 mg/kg group showing mild portal cellular infiltration (arrowed). M x 100, H and E.

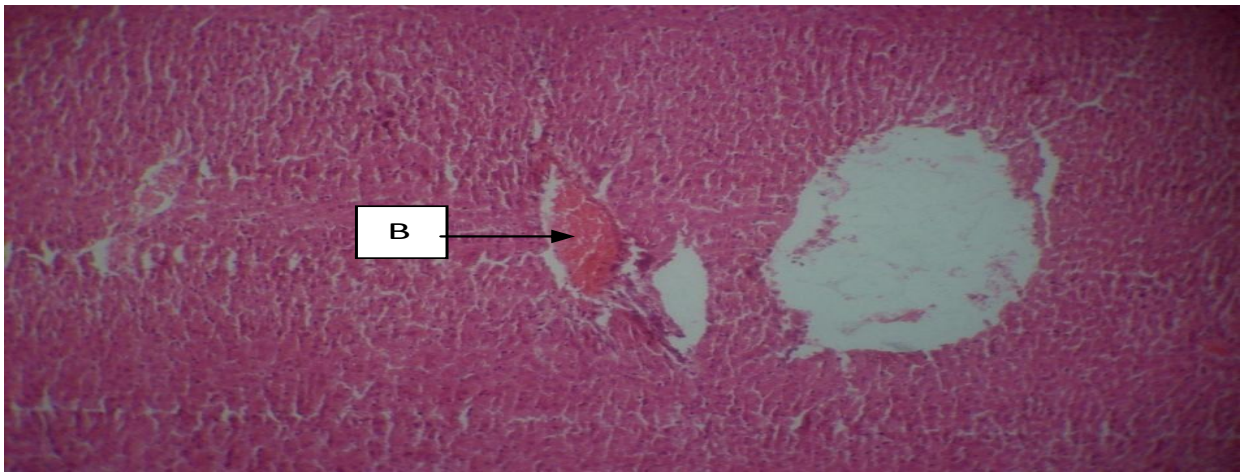

Figure 3. Liver of 800 mg/kg group showing periportal congestion (B) and diffuse hepatic degeneration M x100, H and E.

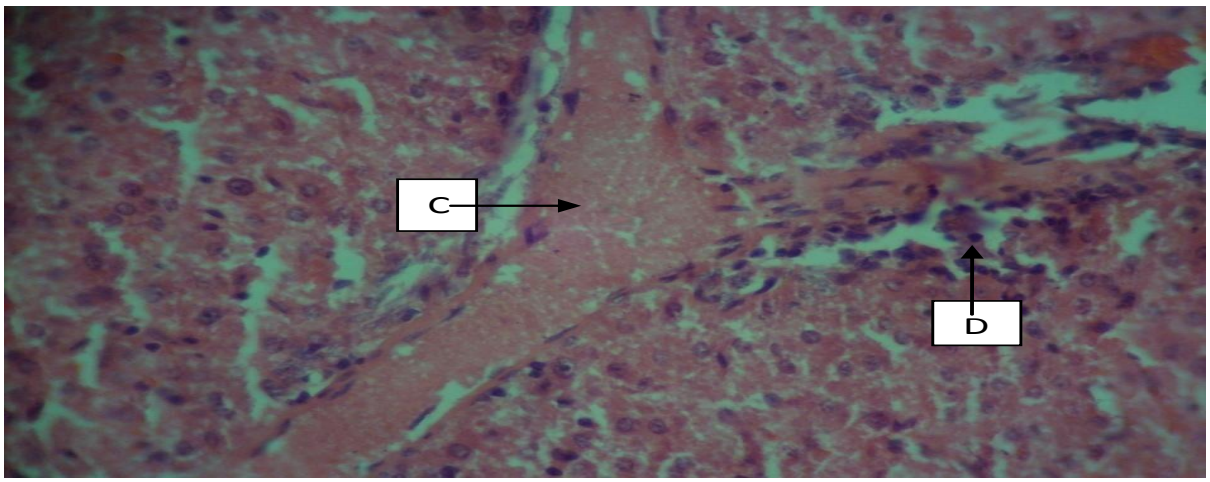

Figure 4. Liver of the 1600 mg/kg group showing severe periportal congestion; **C**, portal cellular infiltration; **D** and diffuse hydropic degeneration of hepatocytes. M x 400, H and E.

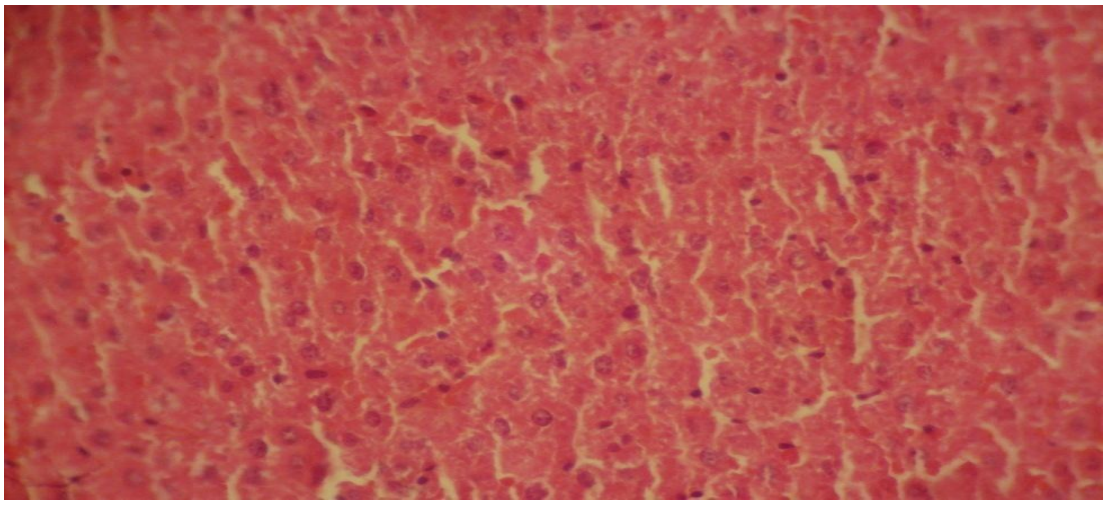

Figure 5. Kidney of the control group with no visible lesion. M x 100 H and E.

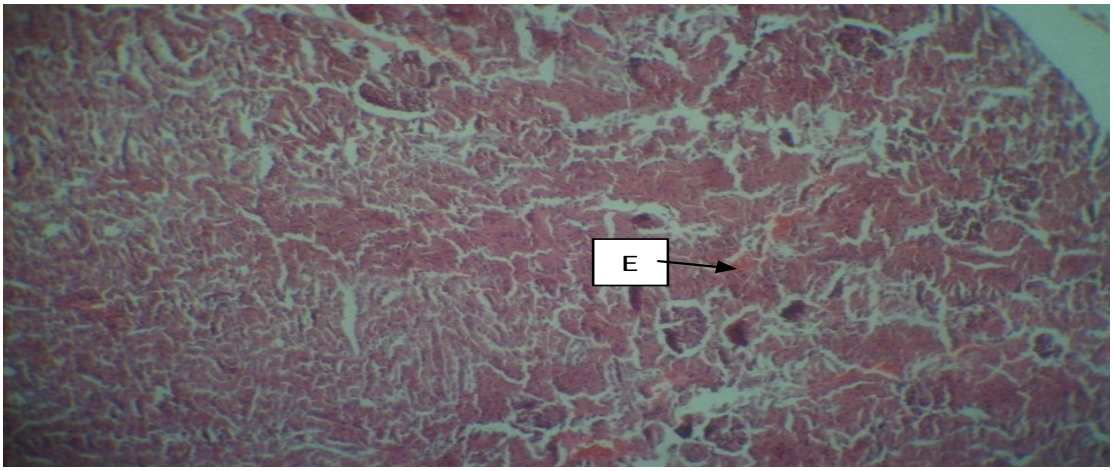

Figure 6. Kidney of the 400 mg/kg b.w. group showing mild renal cortical congestion; **E** M x 100, H and E.

DISCUSSION

The present study shows that *M. oleifera* seed extract did not induce obvious toxic changes in the RBC, Hb, PCV hematocrit and MCHC of rats. The absence of significant changes on these indices may suggest that the extract does not possess toxic substances that can cause an anemic condition in rats. This observation is in agreement with the report of Jahn (1988) who also did not observed any toxic effect of *M. oleifera* in Wistar rats. The significant decrease in the total WBCs, neutrophils and monocytes at 800 and 1600 mg/kg observed in this study contradicts the report of Swenson and Reece (1993), who reported that toxic plants do not produce a direct effect on WBC and its functional indices. The decreased total WBCs and neutrophil counts might have resulted from the suppression of leucopoiesis in the bone marrow and according to Afolayan and Yakubu (2009) may have consequential effects on the immune system and phagocytic activity of the blood cells of the animals. The reductions observed in the platelet count may impair the repair of minute breaks in capillaries and other small vessels (Guyton and Hall, 2006). Therefore, continued administration of the extract at high doses may result in widespread hemorrhages as seen at histology (Figure 7) due to coagulation deficiency because platelets play a crucial role in reducing blood loss and repairing vascular injury (Adedapo et al., 2007; Dahlback, 2008).

Unlike the leaves of *M. oleifera* which have been reported to be hepatoprotective (Gaundar and Uma, 2007), the seeds may be hepatotoxic at very high doses. The increase in the level of ALT and AST (enzymes which are normally present at low levels in the blood) may be due to a leak of the enzymes from damaged

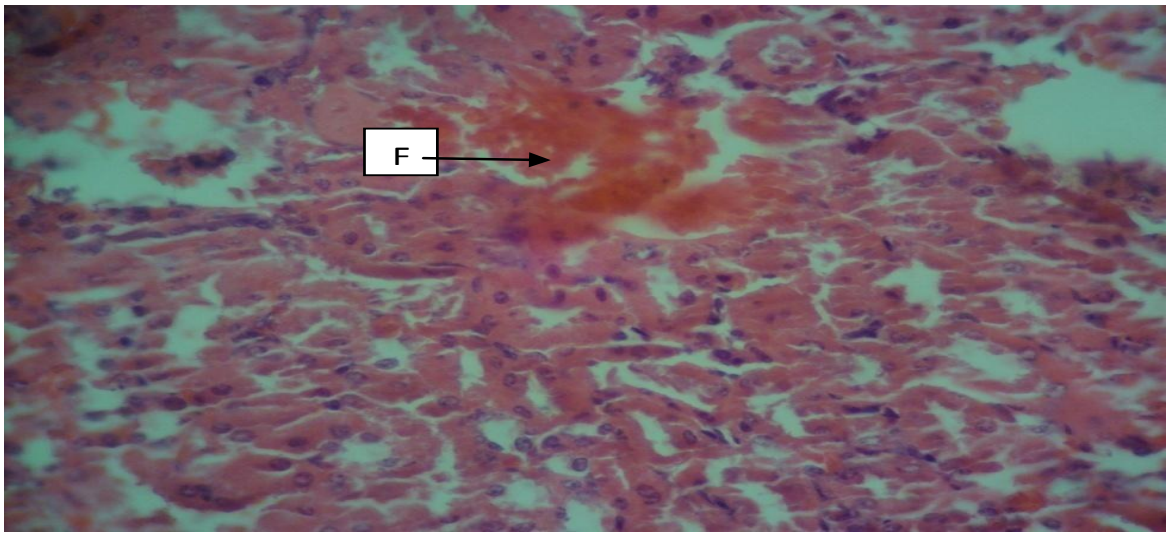

Figure 7. Kidney of the 800 mg/kg b.w. group showing cortical congestion. M x 400, H and E.

hepatocyte into the systemic circulation. This may have accounted for the diffuse hepatic degeneration observed with the 1600 mg/kg body weight dose at histology. Serum ALT is also known to increase in liver disease and it has been used as a tool for measuring hepatic necrosis (Bush, 1991). The administration of *M. oleifera* seed extract appears to be relatively non-toxic to animals at low dosages. This is because there was no apparent damage to the physiology and biochemistry of the blood of rats in this study. However, at high dosages, the alterations observed on rat leucocytes, platelets, AST, ALT, urea and creatinine suggest dose selective toxicity of *M. oleifera* seed extract when repeatedly consumed on a daily basis for a prolonged time.

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