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Abubakar Amali Muhammad
Department of Pharmacology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria

Dallatu Muhammad Kabir
Department of Chemical Pathology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto, Nigeria

Asmau Darma Lawal
Department of Chemical Pathology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto, Nigeria

Correspondence
Abubakar Amali Muhammad
Department of Pharmacology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria

Comparative study of the effect of different extracts of *M. Oleifera* on some biochemical and histological parameters in rats

Abubakar Amali Muhammad, Dallatu Muhammad Kabir and Asmau Darma Lawal

Abstract
Moringa oleifera is a plant whose therapeutic significance has been reported scientifically. However, relatively few studies elucidate the safety of different extraction solvents used for the extraction of the plant bio actives. This study investigated the effect of administration of different solvent extracts of *M. oleifera* on liver and kidney functions of rats. Thirty six adult male rats were used and divided into four groups of nine rats each. Group 1 served as control. Different solvent extracts (aqueous, ethanol, and methanol) were administered to study groups (2, 3 and 4) at different doses of 100, 200 and 400 mg/kg respectively. Biochemical parameters such as total protein, albumin, total and conjugated bilirubin, AST, ALT, urea and creatinine were determined after 3 days (acute) and 28 days (sub-chronic) using standard techniques. Histology of liver and kidney tissues was examined 28 days after administration of the extracts at the highest dose of 400mg/kg. The results showed treatment-related abnormalities in most of the biochemical parameters of methanolic and ethanolic extracts of *M. oleifera*. However, aqueous extracts did not show any abnormality on the biochemical parameters. Methanolic and ethanolic extracts of *M. oleifera* significantly increased ($p<0.05$) the activity of aspartate amino transferase, alanine aminotransferase, total bilirubin, urea and creatinine. It also showed significant decrease ($p<0.05$) in the levels of total protein and albumin. The significant increase and decreases were however, dose dependent. This was supported by the result of histological evaluation of liver and kidney which did not show any abnormal feature following administration of aqueous extract of *M. oleifera*. The present study demonstrated some level of safety in the use of aqueous extract of *M. oleifera* when compared to methanolic and ethanolic extracts that revealed some form of abnormality in the kidney and liver tissues following 28 days of administration. This suggests that, care should be taken in the use of these extracts for their counterproductive consequences resulting possibly from chronic administration of the extract.

Keywords: comparative, *M. Oleifera*, histological parameters, rats

Introduction
Moringa oleifera (Moringaceae) is a valuable plant, distributed in many tropical and subtropical countries. It has variety of medicinal uses. Different parts of this plant contain minerals, proteins, vitamins, B – carotene, amino acids and various phenolics (Bukar *et al*., 2010) [3].

Pharmacologically, *M. oleifera* has been reported to be useful in the treatment and prevention of disease or infection either by using dietary or tropical administration of *Moringa* preparations such as (Extracts, decoction, poultices, creams, oils, emollients, salves, powders and porridges) (Palada, 1996) [14]. *Moringa oleifera* leaves has been reported to possess antimicrobial action (Prashith *et al*., 2010) [16] anti-inflammatory properties (Mahajan, *et al.*,2007) [11], antidiabetic (Jaiswal *et al*., 2009; Edoga, *et al*., 2013) [9, 7], antioxidant (Sultana *et al.*, 2009) [19], and anticancer properties (Parvathy and Umamaheshwari, 2007; Sreelatha, *et al*., 2011) [15, 18] and recently, it has been reported to possess wound healing properties both *in vitro* and *in vivo*.

However, this study was undertaken to compare the toxicological effects of different extraction solvent of *Moringa oleifera* leaves on histological appearance of the vital organs and some biochemical parameters.

Materials and Methods

Collection of Plant material

Fresh leaves of *M. oleifera* were purchased from Kara Market in Sokoto town of Sokoto State, Nigeria. The leaves were air dried for about one week away from direct sunlight to avoid possible damage to their phyto-constituents. The leaves were grinded in to fine powder and constant weight was obtained and stored appropriately until needed for extraction.

Extraction of Plant material

The extraction was carried out by maceration process in which 500g each of the dried leaves powder were soaked in 1000 ml of 70% ethanol. 80% methanol and distilled water using separate 5 litres volumetric flask. The mixture was shaken for 30minutes and then allowed to stay at room temperature overnight. The filtrate was decanted in a separate container. This process was repeated for 3 consecutive days to ensure complete extraction. The mixtures were first filtered with cheese cloth, then with What Man No 1 filter paper. The filtrates were separately concentrated in vacuum using Rotary Evaporator (Model EYELA SB- 1100, China) to 10% of its original volume at 60ºC. This was concentrated to complete dryness in water bath at 45°C in order to obtain the crude extract and the dried extact was stored at 4°C until needed for further use.

Grouping and treatment of animals

Thirty adult male Wistar rats weighing (200-300g) were obtained from the animal house of the Faculty of Pharmaceutical Sciences, Usman Danfodiyo University Sokoto. Nigeria and were allowed to acclimatize for a period of fourteen days in well ventilated room with a temperature and relative humidity of 29±2ºc. They were maintained with commercial rat chow (Vital Feeds LMT, Jos, Plateau, Nigeria) and water and libitum. The animals were housed in a cage and were exposed to 12 hour light-dark cycle and handled according to NIH standard protocol. At the end of the acclimatization period, they were divided into four groups of nine rats per group. Group 1 served as control and were treated with distilled water of treatment equivalence, group 2, 3 and 4, were treated with aqueous, ethanol, and methanol of the crude extract of *M. oleifera* respectively at different doses. The administration of the extract lasted for twenty eight days period.

Group 2, were administered with aqueous extract of *M. oleifera* leaves at different dose of 100 mg/kg, 200 mg/kg and 400 mg/kg. Group 3 were administered with ethanolic extract of *M. oleifera* leaves at different doses of 100mg/kg, 200mg/kg and 400mg/kg. Group 4 were also treated with methanolic extract of *M. oleifera* leaves at the same dosage range as above intra gastric gavage using oral cannula (a feeding needle).

Collection of blood

Three days after administration of the extracts, the animals were anaesthetized using chloroform, and the blood samples were collected from the animals through cardiac puncture, into clean, dry plain containers. The serum of each sample was analysed for biochemical parameters. The procedure was repeated after 28 days administration of the extracts. The blood collected was allowed to clot and then centrifuged at 5000 rpm for 10 minutes. The serum of each sample was used for the assay of aspartate amino transferase (AST), alanine anpmino transferase (ALT), total protein (TP), albumin (ALB), globulin (GLOB), urea and creatinine (Cr).

Histopathological Examinations

The Liver and Kidney of the rats were fixed and processed carefully Sections of 5-6µm in thickness were cut and made onto slides. These tissue sections were then stained using Haematoxyline and Eosin method for photo-microscopic examination of general tissue structures as reported by Orchad and Nation (2012) [10].

Statistical analysis

The data obtained from this study was analyzed using the statistical package for social science (SPSS) for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA). The data was represented as mean ± standard deviation (S.D). ANOVA was used to evaluate the significance of the difference between the mean values of the measured parameters in the respective test and control groups. A mean difference was considered significant when $p < 0.05$.

Results

The results of biochemical analysis of the liver enzymes showing a slight increase in serum levels of alanine amino transferase (ALT) and aspartate amino transferase (AST) following 3 days administration of different solvent extracts of *M. oleifera* at different doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with the control. (Figure 4.1).

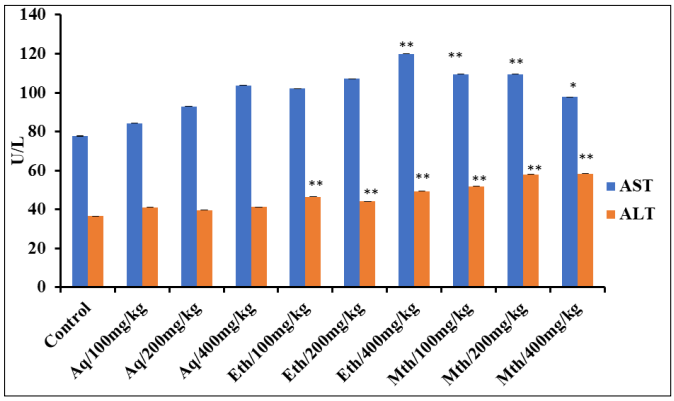

Fig 4.1: Effect of different extracts of *M. oleifera* on AST and ALT, following 3 days administration at different doses. **Significantly different from control (P<0.05)

The results of biochemical analysis of the liver enzymes showing a significant increase in serum levels of alanine amino transferase (ALT) and aspartate amino transferase (AST) following 28 days administration of different solvent extracts of *M. oleifera* at different doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with the control. (Figure 4.2).

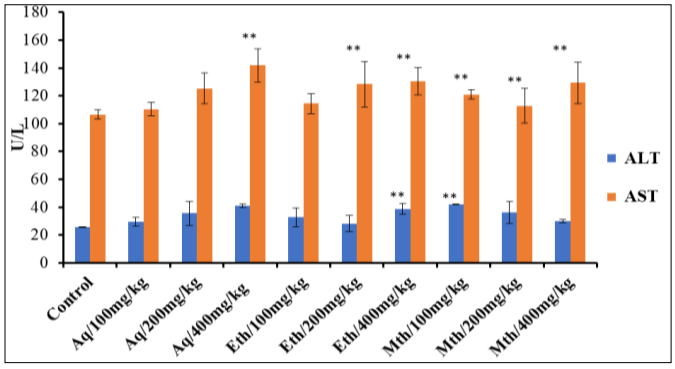

Fig 4.2: Effect of different extracts of *M. oleifera* on AST and ALT following 28 days after administration at different doses. **Significantly different from control (P<0.05)

The result of the biochemical analysis showing a slight increase in serum levels of total bilirubin (TB) and direct bilirubin (DB) following 3 days of administration of aqueous extracts of *M. oleifera* at different doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with their controls (Figure 4.3).

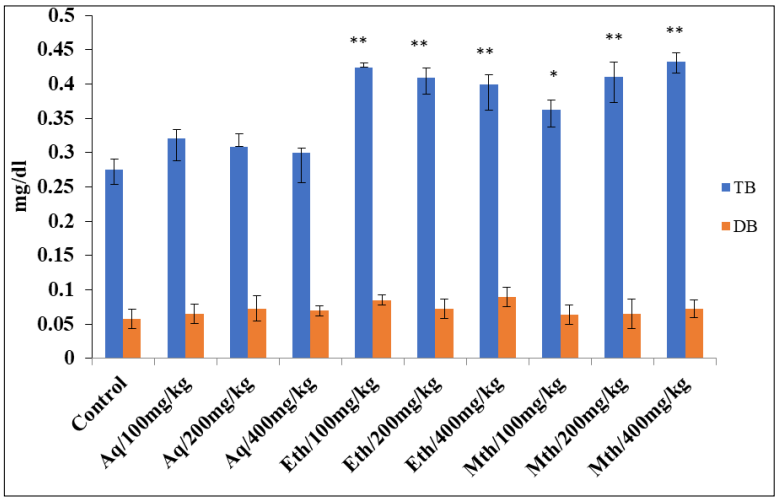

Fig 4.3: Effect of different extracts of M. oleifera on total and direct bilirubin 3 days after administration at different doses. **Significantly different from control (P<0.05)

The result of the biochemical analysis showing a slight increase in serum levels of total bilirubin (TB) and direct bilirubin (DB) following 28 days of administration of aqueous extracts of *M. oleifera* at different doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with their controls (Figure 4.4)

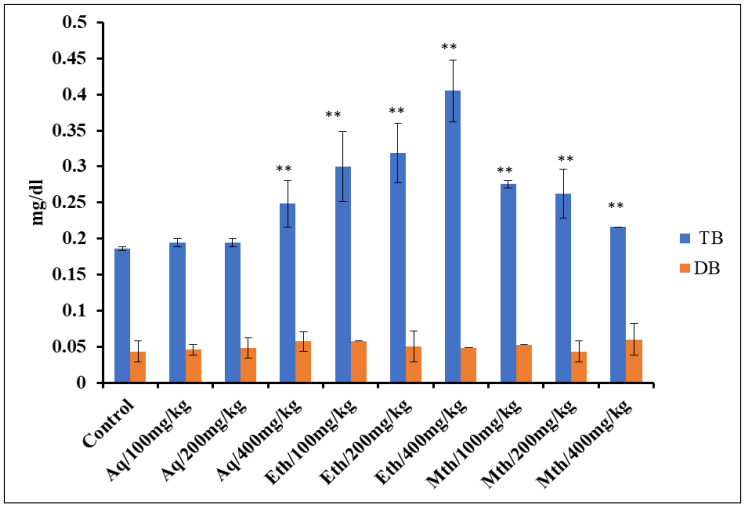

Fig 4.4: Effect of different extracts of M. oleifera on total and direct bilirubin 28 days after administration of different doses. **Significantly different from control (P<0.05)

The result of the biochemical analysis showing a slight increase in serum levels of total protein, Albumin and Globulin) following 3 days administration of aqueous extracts of *M. oleifera* at different doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with their controls (Figure 4.5).

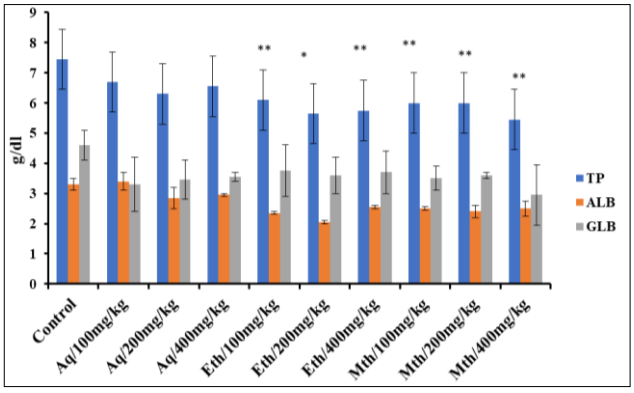

Fig 4.5: Effect of different extracts of *M. oleifera* on total protein, Albumin and Globulin 3 days following administration at different doses. **Significantly different from extract administered groups (P< 0.05)

The result of the biochemical analysis showing a slight increase in serum levels of total protein, Albumin and Globulin) following 28 days administration of aqueous extracts of *M. oleifera* at different doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with their controls (Figure 4.6).

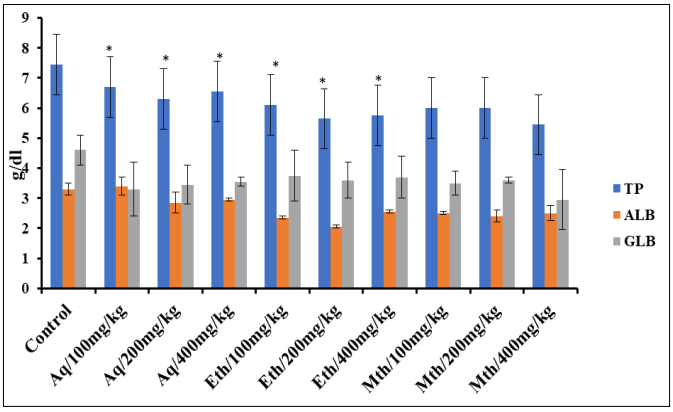

Fig 4.6: Showing the effect of different solvent extract of M. oleifera on total protein, Albumin and Globulin 28 days after administration at different doses. **Significantly different from extract administered groups (P<0.05)

The result of the biochemical analysis showing a slight increase in serum levels of Urea and Creatinine following 3 days administration of aqueous extracts of *M. oleifera* at different doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with their controls (Figure 4.7).

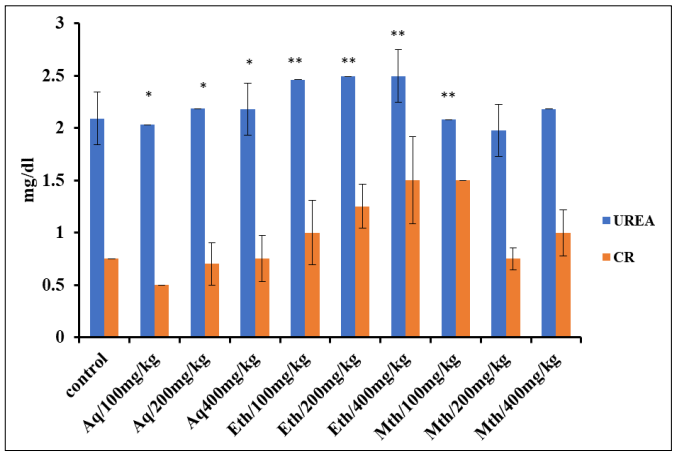

Fig 4.7: Effect of different solvent extract of *M. oleifera* on Urea and Creatinine 3 days after administration at different doses. **Significantly different from control (P<0.05)

The result of the biochemical analysis showing a slight increase in serum levels of Urea and Creatinine following 28 days administration of aqueous extracts of *M. oleifera* at different doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with their controls (Figure 4.8).

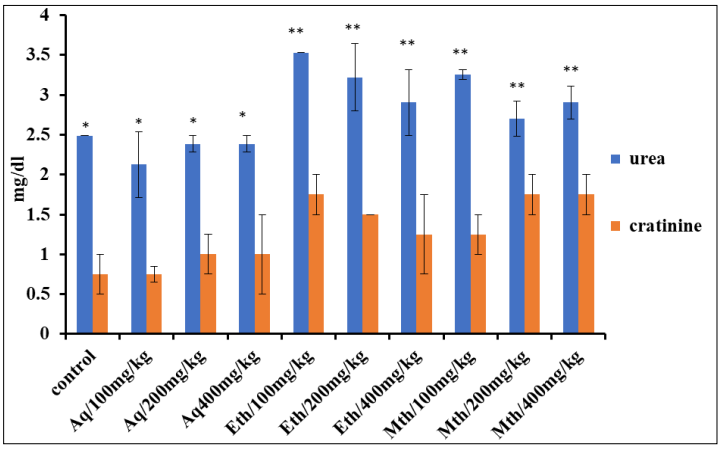

Fig 4.8: Effect of different extracts of *M. oleifera* on Urea and Creatinine 28 days after administration at different doses. **Significantly different from control (P<0.05)

The result of histopathological examinations of the liver sections under the light microscope revealed normal hepatocellular tissue, in normal control group. (Figure 4.9).

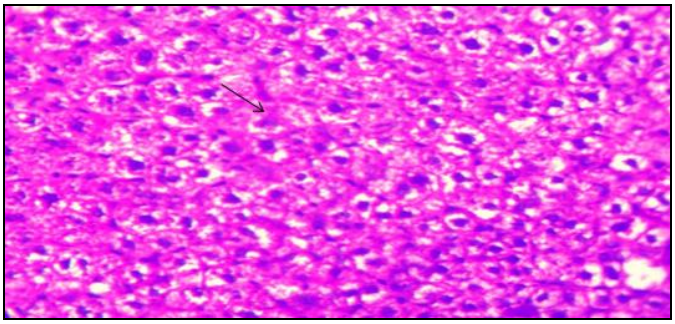

Fig 4.9: Group 1 (Control) Showing normal liver cells (M x 640, H and E)

The result of histopathological examinations of the Kidney sections under the light microscope revealed normal kidney tissue, in normal control group. (Figure 4.9).

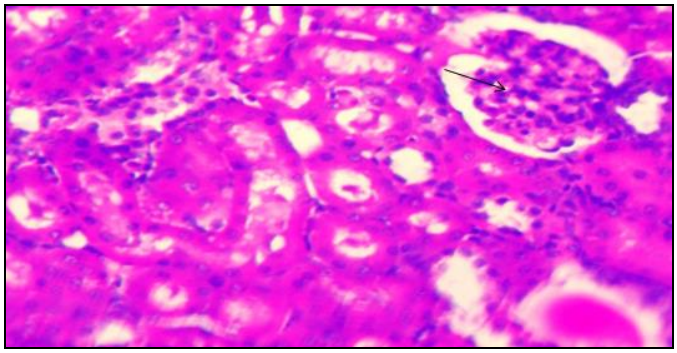

Fig 4.10: Group 1 (Control) Showing normal kidney tissue (M x 640, H and E)

The result of histopathological examinations of the liver

sections under the light microscope revealed normal hepatocellular tissue, 28 days after administration of aqueous extract of *M. oleifera* at 400mg/kg body weight/ day as compared with the control group. (Figure 4.11).

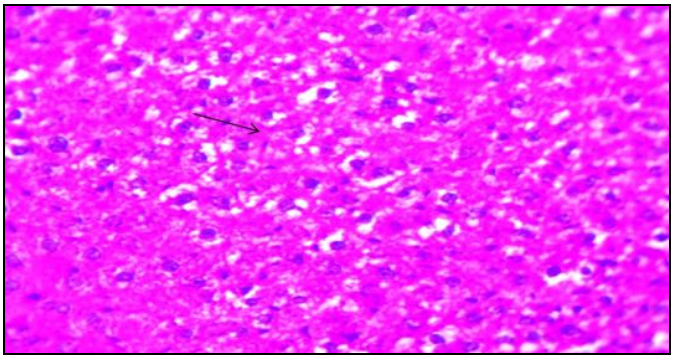

Fig 4.11: showing normal hepatocellular tissue, 28 days after administration of aqueous extract of *M. oleifera* at 400mg/kg (M x 640, H and E)

The result of histopathological examinations of the liver sections under the light microscope revealed normal kidney tissue, 28 days after administration of aqueous extract of *M. oleifera* at 400mg/kg body weight/ day as compared with the control group. (Figure 4.12).

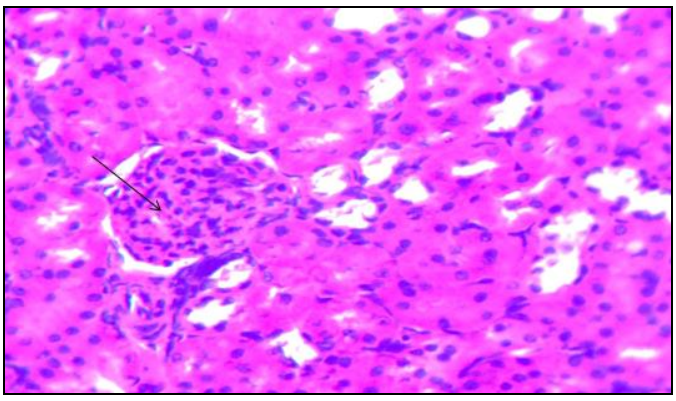

Fig 4.12: showing normal kidney tissue, 28 days after administration of aqueous extract of *M. oleifera* at 400mg/kg (M x 640, H and E)

The result of histopathological examinations of the liver sections under the light microscope revealed cytoplasmic degeneration of hepatic cells, 28 days after administration of aqueous extract of *M. oleifera* at 400mg/kg body weight/ day as compared with the control group. (Figure 4.13).

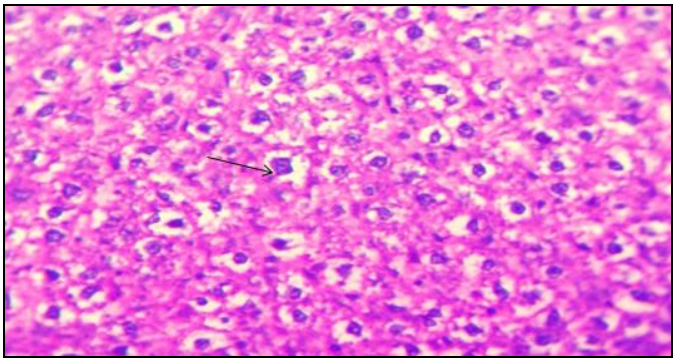

Fig 4.13: showing preserved hepatocellular architecture with cytoplasmic degeneration of hepatic cells hypochromic 28 days after administration of ethanolic extract of *M. oleifera* at 400mg/kg (M x 640, H and E)

The result of histopathological examinations of the liver sections under the light microscope revealed kidney tissue with glomerular inflammation 28 days after administration of aqueous extract of *M. oleifera* at 400mg/kg body weight/ day as compared with the control group. (Figure 4.14).

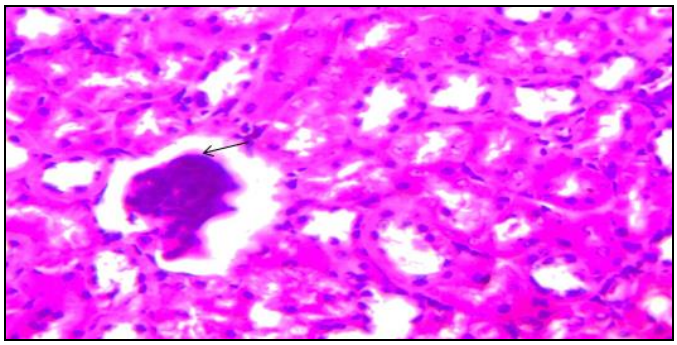

Fig 4.14: showing kidney tissue with glomerular inflammation 28 days after administration of ethanolic extract of *M. oleifera* at 400mg/kg (M x 640, H and E)

The result of histopathological examinations of the liver sections under the light microscope revealed hepatocellular tissue with evidence of intrahepatic hemorrhage following 28 days administration of aqueous extract of *M. oleifera* at 400mg/kg body weight/ day as compared with the control group. (Figure 4.15)

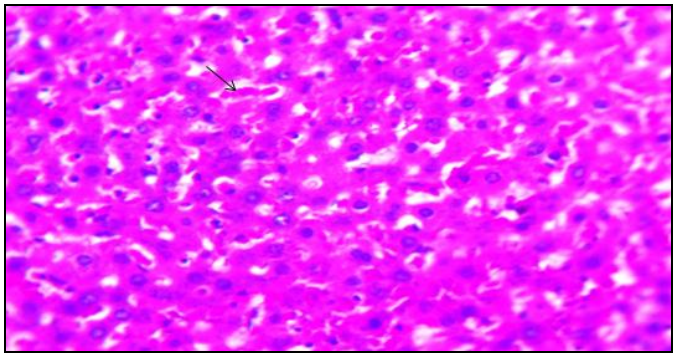

Fig 4.15: showing hepatocellular tissue with evidence of intrahepatic hemorrhage 28 days after administration of ethanolic extract of *M. oleifera* at 400mg/kg (M x 640, H and E)

The result of histopathological examinations of the liver sections under the light microscope revealed mild glomerular congestion 28 days after administration of aqueous extract of *M. oleifera* at 400mg/kg body weight/ day as compared with the control group. (Figure 4.16).

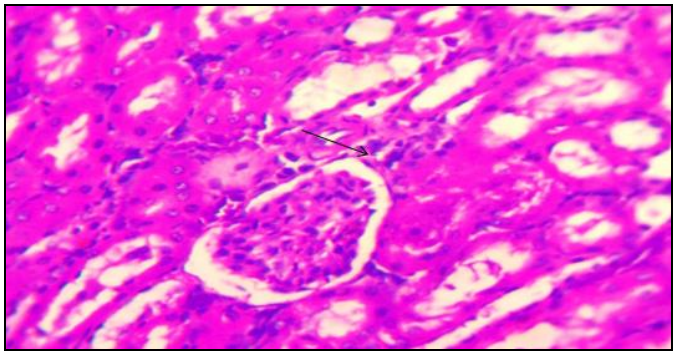

Fig 4.16: showing mild glomerular congestion 28 days after administration of methanolic extract of *M. oleifera* at 400mg/kg (M x 640, H and E)

Discussion

The result of the present study revealed a slight increase in serum levels of alanine amino transferase (ALT), aspartate amino transferase (AST), Total bilirubin (TB) and direct bilirubin (DB) after 3 and 28 days of administration of aqueous extracts of *M. oleifera* at different doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with their controls. This result is in contrast with the study of Adedapo *et al.*, 2009 in which they showed significant increase in the activity of liver enzymes AST, ALT when ethanolic and aqueous leaves extract of *M. oleifera* was administered to rats at the dose of 1.6g/kg. This contrast may be due to variation in the dosage of the extracts administered.

However, following 3 days and 28 days administration of aqueous extract of *M. oleifera*, there was no significant increase or decrease ($p>0.05$) in total protein, albumin, globulins, urea and creatinine at the doses of 100mg/kg, 200mg/kg and 400mg/kg when compared with the control this signifies that, aqueous extract of *M. oleifera* leaves did not interfere with the synthetic functions of the liver. The slight decrease in serum urea and creatinine signifies that it is non-toxic to the kidney as reported earlier by Abdulazeez *et al.*, 2010. This finding from biochemical analysis also supports the result of histology conducted on the liver and kidney which showed no abnormal feature in these organs following administration of aqueous extract of *M. oleifera* at the dose of 400mg/kg body weights of rats.

Administrations of ethanolic extract of *M. oleifera* significantly caused increase ($p<0.05$) in serum levels of ALT, AST and Total Bilirubin after 3 and 28 days at the doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with their controls. These increases may be as a result of cellular leakage and loss of functionality of membrane integrity as reported by Saraswat *et al.*, 1993. These results does not agree with the study of Nwangwu *et al*., 2010 which showed significant reductions in ALT, AST, ALB and T.BIL and increase in TP when 200mg/kg ethanolic extract *Gongronema latifolium* leaves were administered to male rats

However, ethanolic extract of *M. oleifera* also shows a significant reduction ($p<0.05$) in Total protein, albumin and globulin both after 3days and 28day of administration at different doses. The decrease in serum total protein (TP) observed with the ethanolic extract in this study could be as a result of reduced albumin (ALB) which is a marker for diagnosis of liver disease as reported by Benoit *et al*., 2000. This decrease could have resulted from a concomitant decrease in the number of cells responsible for ALB synthesis in the liver which is in agreement with the report of Omotuyi *et al.*, 2008 or a direct interference with the albumin-synthesizing mechanism in the liver as obtainable in mammalian cells or a combination of both.

Creatinine and urea are major catabolic products of creatin and protein metabolism, respectively. Increases in serum urea levels from 28days of administration of the extract can be the effect of nephrotoxicity of *M. oleifera* ethanolic leave extract indicative of impaired kidney function. Afolayan and Yakubu, 2009; Abdulazeez *et al*., 2010 stated that renal failure leads to retention of creatinine and other non-protein nitrogenous constituents of the blood which may be responsible for the increases observed with the ethanolic extract groups in this study.

These increases in serum enzymes level, Creatinine and urea and decrease in the level of protein may be accounted for the changes found in the liver and kidney cells of ethanolic extract of *M. oleifera* at 400mg/kg concentration.

Methanolic extract of *M. Oleifera* significantly increased ($p<0.05$) serum levels ALT, AST and Total Bilirubin after 3 and 28 days of administration of the extracts at the doses of 100mg/kg, 200mg/kg and 400mg/kg as compared with their controls. This is in agreement with the findings of (Abu *et al*., 2013) [2] which showed a significant increase in serum levels ALT, AST and Total Bilirubin when 300mg/kg of methanolic extract of *H. Polyrhizus* fruits was administered to rats. This could be due to leakage of enzyme from the damage hepatocytes into systemic circulation.

A significant reduction ($p<0.05$) in Total protein, albumin and globulin both after 3days and 28day of administration at different doses as above was also seen in methanol extract which could be due to damage of the hepatocytes. The result also revealed significantly increased in serum urea and ceratinine following 3 and 28 days of methanolic extract at different doses when compared with their controls. This happens when kidneys removal cannot match up with the rate of production of this product due to kidney disease, high concentrations are measured. Other factors such as high protein intake, increased protein catabolism, stress and dehydration can be responsible for such increases. This may also be associated with the abnormal features seen in histology of liver and kidney when 400mg/kg of methanolic extract of *M. oleifera* was administered.

Histological analysis of aqueous extract of *M. oleifera* showed no abnormal features in the liver and kidney tissues even at higher dose of 400mg/kg for 28 days. This finding is in accordance with the study of (Adedapo *et al.*, 2009) [4] who observed no abnormal features in histopathology of liver and kidney of rats treated with the dose of 16.1g/kg of aqueous extract of *M. oleifera.*

The ethanolic extract of *M. oleifera* treated rats showed preserved hepatocellular architecture with cytoplasmic degeneration of hepatic cell, hypochromic at the dose of 400mg/ kg after 28 days of administration. However, the kidney tissue showed mild glomerular inflammation at the dose of 400mg/kg after 28days. This is in agreement with the findings of (Josephine *et al.*, 2012) [10]. Which showed congestion with scattered focal necrosis of the liver tissue and kidney tissue with expanded and congested glomeruli after receiving a single oral daily dose of ethanol extract of *M. oleifera for* 30 days.

The methanolic extract of *M. Oleifera* showed normal hepatocellular cells with evidence of intrahepatic haemorrhage. The kidney tissue also showed mild glomerular congestion at the dose of 400mg/kg for 28days. This is in agreement with the findings of Ajibade *et al*., 2011.Which showed mild portal cellular infiltration in the liver cell and kidney tissues showed mild cortical congestion at 400mg/kg of methanol seed extract of *M. oleifera* for 30days.

Conclusion

The ethanolic and methanolic extracts of *M. oleifera* demonstrated nephrotoxic and hepatotoxic potential which was not the case with the aqueous extract. This suggests that, care should be taken in the use of the ethanolic and methanolic extracts of *M. oleifera* for the reasons of its counterproductive consequences resulting possibly from bioaccumulation as a consequence of prolonged administration

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