

Toxicological evaluation of *Moringa oleifera* Lam seeds and leaves in Wistar rats

Ajayi Temitayo Olayemi^{1*}, Moody Jones Olanrewaju¹, Akintayo Christopher Oloruntoba²

¹Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, NIGERIA.

²Department of Physiology, College of Medicine and Health Sciences, AfeBabalola University, Ado-Ekiti, NIGERIA.

ABSTRACT

Miracle tree (*Moringa oleifera*) as it is popularly called, has been found useful both medicinally and economically. Its consumption both in the raw and as processed preparations has increased a great deal thus making the fast growing plant a highly valued and cultivated one in the tropics and sub-tropics. There is however, little reference to its toxicity profile and evaluation. Hence, this study evaluated the toxicity profiles of the leaves and seeds of *M. oleifera* and the corresponding effects on vital organs of Wistar rats using the biochemical, hematological and histopathological indices. Daily doses of 100, 200, 400 and 1000 mg/kg body weight of crude methanol extracts of *M. oleifera* leaves and seeds were administered orally to 8 groups of 5 rats per group each for 28 days. A control group of 5 rats was also included in the experiment. Hematological, biochemical and histopathological indices were evaluated by standard methods. Data were analyzed using one way analysis of variance and statistically significant difference was considered at $p < 0.05$, $p < 0.01$ and $p < 0.001$. Histopathological changes were observed in the heart, liver, lungs, spleen and kidneys of rats treated with the extracts at all doses tested. Some other physical changes like agitation, confusion and disorientation were observed at

the highest dose tested (1000 mg/kg) of the seed extract. A significant increase ($p < 0.05$) in neutrophil, white blood cell (WBC) and platelet were observed. However, a significant decrease in aspartate amino transferase (AST), alanine amino transaminase (ALT), alkaline phosphatase, (ALP) was also observed. The results suggest that the leaf and seed extracts of *M. oleifera* could boost immunity and offer hepatoprotective effects.

Key words: *Moringa oleifera*, Hemolysis, Histopathology, Biochemical, Hematological indices.

Correspondence:

Ajayi Temitayo Olayemi,

Department of Pharmacognosy,
Faculty of Pharmacy, University of Ibadan,
NIGERIA.

Tel: +234(0) 7030308600;

E-mail: tayomiajayi@yahoo.com

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INTRODUCTION

Herbal medicine is one of the oldest forms of treatment for diverse ailments and it has enjoyed a relatively high subscription for obvious reasons like being cost effective, accessible, and it blends with socio-cultural life of the people.¹ WHO has stated that herbal or medicinal plants are the best source to obtain a variety of drugs.² *M. oleifera* popularly known as miracle tree or drumstick tree in Nigeria is highly valued and most cultivated species of the monogenetic family Moringaceae. It is cultivated widely both in the tropics and subtropics.³ The *Moringa* plant has found a great deal of economic, nutritional and medicinal use globally as all its parts contain good sources of proteins, vitamins and minerals and carotenoids.⁴ Ethnobotanical surveys indicate that the root has been used to treat a number of ailments like rheumatism and constipation, while the leaves has been used as purgatives, for treating piles, headache, ear and eye infection.⁵ The gum is used for dental caries, dysentery, asthma, fevers and the seed exerts protective effect by decreasing liver peroxides.⁶ Commercially, it is used in water treatment where crushed seeds are used to clarify turbid and dirty water. The oil can be used as fertilizer and in ointment for skin conditions. It has also found use as fodder for cattle.^{7,8} Medicinally, it is used in the management of hypertension, cholesterol lowering, as diuretics, analgesics, antioxidants and antifungal.^{3,5,9-13} *Moringa* is rich in simple sugars like rhamnose and its bark contain some alkaloids.¹⁴ Several unique compounds such as glucosinolates and isothiocyanates are also components of *M. oleifera* plant.^{10,15} Its phytochemistry also reveal the presence of flavonoids, glycosides, carbohydrates and sterols¹⁶ all of which may contribute to the biocativities attributed to this plant.¹⁷ Previous studies showed no toxicity up to a dose of 5000 mg/kg body weight for the aqueous leaf extract when evaluated for the acute and sub-acute toxicity in male Sprague-Dawley rats.

The possible toxic effects however, may vary and this may be attributable to significant differences which exist between various species of *Moringa* from different locations.¹⁸ Therefore, this present study seeks to assess and compare the sub chronic toxicity profile of the leaf with seed extracts of *Moringa oleifera* by monitoring the histopathological, biochemical and hematological indices aimed at revealing the effect these extracts may have on long term use. The findings in this work will add to the value of this plant nutritionally, medicinally, commercially and ultimately, provide information on its safety profile.

MATERIALS AND METHODS

Plant materials

The leaves and seeds of *M. oleifera* were obtained from Ibadan metropolis, Nigeria in May 2013. The authentication was done in Forest Herbarium Ibadan where a voucher specimen (FHI 110098) was deposited.

Extraction

A mass of 500 g each of the seed and air-dried leaves were blended to produce a coarse powder. Extraction was by cold extraction in 95% methanol for 72 h and concentrated using rotary evaporator to give a 40 g and 62.5 g yield of the seed and leaf methanol extracts respectively.

Experimental animals

Forty-five albino rats of average weight 120-150 g were purchased from the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria and kept under standard environmental conditions (12 h light/12 h dark cycle). They were grouped into two

major groups of twenty rats each for the seed and the leaf extract administration. Sub-groups of five animals were kept in each cage with two groups of five animals per group as controls and they were fed with standard diet and clean water was given *ad libitum*. The animals were allowed to acclimatize for two weeks prior to the experiment. Experimental animals were fasted for 14 h overnight before the experiment and were handled in accordance with the OECD 420 guidelines for testing of chemicals.¹⁹

Treatment protocol

The animals were divided into 8 test groups of 5 rats per group and were administered 100, 200, 400 and 1000 mg/kg body weight of methanol (seed and leaf) extracts of *M. oleifera* daily for 28 days. The control group of 5 rats was also administered distilled water daily.

Blood sample and organ collection

Blood samples were collected into lithium heparinized bottles by capillary tubes from the retro-orbital sinus of the rats. Plasma was obtained by centrifuging the blood at 3000 revolutions/min and stored at -20°C for biochemical assays and the whole blood was used for hematological studies. The organs were collected and placed in 10% formalin to prevent decay.^{8,20}

Biochemical assays

An auto-analyzer (Archem, BM240, Turkey) was used to assay for biochemical parameters which include: aspartate aminotransferase AST;²¹ alanine aminotransferase ALT;²² alkaline phosphatase (ALP),²³ albumin¹⁴ and urea.¹²

Hematological assays

An automated hematology system analyzer (Archem BM240 Turkey) was used to assay for white blood cell count (WBC), red blood cell (RBC), haemoglobin (HB), hematocrit (HCT), mean cell volume (MCV), mean corpuscular neutrophil (NEU).²⁴

Histopathological Examination

The organs were harvested and fixed in normal saline for 72 h and sliced and the tissues were then dehydrated and treated with paraffin wax; sections were then cut on a microtome and were attached to a slide and allowed to dry, stained with hematoxylin-eosin and examined under a light microscope; photomicrographs of the tissues were then recorded.^{19,25}

Statistical Analysis

Data analysis was carried out using one way analysis of variance (ANOVA) followed by Tukeys multiple comparison pair and student t test was used to determine the difference between the control and individual test groups. Results were presented as mean \pm SEM, and the differences were considered significant $p < 0.05$.

RESULTS AND DISCUSSION

Toxicity studies are very important in establishing safety limits for potential drugs and they are also usually used to assess possible health risk posed by plant extracts.²⁶ Our study examined the effect of *M. oleifera* extracts on hematological, biochemical and histopathological indices as specific markers can be used and the evaluation of these indices will establish a safety profile for this plant.

Hematological parameters as shown in Table 1 revealed some changes in its indices. The changes in the effect of these extracts, since this plant has been known to be rich in isothiocyanate, may then be attributable to isothiocyanate producing glycosides.²⁷

A consistent and significant decrease in PCV ($p < 0.05$) was observed in animals treated with 200 mg/kg body weight of the seed extract while

no significant change at other doses including those of the leaf extracts tested was observed. There was a significant decrease in Hb ($p < 0.05$) in animals treated with 1000 mg/kg body weight of the seed extract while there was no significant change in Hb at other doses. The leaf extract, also did not produce any significant decrease in Hb at all the doses tested. The decrease in PCV and Hb indicates that the seed extract at some particular doses can precipitate some degree of anaemia especially if used over a long period of time. The observed increase in neutrophil and white blood cells (WBC) though insignificant, in animals treated with 200 and 1000 mg/kg of the seed extract may be as a result of the ability of the plant to cause some degree of improvement in immunity. This observation supports previous studies where *Moringa oleifera* has been to cause significant increase in white blood cell count.¹⁸

Some biochemical indices are markers of hepatic injury and hepatocellular necrosis.²⁸ In the present study, a significant decrease ($p < 0.05$, $p < 0.01$ and $p < 0.001$) in the liver enzymes, Aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine amino transferase (ALT) in animals treated with both extracts were observed and compared to the control at all doses tested as shown in Figures 1-5 below.

Aspartate aminotransferase

Aminotransferases (ALT and AST) are good markers of liver cell damage though not necessarily indicative of the severity of damage.²⁹ There was a significant decrease of AST levels $p < 0.01$ in the group of rats treated with 100 and 200 mg/kg, $p < 0.001$ and $p < 0.05$ in rats treated with 400 mg/kg and 1000 mg/kg seed extract respectively while only the group treated with 1000 mg/kg leaf extract revealed a significant decrease in AST levels $p < 0.01$. Other doses of the leaf extract tested revealed no significant change in the levels of AST. A decrease of AST observed is an indication that the extracts possess some potential to protect the liver cells. The seed extract at all doses tested can thus be regarded as safe doses at which the extract may be administered.

Alanine aminotransferase

An increased ALT is known to indicate liver disease and has become a tool for measuring hepatic necrosis.³⁰ In this study, the decrease in alanine amino transferase (ALT) levels was significant $p < 0.05$ in the group treated with 200 mg/kg of the seed extract, emphasizing the 200 mg/kg body weight as an effective and safe dose while other doses of both seed and leaf extracts, revealed no significant changes.

Alkaline phosphatase

A significant decrease in the alkaline phosphatase (ALP) levels $p < 0.001$ was observed in rats treated with the seed extract at all doses tested, indicating that the seed extract is safe at all doses tested and effective in lowering the liver enzyme ALP. The occurrence of cholestasis normally associated with increase in serum ALP and which mostly precedes other indicators such as hyperbilirubinemia is thus ruled out. Hence, the seed can be said to be more effective in reducing the liver enzyme ALP when compared with the significant decrease in ALP levels $p < 0.01$ and $p < 0.001$ at 100 mg/kg and 1000 mg/kg body weight respectively which was observed in rats treated with the leaf extract while at other doses of the leaf extract (200 and 400 mg/kg) revealed no significant change.

Blood urea nitrogen

Urea is one of a number of non-protein nitrogenous substances that accumulate in the plasma when renal excretion is reduced. Causes of increased blood urea levels include high protein diet, intestinal hemorrhage, dehydration and shock. Urea level could be decreased as a result of liver failure, low protein diet and presence of anabolic steroids.³⁰

In this study, there was a significant decrease ($p < 0.001$) in blood urea nitrogen levels in animals treated with 100 mg/kg of the seed extract

Table 1: Effects of methanol extract of *Moringa oleifera* seed and leaf on heamatological indices

Parameters	<i>Moringa Seed</i>					<i>Moringa Leaf</i>			
	Control	100 mg/kg	200 mg/kg	400 mg/kg	1000 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	1000 mg/kg
PCV%	53.5±0.71	47.33±5.51	48.00±3.08*	48.33±2.08	46.0±2.83	46.25±5.18	41.80±5.35	44.66±12.74	46.0± 3.00
Hb g/dL	17.5±0.64	15.63±1.85	15.70±1.28	16.30±0.61	5.2±0.71	15.37±1.62	13.78±1.82	14.66±4.41	15.3±1.55
RBC 10 ³ µl	8.3±0.60	7.70±0.64	8.27±0.55	9.95±0.56	7.6±0.09	7.68±0.05	6.96±0.88	7.36±2.42	7.8±0.29
WBC10 ³ µl	6325± 530.33	5600± 507.44	6810± 2016.93	4133.33± 1678.04	6950± 2050.61	5787.5± 766.35	5260± 1386.72	5466.66±2447.62	5600± 507.44
Plat 10 ³ µl	99500± 7778.17	96666.6± 14047.53	110800± 40046.22	68666.66 ± 38070.11	99500± 3905.14	91250± 11644.02	84800± 23910.25	80333.33± 3098.24	116666.6±59315.52
Lymp%	77.0± 2.83	67.25± 14.4	73.60± 4.04	68.33± 26.3	76.5± 0.71	71.66± 15.27	77.60± 7.44	82.00± 5.19	60.0± 24.76
Neut%	18.0± 16.88	23.76± 16.39	22.8± 3.70	28.33± 27.59	21.0± 4.24	24.66± 15.53	17.80± 8.32	13.33± 6.11	37.3± 25.32
Mono%	1.0± 0.00	2.25±2.06	1.60±1.34	2.00±2.00	1.0±1.41	1.33±0.57	1.80±1.30	2.00±1.00	1.0± 1.00
Eosin%	1.50± 0.71	1.75±1.71	2.00±0.71	1.33±1.53	1.5± 2.12	2.30±0.57	2.80±0.84	3.33±1.53	1.7± 0.57

Values are Mean ± SEM (n=5), *p<0.05 as compared to the control.

PCV Packed cell volume
 RBC Red blood cell
 LYMP Lymphocytes
 MONO Monocytes
 Hb Heamoglobin
 WBC White blood cell
 NEUT Neutrophils
 EOSIN Eosinophil

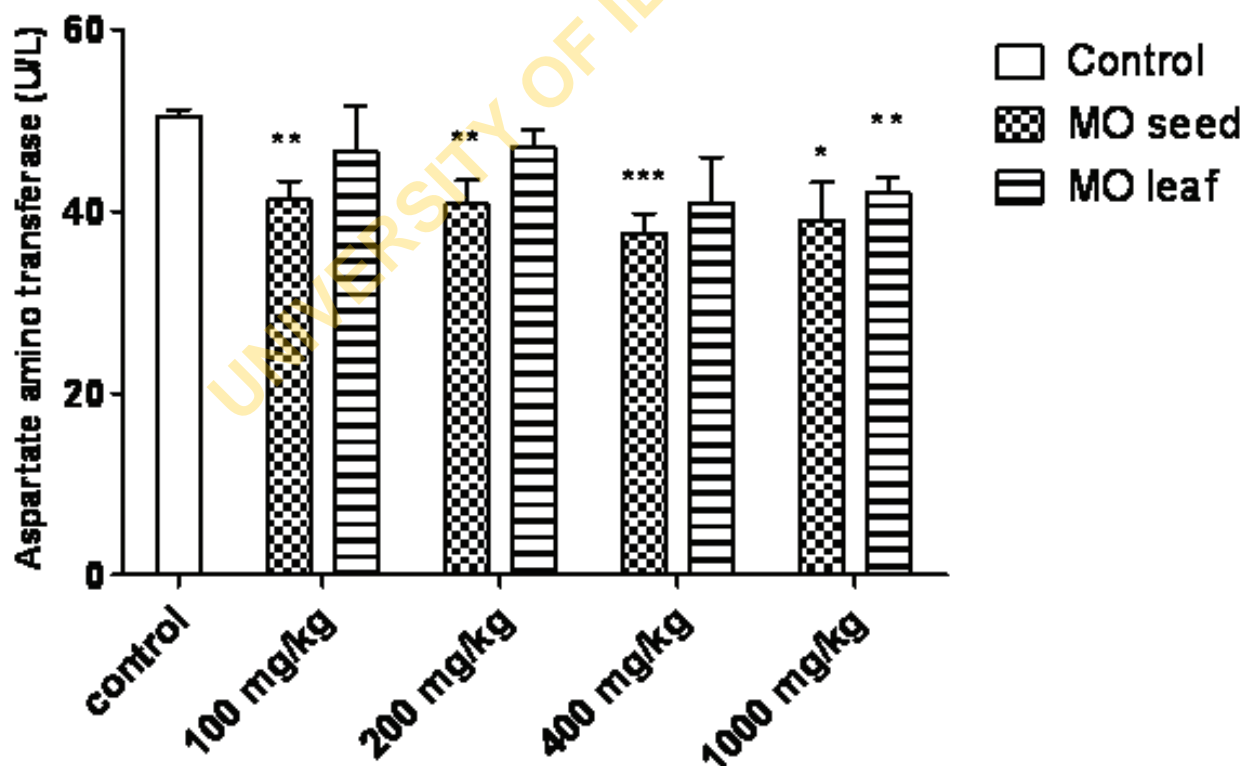


Figure 1: Effects of methanol extract of *Moringa oleifera* seed and leaf on aspartate amino transferase (AST). Values are Mean ± SEM (n=5), *p<0.05, **p<0.01 and ***p<0.001 as compared to the control

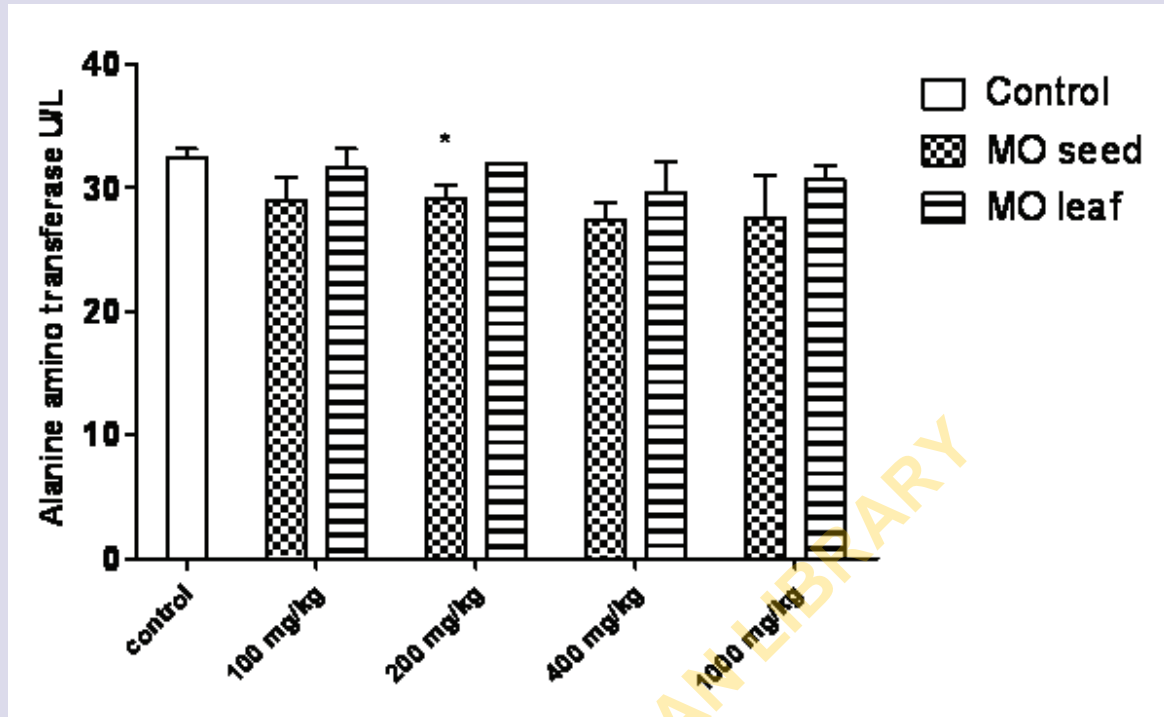


Figure 2: Effects of methanol extract of *Moringa oleifera* seed and leaf on alanine amino transferase (ALT). Values are Mean \pm SEM (n=5), *p<0.05, **p<0.01 and ***p<0.001 as compared to the control

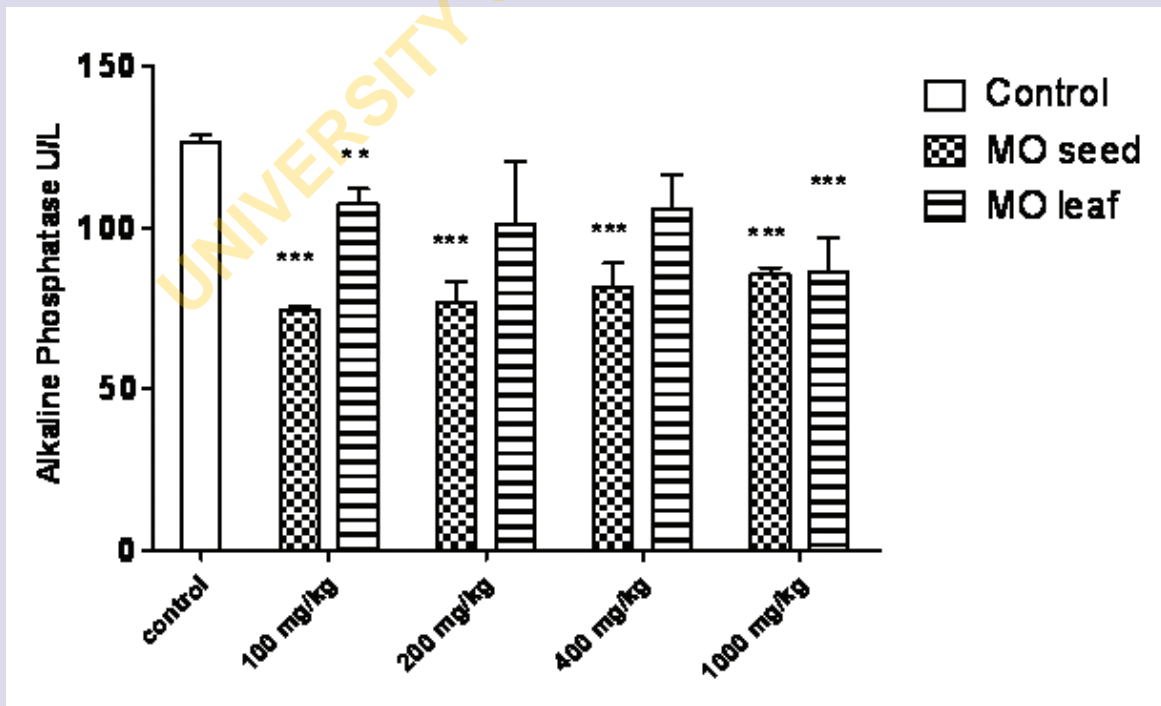


Figure 3: Effects of methanol extract of *Moringa oleifera* seed and leaf on alkaline phosphatase (ALP). Values are Mean \pm SEM (n=5), *p<0.05, **p<0.01 and ***p<0.001 as compared to the control

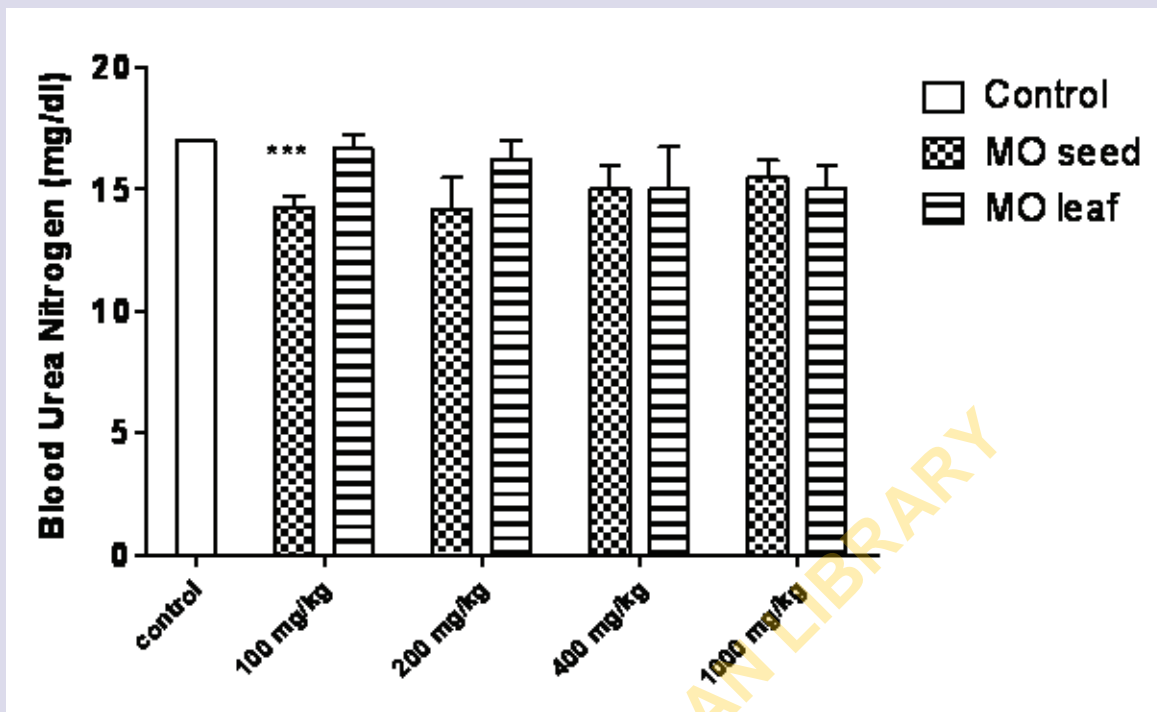


Figure 4: Effects of methanol extract of *Moringaoleifera* seed and leaf on blood urea nitrogen (BUN). Values are Mean \pm SEM (n=5), *p<0.05, **p<0.01 and ***p<0.001 as compared to the control

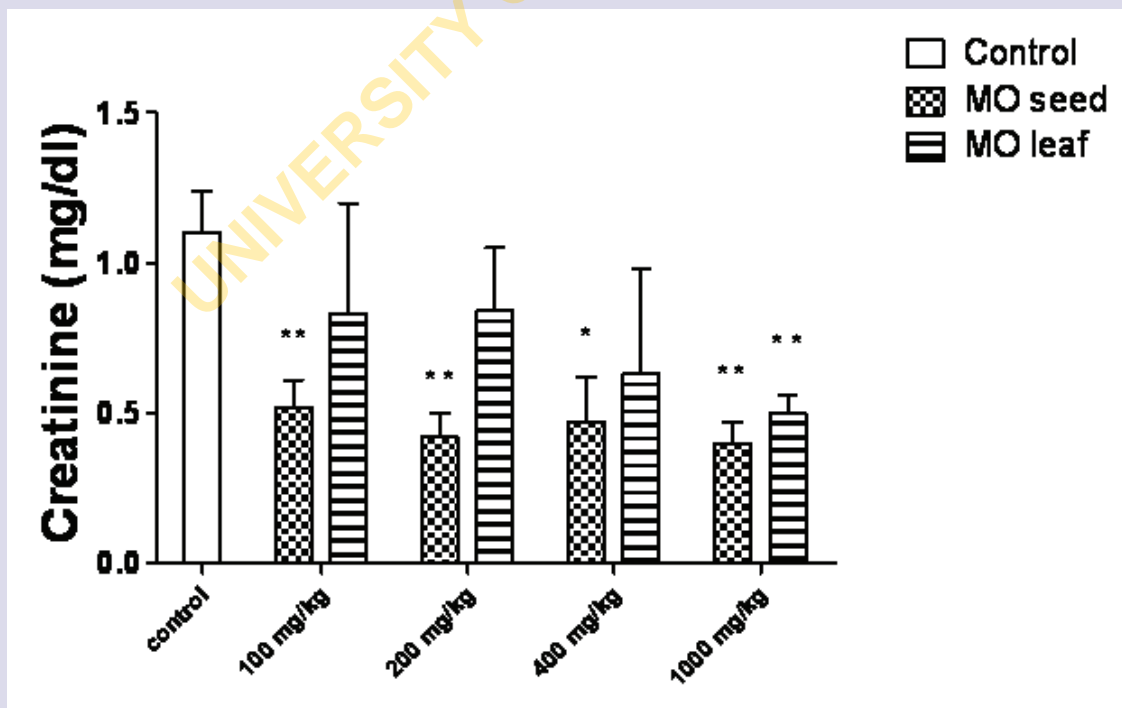


Figure 5: Effects of methanol extract of *Moringa oleifera* seed and leaf on creatinine. Values are mean \pm SEM (n=5), *p<0.05, **p<0.01 and ***p<0.001 as compared to the control

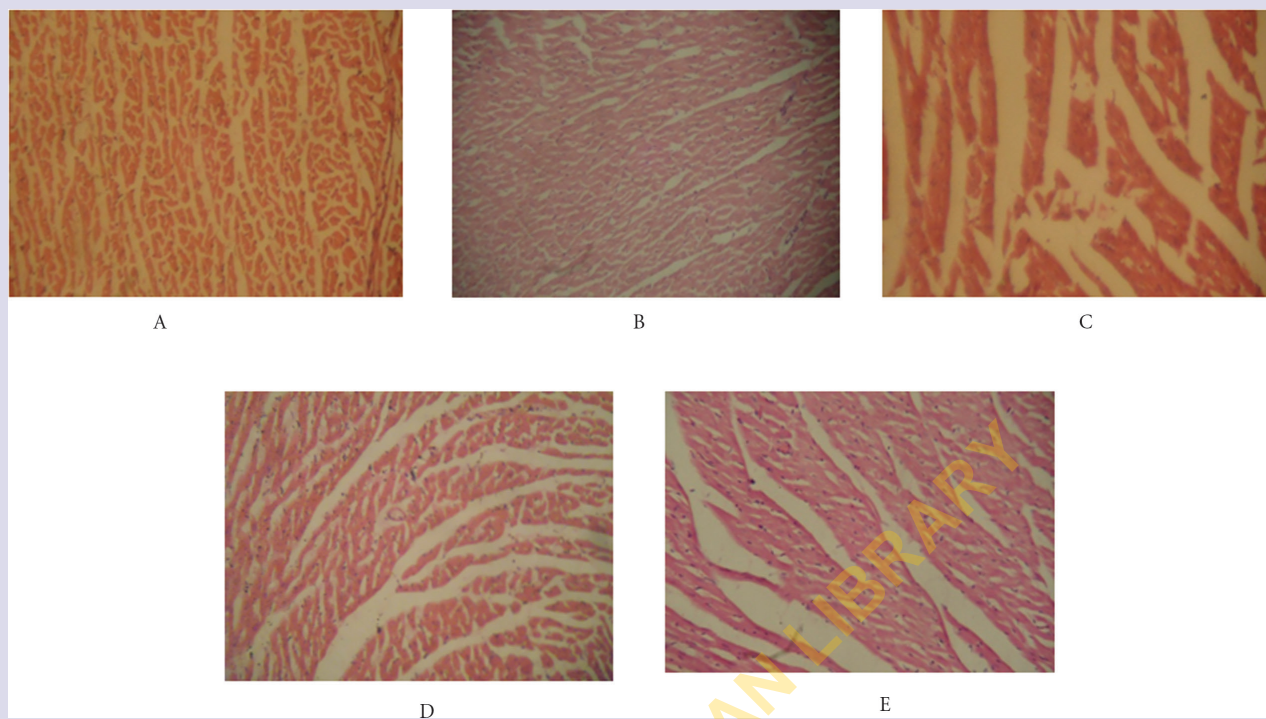


Figure 6: The photomicrographs showed normal morphological cytoarchitecture of rat heart administered graded doses of *Moringa oleifera* seed extract when compared with the control group. Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* seed

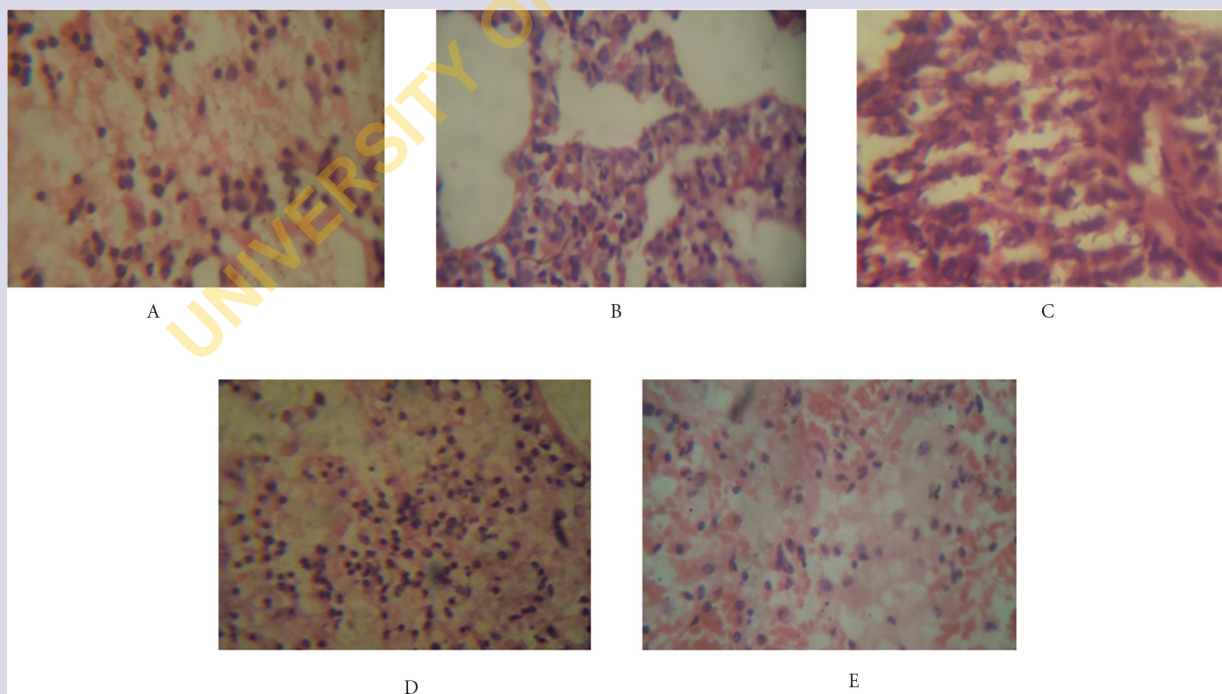


Figure 7: The photomicrographs showed no visible lesions in the morphological cytoarchitecture of rat lung administered lower doses of *Moringa oleifera* seed extract when compared with the control group. However, there is a moderate to severe interstitial thickening probably caused by proliferation of pneumocytes in the one treated with 400 mg/kg. There lung parenchyma is oedematous and diffusely infiltrated in animals treated with 1000 mg/kg.

Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* seed

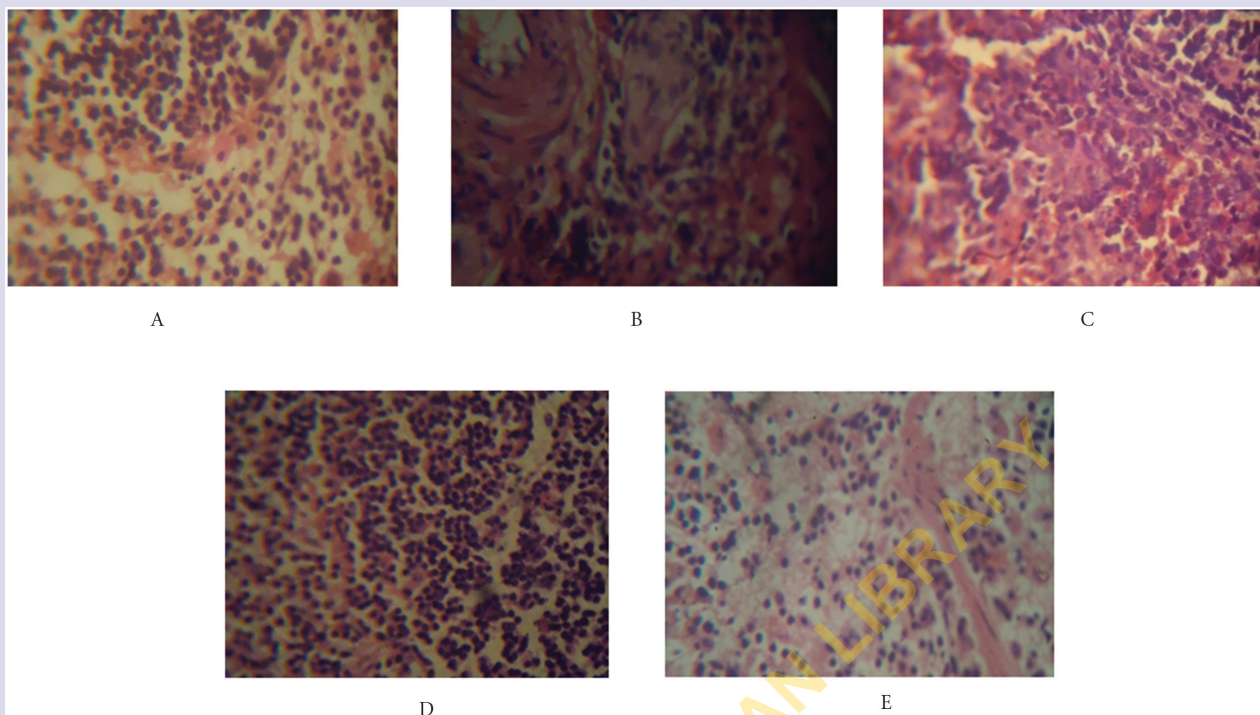


Figure 8: The photomicrographs showed normal morphological cytoarchitecture of rat spleen administered graded doses of *Moringa oleifera* seed extract when compared with the control group. Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* seed

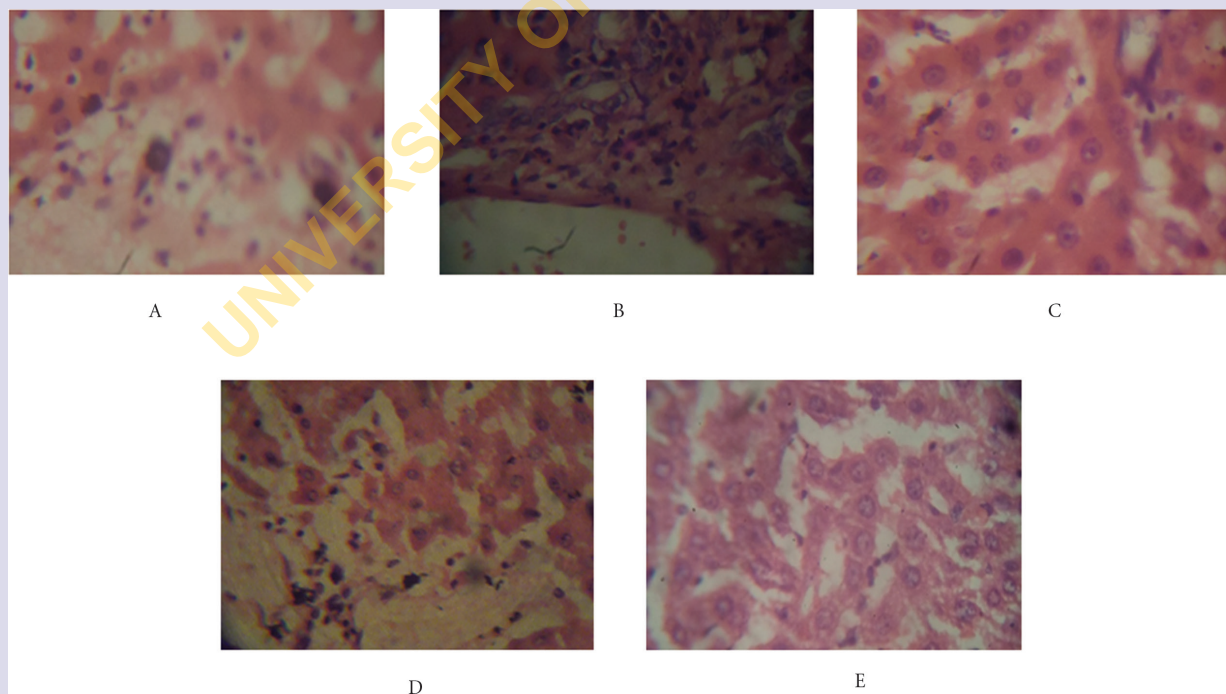


Figure 9: The photomicrographs showed normal morphological cytoarchitecture of rat liver administered graded doses of *Moringa oleifera* seed extract when compared with the control group. However, There is a very mild periportal cellular infiltration in rats treated with the 1000 mg/kg and a moderate periportal hepatic necrosis and cellular infiltration observed in the rats treated with 200 mg/kg body weight of the *Moringa oleifera* seed extract. Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* seed

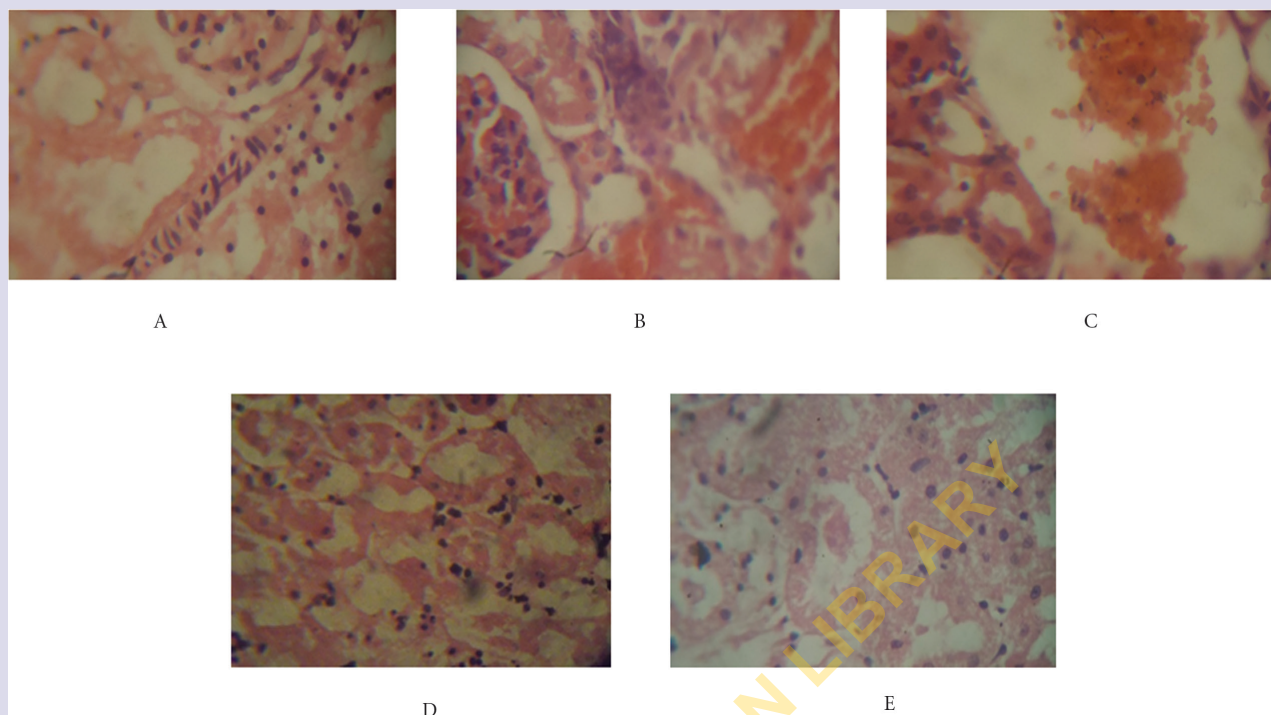


Figure 10: The photomicrographs showed normal morphological cytoarchitecture of rat kidney administered graded doses of *Moringa oleifera* seed extract when compared with the control group. However, renal tubules are severely necrotic and shrunken and the interstitium is infiltrated by inflammatory cells in rats treated with 1000 mg/kg Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* seed

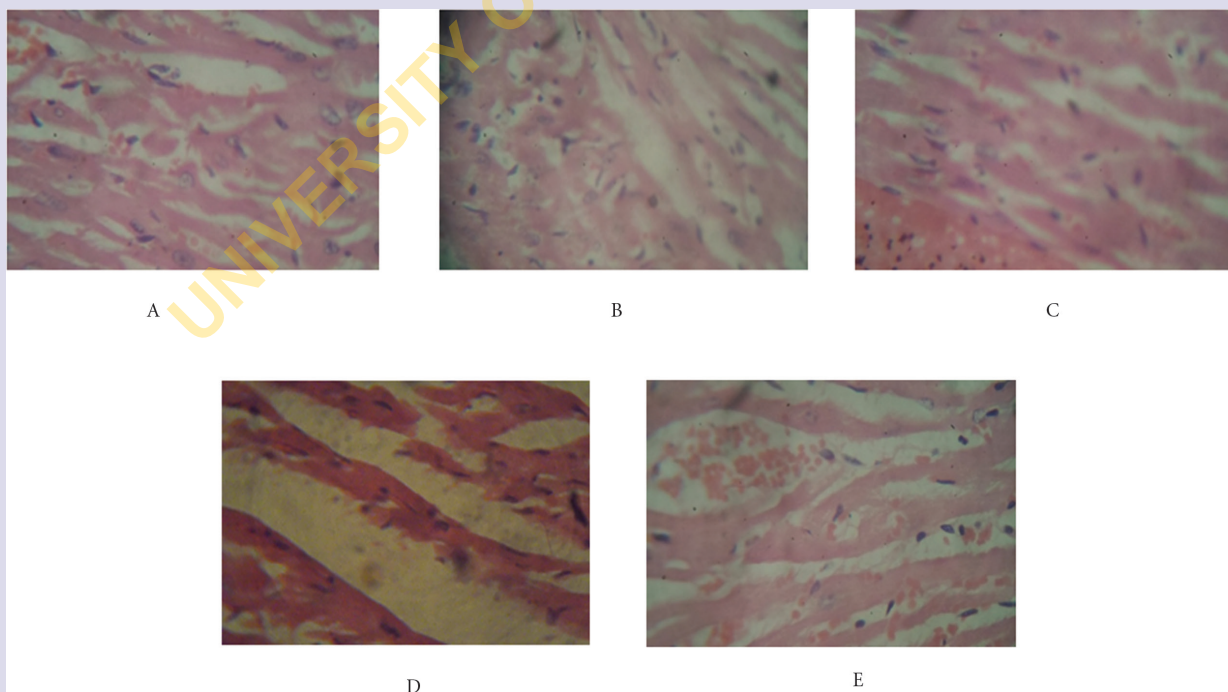


Figure 11: The photomicrographs showed normal morphological cytoarchitecture of rat heart administered graded doses of *Moringa oleifera* leaf extract when compared with the control group. However, There is a moderate congestion of the coronary vessels in animals treated with 400 mg/kg. Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* leaf

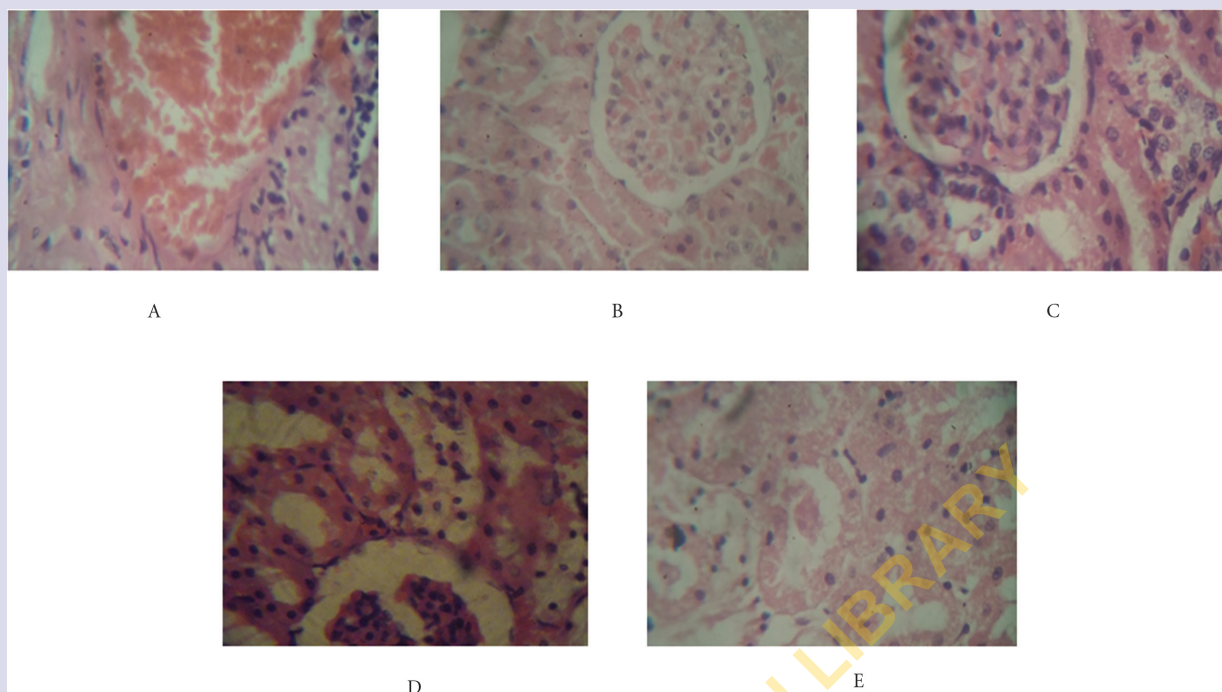


Figure 12: The photomicrographs showed normal morphological cytoarchitecture of rat kidney administered graded doses of *Moringa oleifera* leaf extract when compared to the control group. However, There is generalised interstitial congestion of the renal parenchyma was observed in the one treated with 100 mg/kg (A) and a moderate renal cortical congestion was observed in the 1000 mg/kg body weight (D) of the *Moringa oleifera* leaf extract. Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* leaf

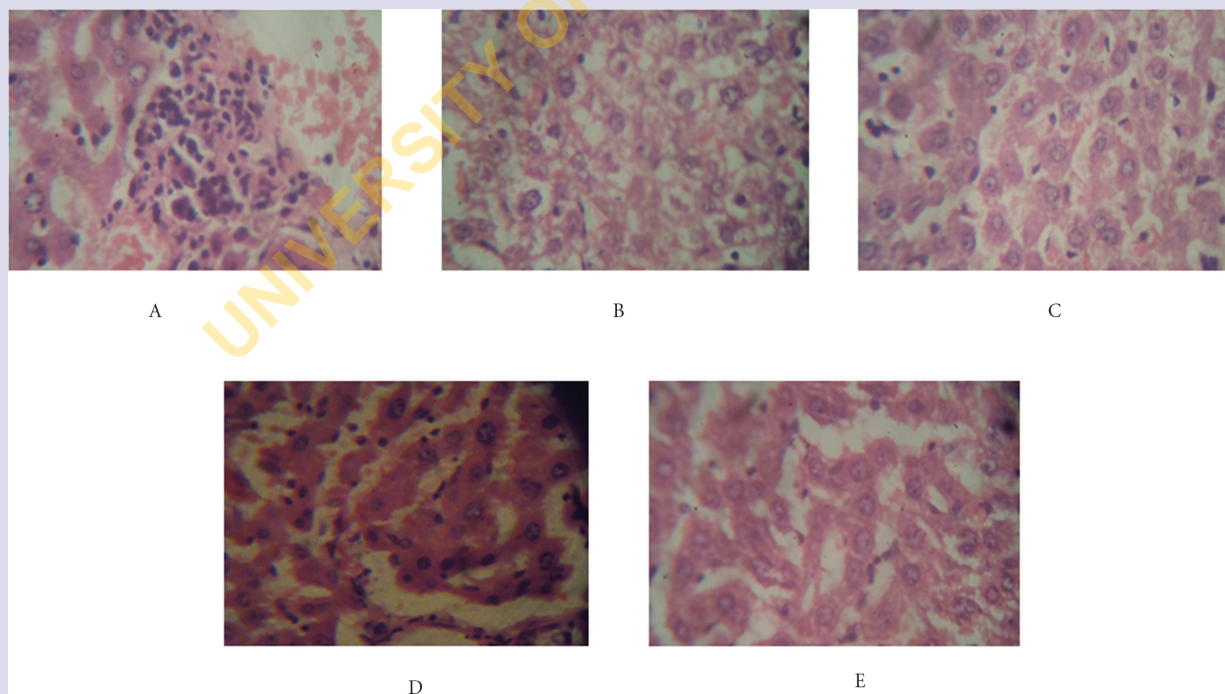


Figure 13: The photomicrographs showed normal morphological cytoarchitecture of rat liver administered graded doses of *Moringa oleifera* leaf extract when compared with the control group. However, There is a severe portal congestion and a periportal cellular infiltration by mononuclear cells 100 mg/kg, There is a severe diffuse vacuolar degeneration of hepatocytes in 200 mg/kg of the *Moringa oleifera* leaf extract. Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* leaf

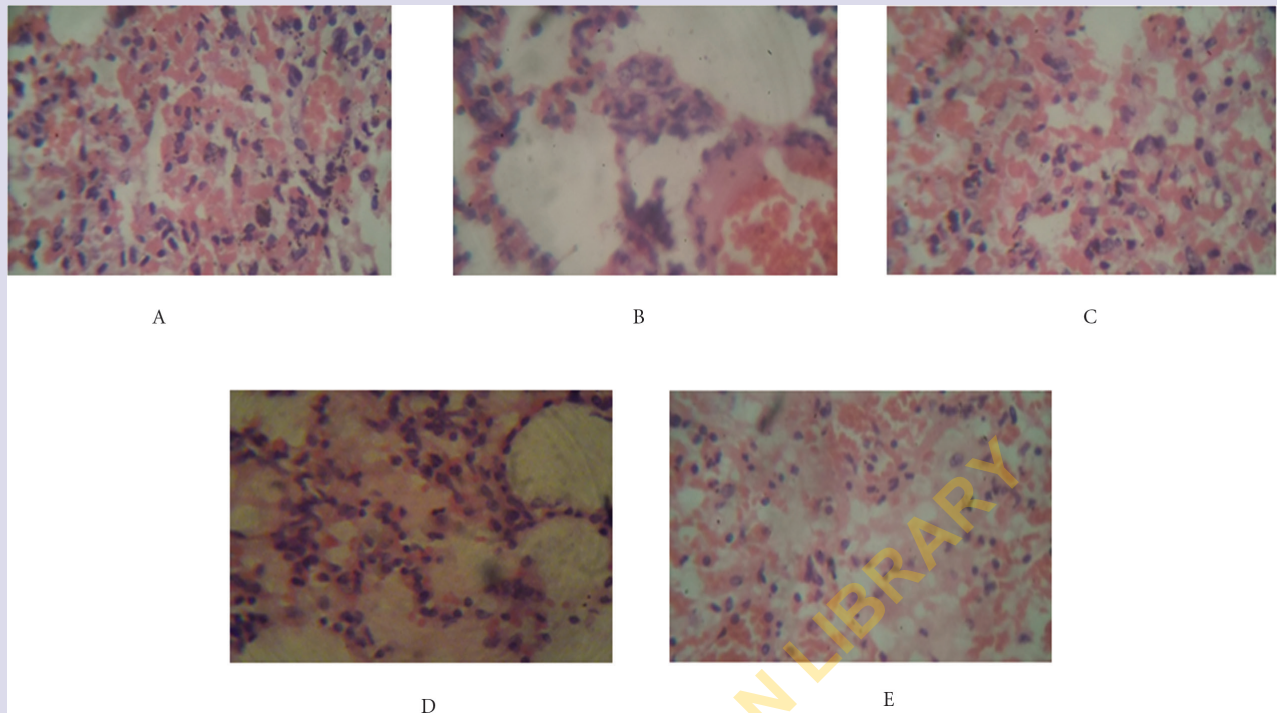


Figure 14: The photomicrographs showed morphological cytoarchitecture of rat lung administered graded doses of *Moringa oleifera* leaf extract when compared with the control group. However, there is a moderate to severe interstitial congestion and proliferation of alveolar pneumocytes in (A), mild interstitial congestion (B), interstitial proliferation of pneumocytes (C) and many alveolar spaces appear filled with pink staining fluid with a mild to moderate interstitial congestion in (D). Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* leaf

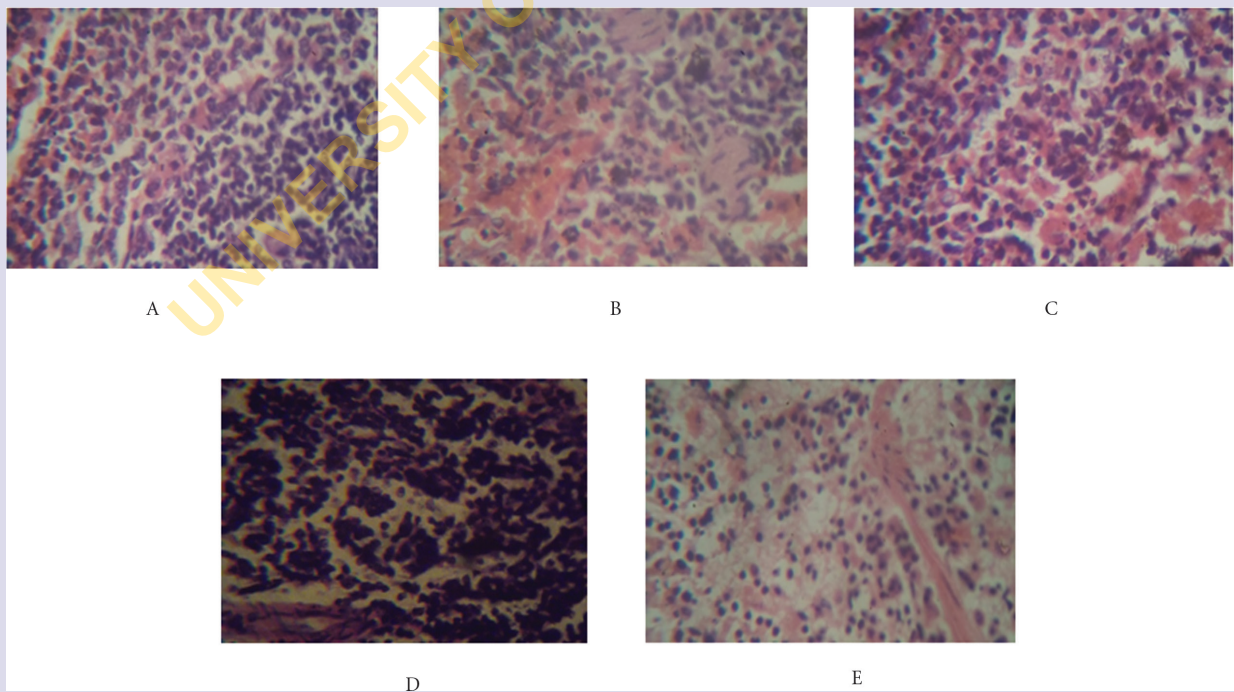


Figure 15: The photomicrographs showed normal morphological cytoarchitecture of rat spleen administered graded doses of *Moringa oleifera* leaf extract when compared with the control group. However, a very mild diffusion was observed generally at all doses tested. Magnification X40. A (100 mg/kg), B (200 mg/kg), C (400 mg/kg), D (1000 mg/kg), E (Control)

Histopathological examination of rats treated with methanol extract of *Moringa oleifera* leaf

while other doses revealed no significant change. The group treated with the leaf extract revealed no significant change at all doses tested. This indicates that the seed extract will be a better option in the control of blood urea nitrogen level.

Creatinine

In this study, the seed extract of *Moringa oleifera* was able to reduce the creatinine levels ($p < 0.01$) at most of doses tested (100, 200 and 1000 mg/kg) apart from at 400 mg/kg body weight where the decrease in creatinine level was significant $p < 0.05$. The leaf extract was only able to significantly reduce the creatinine level $p < 0.01$ in the group of animals treated with the highest dose level (1000 mg/kg). Treatment with leaf extract at other doses revealed no significant change.

Histopathological examination revealed mild to moderate changes in animals treated with *Moringa* seed extract mainly at higher doses when compared to the controls shown in Figures 6-10. All the animals treated with *Moringa* seed at the highest dose of 1000 mg/kg body weight exhibited along side the changes observed in organs, some physical changes like agitation, tremor, confusion and disorientation towards the 25th day of the experiment. These observed effects soon faded as no mortality was recorded throughout the duration of the experiment. The group of rats treated with the leaf extract, however revealed major visible changes in all organs and at all doses tested across the doses tested (even at lowest dose of 100 mg/kg) with exception of the spleen where the change was a very mild diffuse congestion as shown in Figures 11-15. This suggests that the seed extract may be safer across all doses tested even though administration at lower doses will be preferable especially when on a long term use. This is in line with previous studies where no observed overt adverse reactions in the acute and sub-acute studies were observed. It was therefore reported that *Moringa oleifera* dried leaf extract may be reasonably safe for consumption but with recommendation that the consumption of the leaves should not exceed a maximum of 70 g per day to prevent cumulative toxicity over long periods.¹⁸

The results (hematological, biochemical and histopathological) in this study revealed that the seed was able to more significantly alter the levels of liver enzymes when compared to the leaf extract of *Moringa oleifera*. The change in these liver enzymes may confer some protection on the liver and kidney respectively as an elevation is markers of hepatic damage.²⁹ Therefore, the methanol extracts of *M. oleifera* seed and leaf may thus be useful (although to different degree) in the management of acute liver or kidney disease where a reduction in the biochemical indices is required.

CONCLUSION

This study demonstrated that the methanol extract of *Moringa oleifera* seed unlike the leaf extract may not cause significant deleterious effects on the liver and kidney tissues, but both the seed and the leaf extracts however, possess potentials capable of altering some of the biochemical parameters and hematopoietic elements. However, the leaf extract revealed major changes ranging from moderate to severe congestion of coronary vessels across all the doses tested. The seed extract, in this study, revealed more significant changes in all the indices when compared to the leaf extract indicating that the seed may be a better option especially on a long term use. Further investigation is however needed to establish the bioactive compounds involved in mediating these biological effects.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATION USED

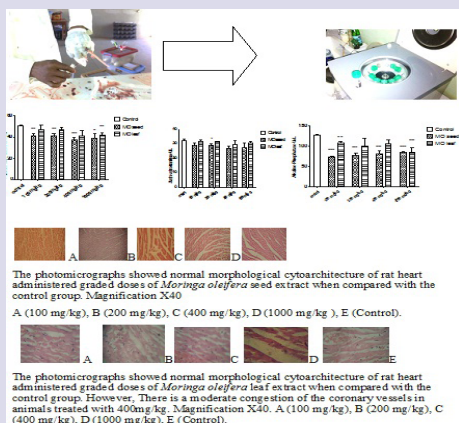
WHO: World Health Organization; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **ALP:** Alkaline phosphatase; **BUN:** Blood urea Nitrogen; **PCV:** Packed cell volume; **WBC:** White blood cell; **RBC:** Red blood cell; **MCV:** Mean cell volume; **HCT:** Heamatocrit; **HB:** Heamoglobin; **MONO:** Monocytes; **NEUT:** Neutrophil; **EOSIN:** Eosinophil; **LYMP:** Lymphocytes; **MO:** *Moringa*; **OECD:** Organisation for Economic Co-operation and development.

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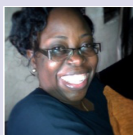
PICTORIAL ABSTRACT



SUMMARY

- Higher doses of *Moringa oleifera* seed extract on a long term use revealed mild to moderate histopathological changes in organs of animals treated.
- Physical changes like confusion, tremor, agitation and disorientation which were disappeared with time was also observed.
- The leaf however, revealed more toxic effects on all the organs of animals treated and at all doses tested which calls for a caution in the use of the leaves especially on a long term.
- Both the seeds and leaves possess potential ability to alter biochemical parameters and so can be a good template for hepatoprotective agents although to different degrees.

ABOUT AUTHORS



Ajayi Temitayo Olayemi: Holds a Bachelor of Pharmacy degree and a masters in Pharmacognosy degree from the University of Ibadan, Nigeria. She is an early career academician on her PhD in the Department of Pharmacognosy, Faculty of Pharmacy of the same University. Her research interests are the natural product chemistry, Natural medicine, Phytomedicine and Phytochemistry. Her research is currently on the Phytochemical and Biological studies of some selected Anti-obesity plants from South-west Nigeria. She has published a number of academic papers to her advantage in peer reviewed journals.