

Original Research Article

Histological evaluation of the effects of *Moringa* leaf extract treatment on vital organs of murine models

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Abstract

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Moringa oleifera has gained popularity especially in recent times due to several publications reporting various nutritional and health benefits of the plant; though it is important to note that most investigations on this plant are basic and the reports would require proper trials to evaluate the exact benefits to human health. This investigation was an attempt to investigate the effects of ethanolic *Moringa oleifera* leaf extract on the histology of vital body tissues. The rationale is that histological observations would provide a more reliable and consistent picture of the effects produced by the interactions of the phytochemicals with the body cells and tissue. It may be helpful in observing the possible toxicological effects on body tissues or on the other hand, the positive effects on the body tissues. A total of twelve Wistar rats ($n=12$) were used for the investigation; divided in two groups of Control (A) and Treated (B). A daily dosage of 200mg/kg body weight of ethanolic *moringa* leaf extract was administered orally to the treated Group B for 28 days. All animals were fed *ad libitum*. Animals were sacrificed by cervical dislocation. The tissues were excised and processed using routine haematoxylin and Eosin staining techniques. Photomicrographs were taken with the aid of the Accuscope Photomicrographic at suitable magnifications. Analysis of each tissue's histo-morphology, general histo-architecture and cytological structures was critically done. The basis of analyses and inferences was clearly defined: whether *Moringa oleifera* leaf extract produced any observable deleterious effects on the tissue [toxicological evaluation]; or whether its effects would improve the tissue's histological architecture especially in manners that can produce improvement in physiological conditions of the individual tissue or general body health [medicinal and nutritional properties]. Extract produced positive effects in most tissues except in the testis and epididymis where the effects were anti-fertility. Various tissues benefited from the positive effects to various extents.

Keywords: Extract, Histology, *Moringa*, Organs, Toxicology

INTRODUCTION

There are several publications, reporting the various benefits of *Moringa oleifera* leaf and other parts of the plant. It is however important to note that there is the need for more specific reports, especially considering the scope of the research activities leading to the presented results. While the plant shows huge potentials to alleviate

hunger and provide herbal and plant-derived medicinal products, especially for the developing nations; it is important to establish the primary effects of the leaf phytochemicals' activities and interactions with the body tissues cum organs. In other words, toxicological research efforts would do well to first establish the safety

of consumption of the plant products and to the various extents; as this will be vital to the establishment of the use of the plant's products as standard nutritional supplements and natural or bio-medicinal products. To this end, we employed histological methods to evaluate the effects of moringa leaf extracts on vital body tissue-organs.

The rationale is that histological methods of observations would provide a more reliable and consistent picture of the effects produced by the interactions of the phytochemicals with the body cells and tissue better than in vitro tests and analysis of the highly dynamic biochemical activities as contained in extracted tissue fluids. Also, the use of Histological methods of assessment of moringa leaf extract effects on body tissues is important because literatures are comparatively scarce on such methods of investigation of the plant's extracts' effects.

Few publications have reported some of the specific effects of *Moringa oleifera* leaf extract on the some body tissues or organs. The cerebroprotective effect of moringa oleifera against focal ischemic stroke has been reported (Woranan *et al.*, 2013). Chatchada *et al.*, (2013) reported that moringa leaf extract was neuroprotectant when administered to animal Models of Age-Related Dementia when their hippocampus was observed. It was also reported that Lyophilized hydroalcoholic extract of *M. oleifera* showed myocardial preservative effect in isoproterenol (ISP)-induced model of myocardial infarction (Nandave, 2009). The report of Ouédraogo (2013) in their efforts to evaluate the protective effect of *Moringa oleifera* leaf extract against gentamicin-induced nephrotoxicity in rabbits stated that histological preparations of the kidney of intoxicated animals treated with moringa leaf extract exhibited reparative tendencies. *Moringa oleifera* ethanolic leaf extract reportedly have hepatoprotective abilities in various induced conditions such as using diclofenac (Hamza, 2007); acetaminophen (Fakurazi *et al.*, 2008) antitubercular drug (Pari and Kumar, 2002) and carbon tetrachloride (Selvakumar and Natarajan, 2008). Awodele *et al.*, (2012) estimated the LD(50) for aqueous extract of moringa leaf and tested various dosages of extract on the sperm, haematological and biochemical parameters as well as histopathological preparations; and they concluded that orally administered moringa leaf extract at their estimated sub-lethal dosages were relatively safe for tested body organs.

The aim of this particular investigation was to observe the effects of *Moringa oleifera* leaf extract on the histological architecture on a dozen vital tissues of the body.

MATERIALS AND METHODS

Twelve male Wistar rats (n=12) were used for the investigation. They were divided in two groups of Control

(A) and Treated (B). A daily dosage of 200mg/kg body weight of ethanolic *moringa leaf* extract was administered orally to the treated Group B for 28 days. All animals were fed *ad libitum*. Animals were sacrificed by cervical dislocation. The tissues were excised and processed using routine haematoxylin and eosin staining techniques. Analysis of tissues was done using qualitative methods with emphasis laid on histo-morphology, general histo-architecture and cytological structures features of the prepared specimen.

RESULTS AND DISCUSSION

Bone Marrow

The bone marrow tissue of the Group A animals [Control] is being illustrated as photomicrographs A₁ and A₂ (Figure 1); the cellular elements are observable [BMC]. Photomicrographs B₁ and B₂ are photomicrographs of the Group B animals administered moringa oleifera extract-the cellular elements are also observable (BMC). In both Groups (A and B), the cells appear relatively normal, and there are no sign to suggest anomalies especially in the untreated Group. In both sets of photomicrographs, the extracellular materials are abundant and well distributed. There is not enough evidence to suggest that *moringa* leaf extract has improved the physiological condition of the bone marrow from the photomicrographs; a deducible fact however, is that the administration of *Moringa oleifera* leaf extract has not produced any observable deleterious effects on the bone marrow tissue in the models employed in this investigation. This strongly suggests that the use of the extract would not compromise vital functions of the active bone marrow which primarily would include haematopoiesis. The report of Adedapo *et al.*, (2009) showed dose pended responses of haematological parameters to moringa extract ingestion; however at 400 mg/kg-bw *significant* increase in packed cell volume (PCV) was recorded; this could be an indication of a positive effect.

Brain Cerebrum

The cerebral cortices of the experimental animals are illustrated at various magnifications in Figure 2; the control Group A cortex is illustrated at various magnifications in A₁, A₂ and A₃. Figure 2- A₁ illustrates the cross section of the cerebral cortex in an attempt to observe the general organisation and arrangement of neurons and other supportive structures across the cortical layers [though this could only be effectively done at such low magnification]. Figure 2- A₂ and A₃ are larger illustrations of the cerebral cortex of the Group A models-deeper and superficial layers respectively. Neurons are clearly observable as well as the glia in their various

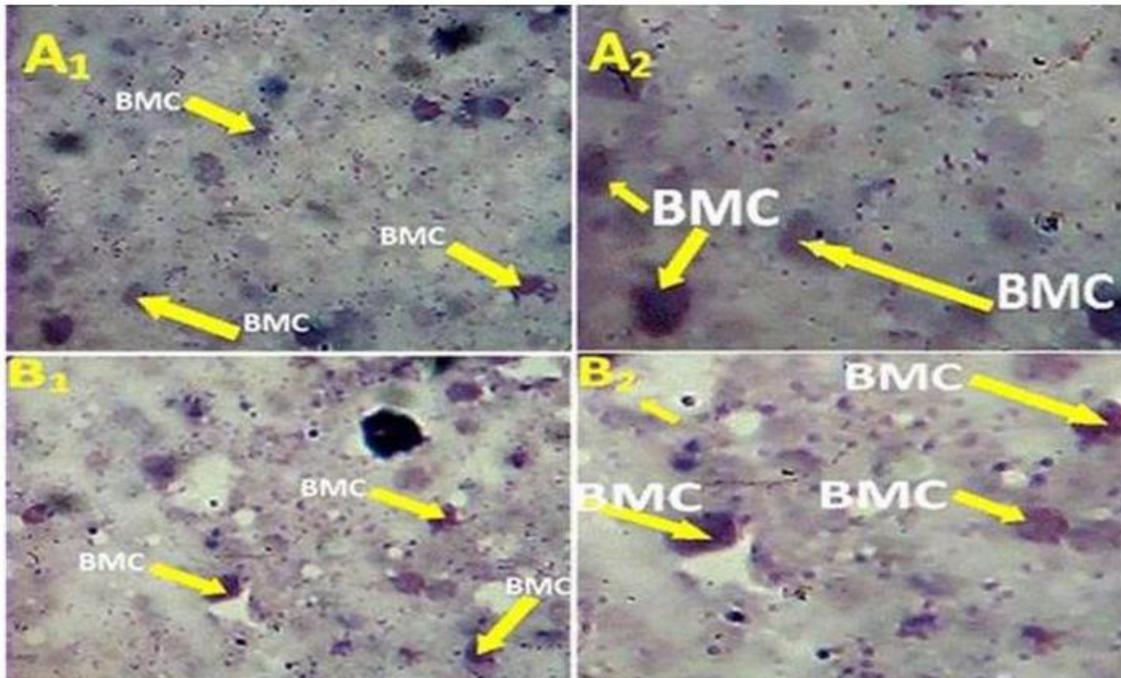


Figure 1. Photomicrograph of the bone marrow of experimental animals; A₁ and A₂ are illustrations of the Control Group A bone marrow cells at X160 and X640. Bone marrow cells are identifiable; B₁ and B₂ are photomicrographs of the Group B administered *moringa* leaf extract at X160 and X640; bone marrow elements are normal relative to control. (BMC = Bone Marrow Cells).

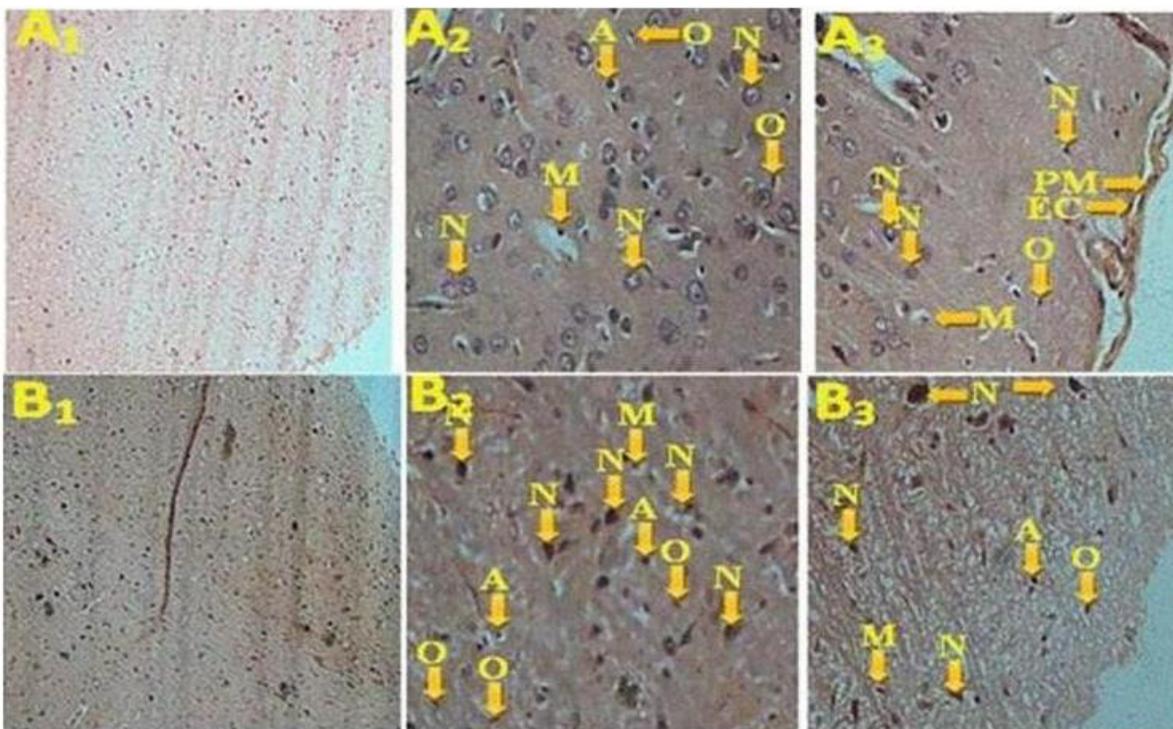


Figure 2. Photomicrograph of the cerebrum of experimental animals; A₁, A₂ and A₃ are illustrations of the Control Group A cerebral cortex at X160 [cross section] X640 deeper layers and X640 (superficial layers). All photomicrographs portray features of normal cortex; neurons and glia are identifiable and the neuropil is defined. B₁, B₂, B₃ are photomicrographs of the Group B administered *Moringa oleifera* extract at X160 [cross section]; X640 [deep layers] and X640 [superficial layers]; histological features are largely normal, there are no features portraying tissues damage or much improvement over control. (N=Neuron; A = Astrocyte; O= Oligodendroglia; M= Microglia).

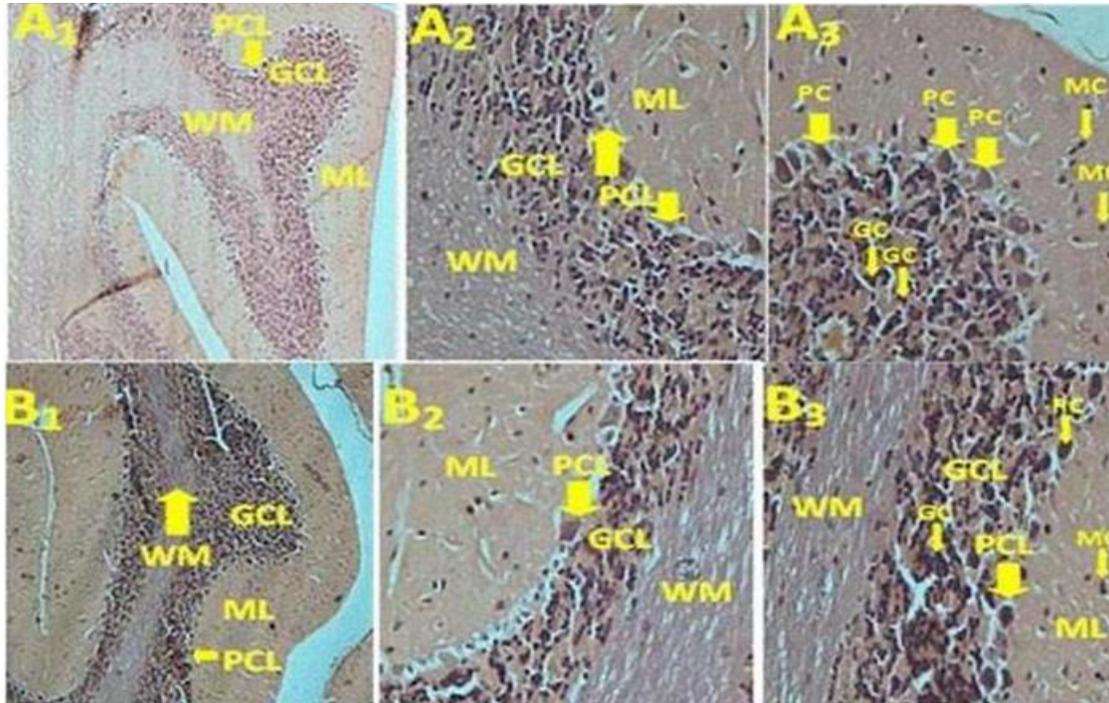


Figure 3. Photomicrograph of the cerebellar cortex of experimental animals; A₁, A₂ and A₃ are illustrations of the Control Group A cerebellar cortex at X160 [cross section], X640 [molecular and granular layers and core white matter] and X640 [molecular and granular layers]. All photomicrographs portray features of normal cortex; cerebellar layers are well defined. B₁, B₂, and B₃ are photomicrographs of the Group B administered *Moringa oleifera* leaf extract at X160 [cross section], X640 [molecular and granular layers and white matter core] X640 [granular and Purkinje cells]; cerebellar cortex layers and elements are well defined.

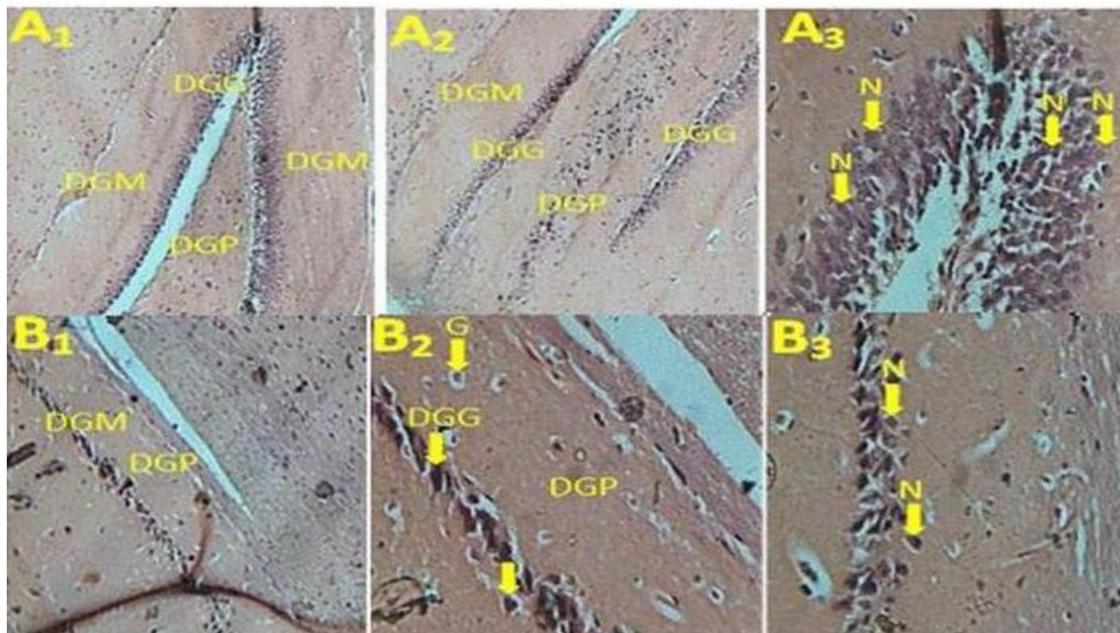


Figure 4. Photomicrograph of the hippocampus of experimental animals; A₁, A₂ and A₃ are illustrations of the Control Group A hippocampus at X160, X160 (cross sections) and X640 (dentate gyrus). All photomicrographs portray features of normal cortex; layers and parts of the hippocampus are well defined. B₁, B₂, B₃ are photomicrographs of the Group B administered *Moringa oleifera* leaf extract at X160 (cross sections), X640 and X640 [neurons morphology]; there is no evidence of altered cellular morphology or extensive tissue damage. (DGM = Dentate Gyrus Molecular Layer; DGP = Dentate Gyrus Polymorphic Layer; DGG = Dentate Gyrus Granular Layer; N = Neuron; G = Glia)

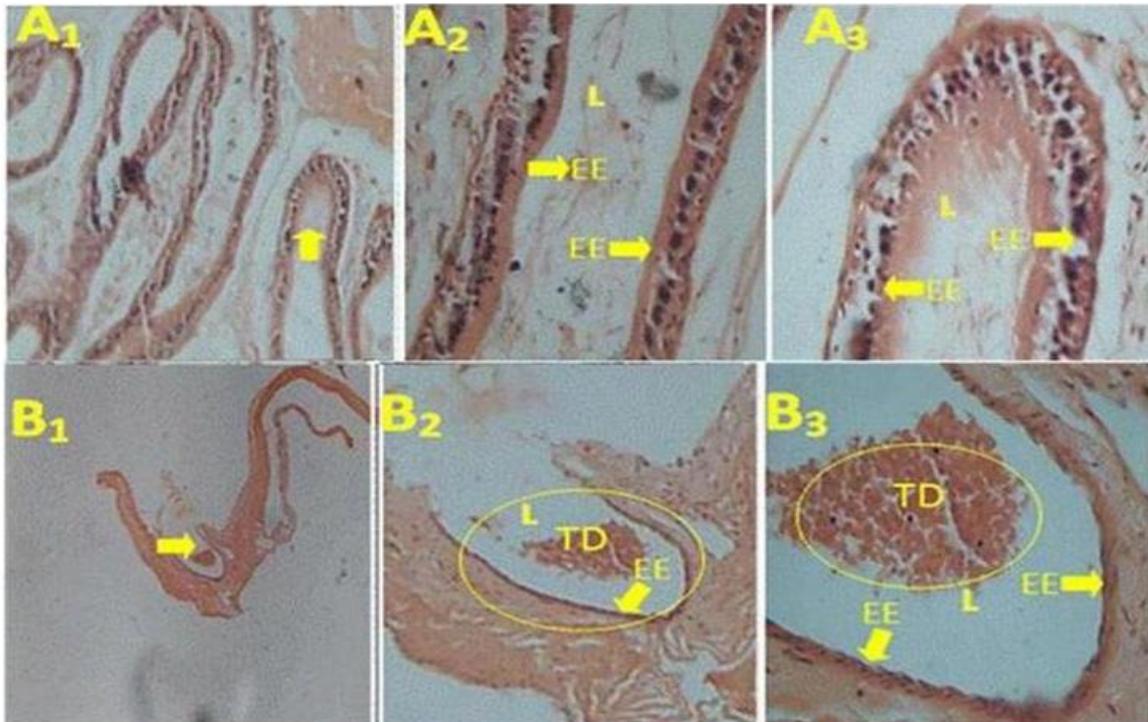


Figure 5. Photomicrograph of the epididymis of experimental animals; A₁, A₂ and A₃ are illustrations of the Control Group A epididymis at X160 (cross sections), X640 (epithelium and lumen) and X640 [epithelium and lumen]. All photomicrographs portray normal features; epithelium is well defined. B₁, B₂, B₃ are photomicrographs of the Group B epididymis of animals administered *Moringa oleifera* leaf extract at X64 [cross sections], X160 [epithelium and lumen] and X640 [epithelium, lumen and tissue debris]; there is observable epithelial disruption and unusual debris accumulation within the lumen. (EE = Epididymis Epithelium; L = Lumen; TD = Tissue Debris).

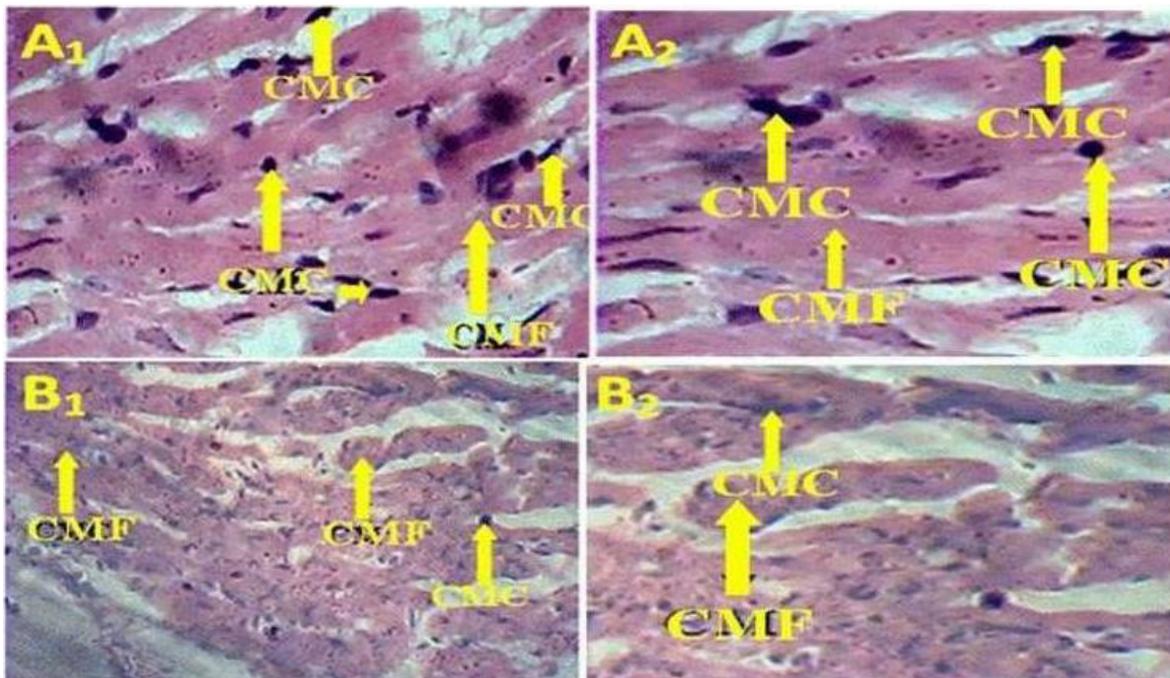


Figure 6. Photomicrograph of the cardiac muscle of experimental animals; A₁ and A₂ are illustrations of the Control Group A cardiac muscle at X160 and X640. Photomicrographs portray features of normal cardiac muscle- cells are well defined; B₁ and B₂ are photomicrographs of the Group B administered *Moringa oleifera* leaf extract X160 and X640; tissues appear normal. (CMC = Cardiac Muscle Cell; CMF = Cardiac Muscle Fibre)

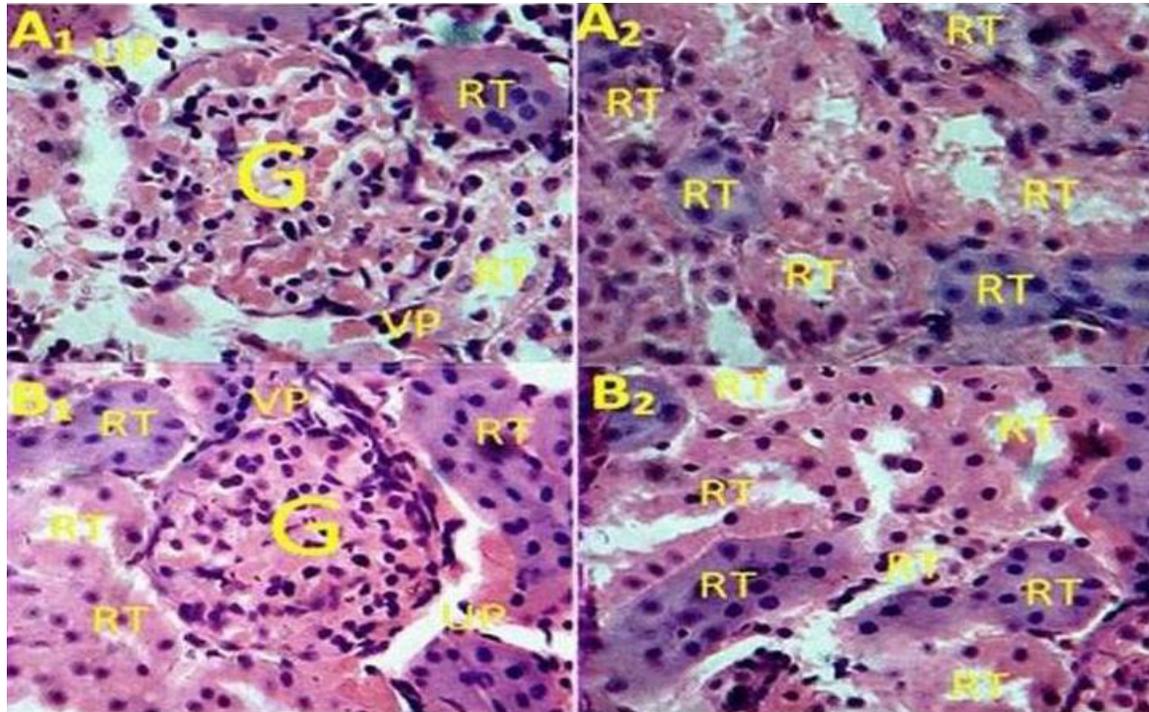


Figure 7. Photomicrograph of the kidney of experimental animals; A₁ and A₂ are illustrations of the Control Group A kidney at X640 (glomerulus) X640 (renal tubules). All photomicrographs portray features of normal kidney; tubules and glomeruli are identifiable and well defined. B₁ and B₂ are photomicrographs of the Group B animals administered *Moringa oleifera* leaf extract at X640 (glomerulus) X640 [renal tubules]; tissues portray normal features, glomerular elements appears more prominent and better defined relative to the control. (G= Glomerulus; RT = Renal Tubules; VP= Vascular Pole; UP= Urinary Pole).

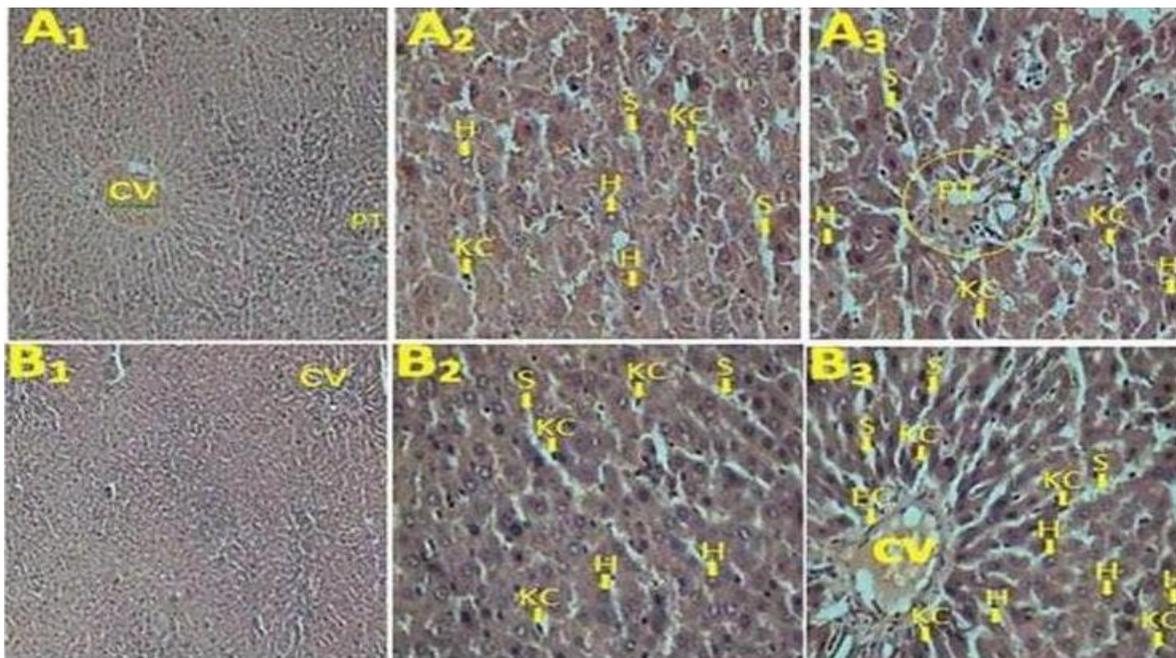


Figure 8. Photomicrograph of the liver of experimental animals; A₁, A₂ and A₃ are illustrations of the Control Group A liver X160 (general histo-architecture) X640 (cellular organisation) and X640 (characteristic features). All photomicrographs portray features of normal liver; histo-architecture is well defined. B₁, B₂, B₃ are photomicrographs of the Group B exposed to lead poisoning at X160 (general histo-architecture) X640 (cellular organisation) and X640 (characteristic features); basic features are normal and general histoarchitecture suggest improved organisation. (H= Hepatocytes; KC= Kupfer Cells; S= Sinusoids; CV = Central Vein; PT = Portal Triad; EC = Endothelial Cell)

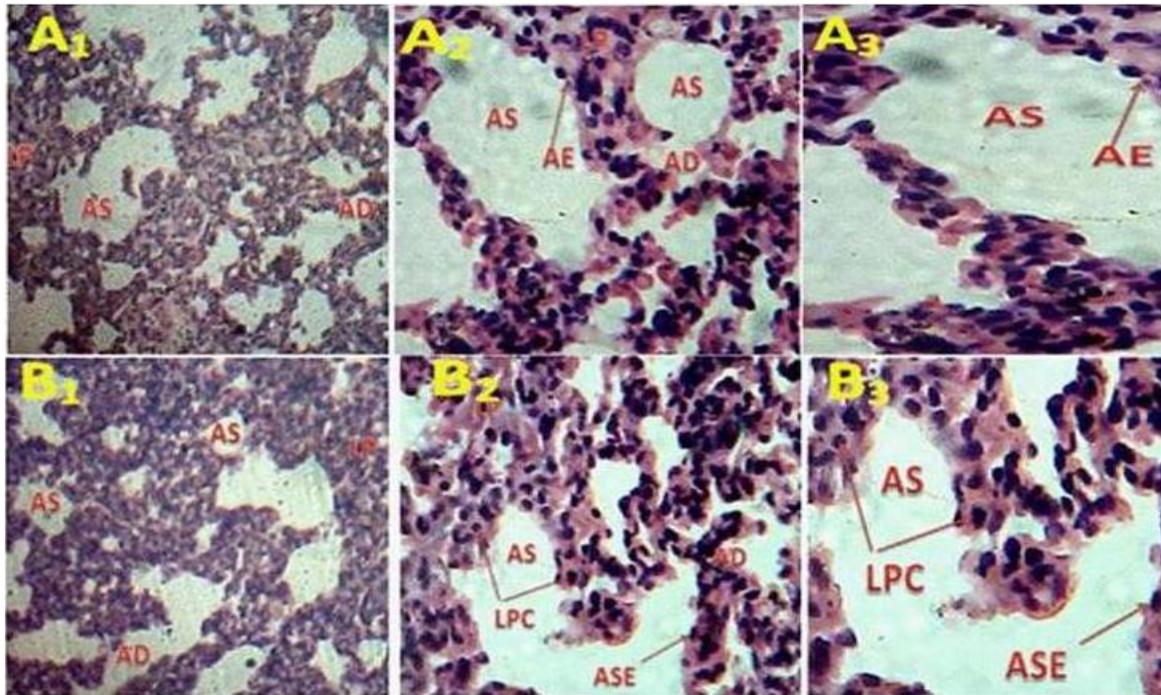


Figure 9. Photomicrograph of the lung of experimental animals; A₁, A₂ and A₃ are illustrations of the Control Group A lung X160 (general histo-architecture) X640 (alveolar organisation) and X640 (alveolar epithelium). All photomicrographs portray features of normal lung; histo-architecture is well defined. B₁, B₂, B₃ are photomicrographs of the Group B administered *Moringa oleifera* extract at X160 (general histo-architecture) X640 (alveolar organisation) and X640 (alveolar sac and epithelium); basic features are normal and general histoarchitecture suggest healthy tissue. (AS= Alveolar Sac; AD= Alveolar Duct; LP= Lung Parenchyma)

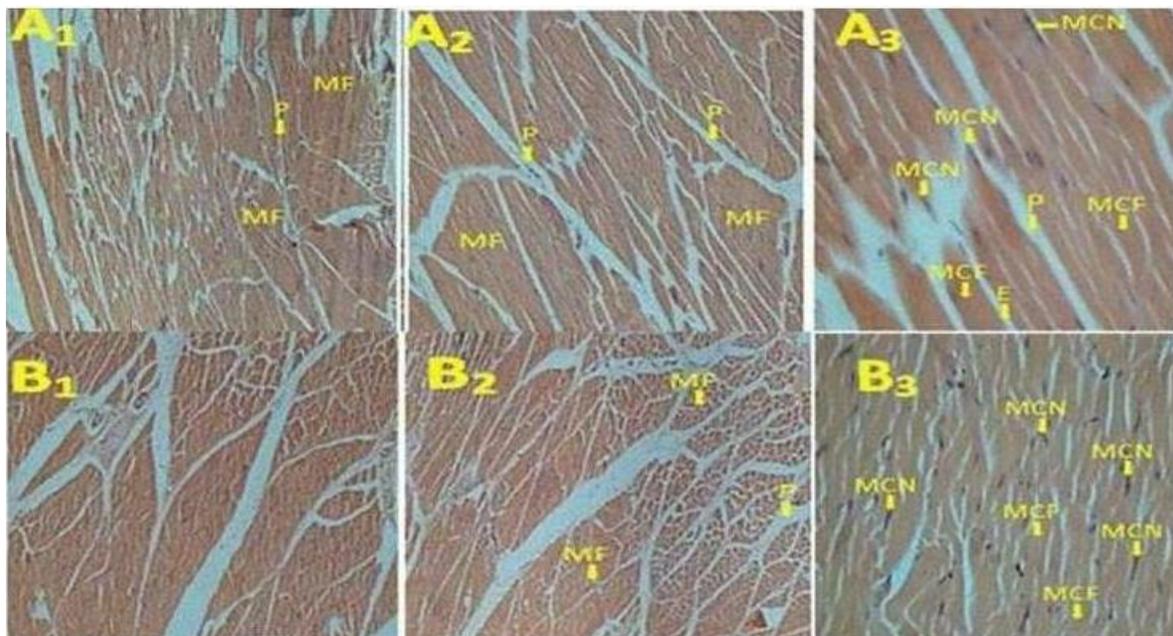


Figure 10. Photomicrographs of the skeletal muscle of experimental animals; A₁, A₂ and A₃ are illustrations of the Control Group A skeletal muscle X160 (general histo-architecture) X160 (fibre organisation) and X640 (myocytes and fibres). All photomicrographs portray features of normal lung; histo-architecture is well defined. B₁, B₂, B₃ are photomicrographs of the Group B exposed to lead poisoning at X160 (general histo-architecture) X160 (fibre organisation) and X640 (myocytes and fibres); features suggest normal tissue while cell prominence and relative abundance are pointers to mild hyperplasia. (MF = Muscle Fascicle; P= Perimysium; MCN= Muscle Cell Nucleus; MCF= Muscle Cell Fibrils; E= Endomysium)

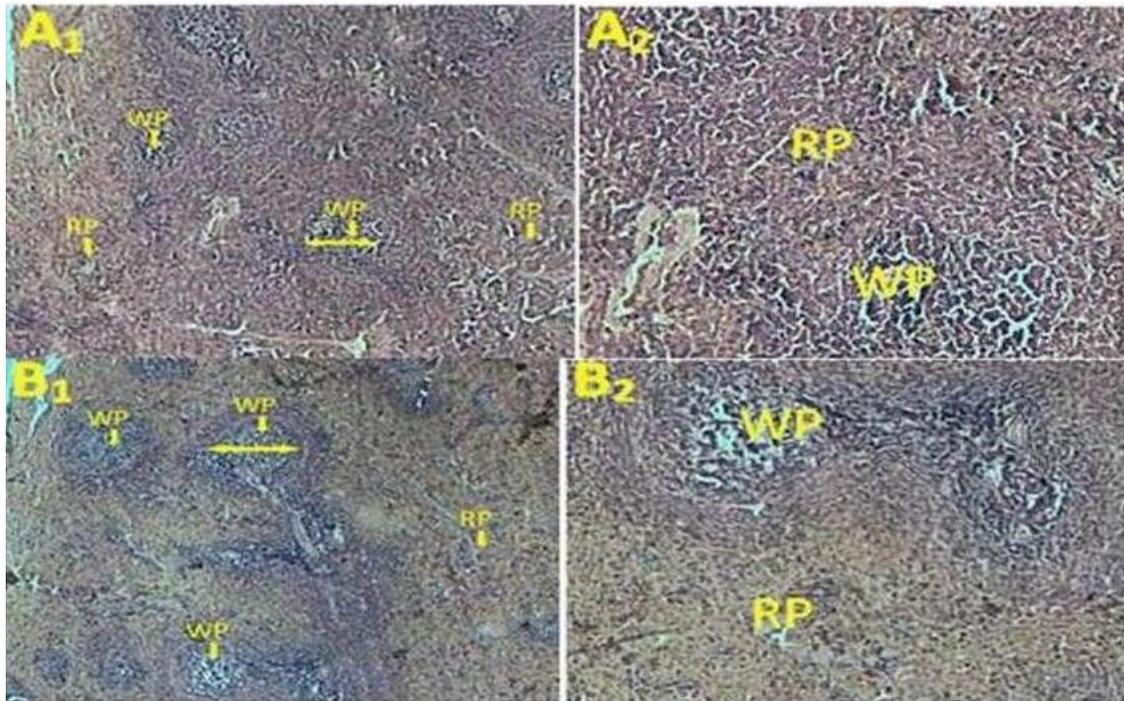


Figure 11. Photomicrograph of the spleen of experimental animals; A₁ and A₂ are illustrations of the Control Group A spleen at X160 (general histo-architecture) and X640 (white and red pulps). All photomicrographs portray features of normal spleen; histo-architecture is well defined. B₁, B₂ are photomicrographs of the Group B administered *Moringa oleifera* leaf extract at X160 (general histo-architecture) X640 (white and red pulps); overall histo-architecture is normal; white pulps are prominent. (WP = White Pulp; RP= Red Pulp)

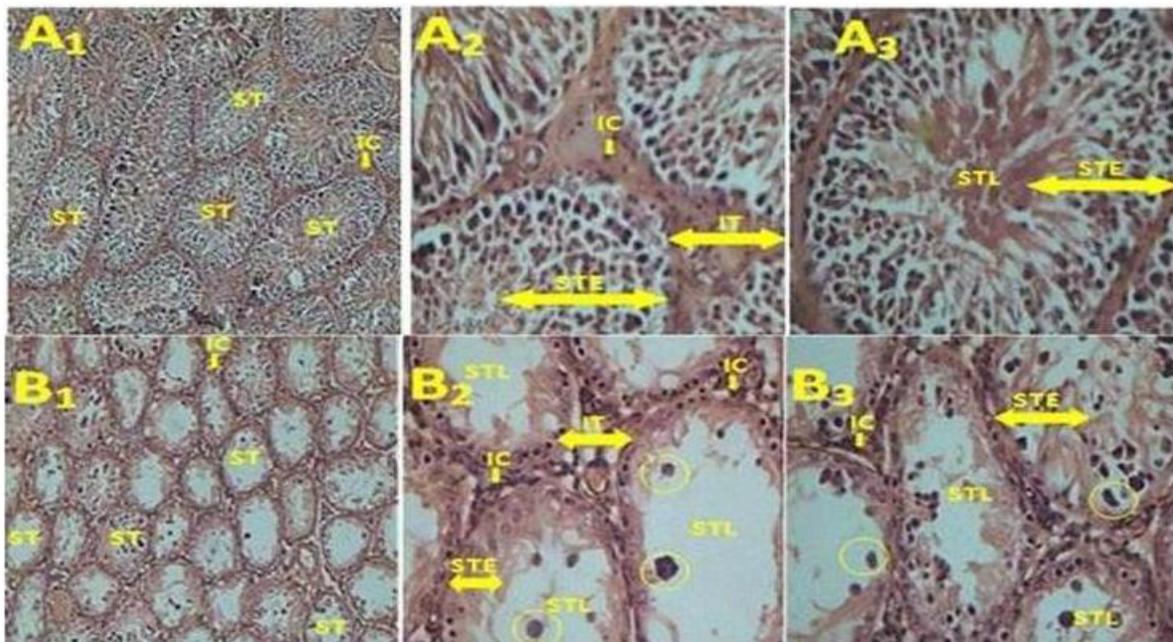


Figure 12. Photomicrograph of the testis of experimental animals; A₁, A₂ and A₃ are illustrations of the Control Group A testis at X160 (general histo-architecture) X640 (seminiferous tubule cross section) and X640 (tubules-interbubular organisation). All photomicrographs portray features of normal testis; histo-architecture is well defined. B₁, B₂, B₃ are photomicrographs of the Group B administered *Moringa extract* at X160 (general histo-architecture) X640 (seminiferous tubule cross section) and X640 (tubules-interbubular organisation); there are disruptions to the seminiferous tubules epithelium and the interstitial tissues. (ST= Seminiferous Tubule; IC= Interstitial Cells; IT= Interstitial Tissues; STE= Seminiferous Tubule Epithelium; STL= Seminiferous Tubule Lumen).

peculiar forms. While glia could be differentiated using their basic forms, neurons as well could be seen as they assume various shapes and forms across the various layers of the cerebral cortex. The neuropil is also normal in appearance. These observations altogether show that the Control Group A cerebral cortex is normal and could serve as a suitable reference. Figure 2- B₁ provides a cross-sectional observation of the brain cortex of the treated Group B at the lowest suitable magnification. The neurons appear well distributed across the layers. At the larger magnifications- B₂ and B₃; the neurons and the glia are also well defined and observable in their various forms and shapes. There are no abnormal observations that could suggest a damaging or deleterious effect of the administered substance on the tissue as illustrated on the photomicrographs. In other words, moringa leaf extract administration did not produce any histologically observable disrupting or damaging effects on the tissue of the cerebral cortex in this investigation. Ganguly *et al.*, (2005) reported positive effects of moringa extracts on the cerebral cortex and suggested it could give protection against devastating disease like Alzheimer's; it has also been shown to have neuroprotective effects in focal cerebral ischemia (Kirisattayakul). Moringa also has positive anti-lead ameliorative effects (Owolabi *et al.*, 2014). There are however very scarce literatures on the specific effects of the extract on tissue morphology and histo-architecture.

Brain Cerebellum

The brain cerebellum of the control Group A animals models is histologically illustrated in the photomicrographs labelled A₁, A₂ and A₃; the basic layers or regions of the cerebellum are clearly illustrated (Figure 3- A₁), so also the primary elements of the layers or regions (Figure 3- A₂ and A₃). The cells of the molecular layer [MC], Purkinje Cells [PC] and the granular layer cells [GC] are clearly defined. The layers are also identified and labelled- molecular layer [ML], most peripheral, the middle layer of Purkinje cells [PCL] as well as the inner granular cells layer (GCL) together with the white matter [WM] core. These are obviously normal features of a healthy cerebellum and as such the control is considered a suitable reference for the study. All the aforementioned features are also well defined and clearly observable in the treated Group B model cerebellum as illustrated in the photomicrographs labelled B₁, B₂ and B₃. All evidences point to a conclusion that the extract did not produce any histologically observable deleterious effect on the cerebellum of the treated Group B animal models. Moringa's anti-toxicity effect in the cerebellum has been previously reported (Owolabi *et al.*, 2014). The brain tissue remains a tissue on which more specific reports about the results of moringa phytochemicals' interactions with the tissue should be properly documented.

Brain Hippocampus

The hippocampus of the control Group A animal is represented in photomicrographs in Figure 4. Photomicrographs A₁, A₂ and A₃ are illustrations of the control Group animals (dentate gyrus); the various regions or zones of the dentate gyrus are observable- the molecular granular and polyform layers are all observable. The neurons and glia are also present- all are morphologically normal. The treated Group B hippocampus tissue is illustrated in B₁, B₂ and B₃. Tissue has no sign of disruption or damage. The granular cells are clearly defined and compact in organisation, suggesting they are also normal. The dentate gyrus granular layer of cells appears relatively thin; this is however not enough to infer a compromising consequence. It could rather be an observation to be investigated furthermore. Moringa leaf extract as administered in this investigation did not produce any histologically compromising effects on the hippocampus of the treated animals. Not many reports are available on the particular effects of *Moringa oleifera* on the hippocampus; our previous report however showed that it could produce an anti-lead toxicity in the hippocampus (Owolabi *et al.*, 2014).

Epididymis

The epididymis of the control Group A animals is being illustrated in Figures A₁, A₂ and A₃ at various suitable magnifications. The epithelium of the epididymis could be observed (EE) as well as the lumen of the tubular structure (L). The observations could be correctly used to adjudge the epididymis in this group to the normal. Figure 6- B₁, B₂ and B₃ are histological representation of the epididymis of the treated Group B animals at three various magnifications. Figure 6- B₁, B₂ and B₃ show the epididymis of the treated Group B models obviously with certain anomalies: the epithelium for the entire structure is grossly disrupted and there is an abnormal accumulation of tissues- tissue debris (TD)- in the lumen. The loss of the epithelium is no doubt an indication that the functions of this organ as a store and nurture chamber for produced spermatozoa until copulation and ejaculation is seriously compromised. Worst still, the lumen appears to contain more than the usual and normal germ cells or spermatozoa. These could possibly be an aggregate of deformed spermatozoa and in addition, the lost epithelial cells. Even if spermatozoa are produced normally in the testis and stored in the epididymis, there are indications that their forms and functions or viability could be seriously compromised. How effective the damaged tissue can respond to reparative stimuli cannot however be measured; but the signs suggest that in cases of continuous administration, the epididymis might have its form and

functions disrupted by the effects of moringa extract phytochemicals. This supports pre-existing reports that moringa plant parts could produce anti-fertility effects when ingested. Lilibeth and Glorina (2010) had earlier reported moringa extract producing unusual effects of the mice epididymis including unusual thickening of the wall and epidermis inactivity; this shares similarities with the current investigation.

Cardiac Muscle

The photomicrographs in Figure 5- A₁ and A₂ illustrate the cardiac muscle tissue of the control Group A animals. The cardiac muscle cells or myocytes (CMC) nuclei as well as the fibrils (CMF) are observable at both magnifications. The organisation of the cells into tissue also appears normal for a healthy heart muscle. Figure 5- B₁ and B₂ are histological illustrations of the heart muscle of the Group B animals treated with moringa leaf extract; the basic elements are also observable as labelled and they are all normal in form, organisation and distribution. Moringa leaf extract therefore did not produce any histologically observable deleterious effects on the heart muscle of the treated animals. While it may be ambiguous to suggest from observations in this context that the extract effects could improve the structure of the heart muscle; it may however be adjudged to be safe for the heart tissue as used in the investigation. Positive effects of moringa extract against isoproterenol-induced myocardial damage in rats has been reported (Nandave, 2009). Davis (2010) also reported moringa to have affected heart muscle contractility in a way that could produce positive effects to counter anomalies such as hypertension while Gunjal *et al.*, (2010) reported that the stem bark extract could have protective effects on myocardial tissue.

Kidney

The renal tissues of the experimental animals are being illustrated histologically in the photomicrographs presented in Figures 7. Figure 7- A illustrates the cortical structures especially the glomerulus (G) and the neighbouring renal tubules (RT). The renal corpuscle that contains the glomerulus as a whole is quite well defined and the constituting elements are clearly observable.

There is the Bowman's capsule; the glomerulus with its capillaries and their constituting elements (endothelial cells); the terminal ends of the afferent arteriole and the beginning of the efferent arteriole; the mesangium partly within the glomerulus partially and extending outside the glomerulus. The constituent cells of the elements are not distinguished by the staining mechanism of the dye employed, understandably a dye for general and

proper demonstration of tissues' histo-architecture; the morphology of the cells however suggest that they are normal and properly organised as they should be in a functional kidney cortex. The podocytes are cells that wrap their processes round the capillary tufts to achieve effective ultrafiltration in the process of urine formation- they are prominent in glomerular presentations. The endothelial cells also form primarily the walls of the capillaries; and there is the mesangial cell. The thin epithelium- simple squamous that forms the Bowman's capsule is equally observable.

Figure 7- B1 is a demonstration of the kidney cortex of the Group B animals treated with the moringa leaf extract. All the aforementioned features of the renal corpuscle (for the control) are present and appear normal. In addition, the glomerulus appear quite well defined, relatively better compactly organised with the constitute cells being more relatively prominent. While there are no usual disadvantages reported in association with better corpuscular element organisation, disorganisations or disruptions could easily be linked with renal malfunctioning; therefore improved organisation of this structure as observed could most likely be of an additional physiological advantage for the kidney, especially with respect to ultra filtration cum urine production- the primary activities that take place within the cortex. In both groups, the photomicrographs portray healthy renal tubules- both proximal and distal convoluted. Renal tubules are normal in the treated animals' photomicrographs; like the glomerulus relative to the control, they also appear better defined.

Paliwal *et al.*, (2011) reported the anti-nephrotoxic effect of Moringa oleifera Lam; Ezejindu *et al.*, (2014) reported that Moringa oleifera leaf extract would not produce any deleterious effects on the kidney of experimental animals even in cases of chronic administration. Awodele *et al.*, (2012) reported moringa leaf consumption to be relatively safe at sub-lethal doses especially with respect to its effects on the kidney and liver tissues. Oyagbemi *et al.*, (2013) however suggested that chronic use could predispose animals to hepatic and kidney damage. The current finding however shows that at moderate doses, moringa leaf extract ingestion is safe for the renal tissues.

Liver

The liver tissue of the Control Group A is being illustrated at various suitable magnifications in photomicrographs A₁, A₂ and A₃ of the Figure 8. The lowest magnification shows a normally organised liver tissue with the plates of hepatocytes being separated by sinusoids. The central vein is also observable. At the higher magnifications- A₂ and A₃; the hepatocytes are observable (H), arranged in plates as well as the sinusoids (S) separating them. A few Kupfer cells (KC) are also observable. The portal

triad- artery, vein and bile duct branches are also observable (PT). The liver tissue as a whole is healthy and normal. Group B photomicrographs (B₁, B₂ and B₃) are illustrations of the Group B animals' liver tissues. All the basic features of a normal liver tissue as found in the control are present. The hepatocytes are however quite prominent and they appear better defined. While these observations show that the administered moringa leaf extract would not produce deleterious effects on the liver tissues; it could also suggest that it could stimulate better state of health and functional status of the hepatocytes; and consequently, improve the functions. It should be noted that hepatocytes acute states of health depend greatly on their functional response to systemic bio- and chemo-assaults. Moringa could therefore have produced synergistic anti-toxicity [such as anti-oxidant actions] or effects to either complement the liver's similar functions, provide prophylactic effects against the consequences of cellular (hepatocytes) activities or help the liver cells improve their state of health. It is important to note that a number of murine model investigations have reported the potency *Moringa oleifera* leaf extracts in protecting the liver from chemical toxicity and damage (Pari and Kumar, 2002; Ndong *et al.*, 2007; Fakurazi *et al.*, 2008; Buraimoh, 2011).

Lung

The lung tissue is being illustrated in Figure 9; A₁, A₂ and A₃ are photomicrographs of the control Group of experimental animals. The alveolar sacs (AS) and the alveolar ducts (AD) leading to them are labelled. The supportive tissues are also of adequate structural integrity. The lung tissue of the control group is therefore normal and well defined. The treated Group B lung tissue is also normal and all the basic features as mentioned for the Group A are also observable and they appear normal. There is therefore histological evidence that moringa extract treatment does not produce any form of deleterious effect on the lung tissue of the treated animals; there is however not adequate evidence to suggest a structural or functional improvement of the Group B lung tissue over the Group A- control. Only a few publications have reported the effects of *moringa* leaf extracts on its own on the lung tissue; however, the antiproliferative effects of moringa leaf extract against alveolar epithelial cell cancer was reported by Tiloke *et al.*, (2013). Dany *et al.*, (2012) and Jung (2014) also suggested that the leaf contains specific anticancer (active for the lung) agent; Owolabi *et al.*, (2012) reported the anti-lead toxicity effects of then leaf on the lung tissue while Yahya *et al.*, (2014) reported that the antioxidant properties of moringa could make it ameliorate the effects of cement dust exposure.

Skeletal Muscle

Figure 10- A₁, A₂ and A₃ are photomicrographs illustrating the skeletal muscle of the control Group A animals. The general histological architecture is normal in cross sections and the fibre bundles or fascicle [MF] are observable even at the lowest magnification employed. The perimysium [P] bundling the fibres are also observable especially at the medium, magnification. In B₃, the myocytes nuclei [MCN] are observable as well as the endomysium [E] surrounding the syncytium-fibre. The treated Group B skeletal muscles have all the basic histological features of a normal skeletal muscle tissue. An important observation worth of attention is the prominence and the relative abundance of myocytes nuclei. This observation suggests hyperplasia- a condition that could build muscle tissues via stimulated or increased cell division, hence producing more cells and subsequently, more fibres. It could be surmised therefore that moringa extract treatment improved muscle volume by inducing hyperplasia in the skeletal muscle; this is normal of a physiological implication. Bhattacharya (2014) reported moringa effect as muscle relaxant; general body growth effects in poultry- including muscles- has also been reported by Teteh *et al.*, (2013)

Spleen

The spleen tissue of the control animals is illustrated in Figure 11- A₁ and A₂; the white pulps (WP) and the red pulps (RP) are clearly defined at both magnifications used. This spleen tissue is normal in histological presentation and could serve the purpose of a suitable reference. In Figure B₁ and B₂; the photomicrographs illustrate the spleen issue of the Group B animals administered moringa leaf extract. The pulps are also well defined and the entire tissues structural integrity is normal. The white pulps appear relatively quite prominent. This structure is quite important for the immune roles of the spleen. Observations therefore strongly suggest that *moringa* leaf extract treatment did not produce any deleterious effect on the spleen tissue structure; there however could have been structural improvement that could translate into improved physiological functions of the tissue. *Moringa* has been reported to produce positive effects on the body's immunity (Gupta, 2010); while Ogunlade *et al.*, (2013) observed that moringa leaf extract could ameliorate the toxicity of aniline on the spleen, thus preventing splenomegaly and splenic hyperplasia.

Testis

The testicular tissues of the experimental animals are histologically illustrated in photomicrographs in Figure 12;

A₁, A₂ and A₃ are photomicrographs of the tissue of the control Group A animals. The seminiferous tubules (ST) are well defined and the interstitial tissues are also defined. The seminiferous tubule epithelium (STE) is well defined and has a normal histo-architecture; so also the central lumen (STL; Figure 12- B₃). The control Group testicular tissue is therefore normal. Figure 10- B₁, B₂ and B₃ are the histological representations of the Group B animals' testis. Extensive disruption of the histo-architecture is observable at all magnifications. The seminiferous tubules have grossly disrupted epithelium and consequently, large lumens. At the higher magnifications, it is evident that the disruptions cut across the various zones or layers of germinal cells which arrangement also correspond with the stages of development. Consequently, spermatogenesis would be generally compromised irrespective of the stage of the maturation of the cells. Only a few isolated and deformed cells are left of the epithelia. A few basal cells in the epithelium appear to remain [B₃]; this could be an indication for possible regeneration upon withdrawal of extract administration or introduction of therapeutic agents. The interstitial structures are also damaged by the effects of the administered substance. All observations as stated point to compromises in the process of producing spermatozoa- spermatogenesis. This is evidence that moringa leaf extract has anti-fertility effects in the male. Paul and Didia (2012) had earlier reported the deleterious effects of the root extract on the testis. Traditional and early experimental reports have implicated this plant product has having fertility influencing effects such as being abortifascient (Nath *et al.*, 1992; Tarafder 1983), and as a birth control (Shukla *et al.*, 1988a, b, c, 1989; Faizi *et al.*, 1988). While moringa leaf extract would produce deleterious effects on the testis; it appears to ameliorate certain induced deleterious effects on the same organ by certain other agents such as diabetes induced testicular damage (Ebong, 2014); lead induced testicular damage (Owolabi *et al.*, 2012); mercury (Asomugha, 2014) and alcohol-induced testicular toxicities (Bassey *et al.*, 2013).

CONCLUSION

Moringa oleifera leaf extract did not produce histologically observable deleterious effects on the brain- cerebrum, cerebellum and hippocampus, kidney, liver, bone marrow elements; however, it produced extensive histological disruptions of the reproductive organs- testis and epididymis, thus acting as an anti-fertility agent. Certain tissues assumed improved Histomorphology due to the effects of moringa extract treatment; these include the kidney, spleen and liver. In conclusion, *Moringa oleifera* extract as used is not toxic; it is rather anti-fertility.

REFERENCES

- Adedapo AA, Mogbojuri OM, Emikpe BO (2009). Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats. J. Med. Plant 3, 586–591.
- Asomugha AL, Ezejindu DN and Nwabekee PO (2014). The protective effect of *Moringa oleifera* leaf extract on mercury induced testicular toxicity in adult wistar rats. Unique Research Journal of Medicine and Medical Sciences. 2(4): 043-051.
- Awodele S, Oreagba IA, Odoma S, da Silva TJA, Osunkalu VO (2012). Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). J. Ethnopharmacol. 139(2): 330-336.
- Bassey RB, Bala DN, Edagha IA, Peter AI (2013). The effect of ethanolic extract of *Moringa oleifera* on alcohol-induced testicular histopathologies in pre-pubertal albino Wistar rats. Biology and Medicine 5:40-45.
- Bhattacharya A, Naik MR, Agrawal D, Sahu PK, Kumar S, Mishra SS (2014). CNS Depressant and Muscle Relaxant effect of Ethanolic Leaf Extract of *Moringa oleifera* on Albino Rats. Int. J. PharmTech Res. 6 (5): 1441-1449.
- Buraimoh AA (2011). Hepatoprotective effect of ethanolic leave extract of *Moringa oleifera* on the histology of paracetamol induced liver damage in Wistar rats. Int. J. Anim. Vet. Adv. 3, 10–13
- Chatchada S, Jintanaporn W and Wipawee T (2013). *Moringa oleifera* Mitigates Memory Impairment and Neurodegeneration in Animal Model of Age-Related Dementia. Oxidative Medicine and Cellular Longevity 2013: 695936.
- Dany M, Madi N, Nemer N, Beyrouthy M, Abdoun S, Usta J (2012). *Moringa oleifera*: Natural leaf extract with potential anti-cancerous effect on A549 lung cancer cells. Lung Cancer, 77 (1): S22.
- Davis NR (2010). Effect of moringa oleifera leaf extracts on the isolated rabbit heart and uterus. Makerere University Institutional Repository; School of Health Sciences (Health-Sciences) Collections
- Ebong PE, Efiang EE, Mgbeje BIA, Igile GO and Itam EH (2014). Combined Therapy of *Moringa oleifera* and Ocimum gratissimum Reversed Testicular Damage in Diabetic Rats. Brit. J. Med. Med. Res. 4(1): 27-290.
- Ezejindu DN, Udemezie OO, Akingboye AJ (2014). Protective Effects of *Moringa oleifera* Leaf Extract On the Kidneys of Adult Wistar Rats. American Journal of Engineering Research (AJER). 3 (2): 157-161.
- Faizi S, Siddiqui BS, Saleem R, Aftab K and Shaheen F, (1988). Bioactive compounds from the leaves and pods of *Moringa oleifera*. N. Trends in Natural Products Chemistry, pp: 175-183.
- Fakurazi S, Hairuszah I, Nanthini U (2008). *Moringa oleifera* Lam prevents acetaminophen induced liver injury through restoration of glutathione level. Food Chem. Toxicol. 46, 2611–2615
- Ganguly R, Hazra R, Ray K and Guha K (2005). Effect Of *Moringa Oleifera* In Experimental Model Of Alzheimer's Disease: Role Of Antioxidants. Annals of Neurosciences. (12):3.
- Ghazal OK, Owolabi JO, Williams FE, Lambe E. (2012). Effects of Ethanolic Extract Of *Moringa Oleifera* Leaves on Lead Acetate Induced Liver Damage in Adult Wistar Rats Int. Jour Biotech Biomed Res; 2: 12: 4009
- Gunjal MA, Shah AS, Wakade AS and Juvekar AR (2010). Protective effects of aqueous extract of *Moringa oleifera* Lam. Stem back on serum lipids, marker enzymes and heart antioxidants parameters in isoproterenol-induced cardio toxicity in Wistar rats. Indian Journal of Natural Products and Resources 1(4): 485-492.
- Gupta A, Gautam MK, Singh RK, Kumar MV, Rao ChV, Goel RK, Anupurba S (2010). Immunomodulatory effect of *Moringa oleifera* Lam. extract on cyclophosphamide induced toxicity in mice. Indian J Exp Biol. 48(11):1157-60.
- Hamza AA(2007) Curcuma longa, Glycyrrhiza glabra and *Moringa oleifera* ameliorate diclofenac-induced hepatotoxicity in rats. Am J Pharmacol Toxicol 2: 80-88.
- Jung IL (2014). Soluble Extract from *Moringa oleifera* Leaves with a New Anticancer Activity. PLoS ONE 9(4): e95492.
- Kirisattayakul W, Wattanathorn J, Tong-Un T, Muchimapura S and Wannanon P (2012). *Moringa Oleifera* Lam Mitigates Oxidative Damage and Brain Infarct Volume in Focal Cerebral Ischemia. Ame. J. Appl. Sci. 9 (9): 1457-1463.

- Lilibeth, AC and Glorina LP (2010). Effects Moringa oleifera lam (moringaceae) on the reproduction of male mice (mus musculus). J. Med. Plant Res., 4(12): 115-1121.
- Nandave M, Ojha SK, Joshi S, Kumari S and Arya DS (2009). Moringa oleifera leaf extract prevents isoproterenol-induced myocardial damage in rats: evidence for an antioxidant, antiperoxidative, and cardioprotective intervention 12 : 47-55.
- Nandave M, Ojha SK, Joshi S, Kumari S, Arya DS (2009). Moringa oleifera leaf extract prevents isoproterenol-induced myocardial damage in rats: evidence for an antioxidant, antiperoxidative, and cardioprotective intervention. J Med Food. 12(1):47-55.
- Nath D, Sethi N, Singh RK and Jain AK (1992). Commonly used Indian abortifacient plants with special reference to their teratologic effects in rats. J. Ethnopharmacol., 36: 147-154.
- Ndong M, Uehara M, Katsumata S, Sato S, Suzuki K. (2007). Preventive effects of *Moringa oleifera* (Lam) on hyperlipidemia and hepatocyte ultrastructural changes in iron deficient rats. Biosci. Biotechnol. Biochem. 71, 1826-1833
- Ogunlade B, Akunna GG, Saalu LC, Aniah JA and Enye LA (2013). Haematological indices and splenic histo-architecture of wistar rat treated with aniline: supplementary role of moringa oleifera leave extracts. International Journal of Biotechnology and Allied Fields 1(3): 136-144.
- Ouédraogo M, Lamien-Sanou A, Ramdé N, Ouédraogo AS, Ouédraogo M, Zongo SP, Gombri O, Duez P, Guissou PI (2013). Protective effect of Moringa oleifera leaves against gentamicin-induced nephrotoxicity in rabbits. Exp Toxicol Pathol. 2013; 65(3):335-9.
- Owolabi J and Williams F (2014). Histological Assessment of Moringa oleifera Anti-Toxicity Actions Against Lead-Induced Damage to the Cerebellar Cortex in Animal Models. International Journal of Moringa and Nutraceutical Research. 1(1): 75-84.
- Owolabi J, William F, Olanrewaju J, Etibor T, Fabiyi O (2014) Histomorphological Evidences of Moringa oleifera's Ameliorative Effects against Lead Toxicity in Cerebral Cortex. World Journal of Life Sciences and Medical Research; 3(2):53-8.
- Owolabi J, Williams F, Fabiyi O (2014). Evaluation of Moringa's Effects Against Lead- Induced Disruption of the Hippocampus in Animal Models. World Journal of Life Sciences and Medical Research 3(2):39-45.
- Owolabi JO, Ghazal OK, Willaims FE and Ayodele EO (2014). Effects Of Moringa Oleifera (Drumstick) Leaf Extracts On Lead-Induced Testicular Toxicity In Adult Wistar Rat (Rattus Novergicus). Int. J. Biotechnol. Biomed. Res. 2: 12:4003 12/2012; 2(1).
- Owolabi JO, Ghazal OK, Dare M, Olanrewaju JA, Caxton-Martins AE, William FE (2013). Prophylactic And Regenerative Effects of Alcoholic Extract Of Moringa Oleifera On Lead-induced Lung Tissue Damage in Adult Male Wistar rats Models. *European Journal of Anatomy* 17(2): 115-122.
- Oyagbemi AA, Omobowale TO, Azeez IO, Abiola JO, Adedokun RA, Nottidge HO (2013). Toxicological evaluations of methanolic extract of Moringa oleifera leaves in liver and kidney of male Wistar rats. J Basic Clin Physiol Pharmacol. 2013;24(4):307-12.
- Paliwal R, Sharma V, Pracheta, Sharma S, Yadav S, Sharma S (2011). Anti-nephrotoxic effect of administration of Moringa oleifera Lam in amelioration of DMBA-induced renal carcinogenesis in Swiss albino mice. Biology and Medicine, 3 (2): 27-35.
- Pari L., Kumar N. A. (2002). Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. J. Med. Food 5, 171-177.
- Paul CW and Didia BC (2012). Methanolic Extract of Moringa oleifera Lam Roots is Not Testis-Friendly to Guinea Pigs. Asian Journal of Medical Sciences 4(1): 47-54.
- Selvakumar D, Natarajan P (2008) Hepatoprotective activity of Moringa oleifera Lam. leaves in carbon tetrachloride induced hepato-toxicity in albino rats . Pharmacognosy Magazine 4: 97-98.
- Shukla S, Mathur R and Prakash AO (1988a). Biochemical and physiological alteration in female reproductive organs of cyclic rats treated with aqueous extract of Moringa oleifera lam. Acta Europ. Fert., 19: 225-232.
- Shukla S, Mathur R and Prakash AO (1988b). Anti-implantation efficiency of *Moringa oleifera* lam and moringa concanensis nimmo in rats. Int. J. Crude Drug Res., 26 (1): 29-32
- Shukla S, Mathur R and Prakash AO (1988c). Anti-fertility profile of the aqueous extract of *Moringa oleifera* root. J. Ethnopharmacol., 22:51-62.
- Shukla S, Mathur R and Prakash AO (1989). Biochemical alterations in the female genital tract of ovariectomized rats treated with aqueous extract of Moringa oleifera lam. Pak. J. Scient. Indust. Res., 32(4): 273-277.
- Taraferder, CR (1983). Ethnogynaecology in relation to plants: 2 plants used for abortion. J. Econ. Taxon. Bot., 4(2):507-516.
- Teteh A, Lawson E, Tona K, Decuypere E and Gbeassor M (2013). *Moringa Oleifera* Leave: Hydro-Alcoholic Extract and Effects on Growth Performance of Broilers. *International Journal of Poultry Science*, 12: 401-405.
- Tiloke C, Phulukdaree A and Chuturgoon AA (2013). The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on cancerous human alveolar epithelial cells. BMC Complementary and Alternative Medicine , 13:226
- Woranan K, Jintanaporn W, Terdthai T, Supaporn M, Panakaporn W and Jinatta Jittiwat (2013). The Cerebroprotective Effect of Moringa oleifera against Focal Ischemic Stroke Induced by Middle Cerebral Artery Occlusion. *Oxidative Medicine and Cellular Longevity* Volume 2013: 951415
- Yahaya T, Okpuzor J and Ajayi T (2014). Antioxidant Activity of Rosele (Hibiscus sabdarifa), Moringa (Moringa oleifera), Ginger (Zingiber officinale) and 'Ugwu' (Telfairia occidentalis) in the Lungs of Albino Rats (Ratus norvegicus) Exposed to Cement Dust. Annual Research & Review in Biology. 4(5): 736-746.