HISTOMORPHOLOGICAL STUDY OF LEAD-INDUCED LUNGS LESION OF MICE TREATED WITH *MORINGA OLEIFERA* LEAVES EXTRACTS

Zakariyya A. A.[1*],Muhammad, I.H. [1], Shema F. B.[1], Ibrahim, D. F.[1,2], David T. N.[1, 2], Fasogbon, S. A.[3], Olayera, A. B.[4], Egbo, C. A.[5], Adeyinka, S. A.[6]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Health Sciences, Bayero University, Kano

[2]Histopathology Department, Aminu Kano Teaching Hospital, Kano

[3]Public Health In-vitro Diagnostic Control Laboratory, Medical Laboratory Science Council of Nigeria, Lagos, Nigeria

[4]Department of Haematology and Immunology, University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State, Nigeria

[5]Department of Community Health, College of Medicine, University of Benin, Benin City, Nigeria.

[6]Department of Anatomic Pathology, Irrua Specialist Teaching Hospital, Irrua, Edo state, Nigeria

***Corresponding Author:** Email: azakariyya.mls@buk.edu.ng; +2348024693838.

ABSTRACT

Background: *Moringa oleifera* leaves extracts are currently among the most common medicinal plants used to cure some diseases. Many studies have been conducted on the benefits of *Moringa oleifera* leaves extracts but only few studies focused on its Anti-fibrotic effects on lung tissue.

Aim: To study the Histomorphological changes associated with *Moringa oleifera* Leave extracts on lead-induced lung lesion of Mice.

Materials and Method: Twenty adult mice were divided into four groups, three test groups (A, B and C) and a control group (D). The test groups were administered orally with 5mg/kg lead acetate and 10mg/kg *Moringa oleifera* leaves extract concurrently; 5mg/kg lead acetate only; 10mg/kg *Moringa oleifera* leaves extract only; while the control group did not receive any intervention. The intervention period lasted for three weeks. On the twenty-two day of the research, the animals were sacrificed; lung tissues were harvested, processed for histological examination and stained using Hematoxylin and Eosin staining technique.

Result: Group A animal administered with lead acetate and *Moringa oleifera* leaves extract concurrently, shows that the deleterious effects of Lead were ameliorated, with an improve histological features of the lungs when compared with that of the Group B. while an Ill defined alveoli, thinned alveoli wall, sacs emphysema and distortion of the parenchyma cells that constitute the lung tissue are seen in test Group B, administered with lead acetate only. A remarkable, intact lung alveoli, lung parenchyma cells were observed to in test Group C and very much correlated to that of the control group.

Conclusion: *Moringa oleifera* administration reduced the severity of lead's damaging effects to the lungs, and may likely exhibits anti-fibrotic as well as prophylactic effects by repairing damaged alveoli and in the latter case by preserving and /or regenerating the lung tubules.

Key words: Anti-fibrotic, lead-induced, lung, lesion, *Moringa oleifera*

Citation: Zakariyya A. A. ,Muhammad, I.H., Shema F. B., Ibrahim, D. F., David T. N., Fasogbon, S. A., Olayera, A. B., Egbo, C. A, Adeyinka, S. A. (2020): Histomorphological Study of *Moringa oleifera* Leaves Extracts On Lead-Induced Lungs Lesion of Mice· *BJMLS. 5(2): 236 - 244*

INTRODUCTION

Moringa oleifera has been a model source of medicines as it contains chemical agents with therapeutic properties. *Moringa oleifera* is the most widely cultivated species of a monogeneric family, the *Moringaceae*, that is native to the sub-Himalayan tracts of India, Sri lanka, Malaysia, Pakistan, Bangladesh and Afghanistan (Fahey, 2008). The plant has a very wide range of traditional uses. It has been cultivated in tropical regions all over the world for reasons that include its high protein, vitamin, mineral and carbohydrate content, high value of fat for humans and livestock, high oil contents with medicinal uses and coagulation of water particle impurities (Foidl *et al*., 2001). *Moringa oleifera* contains high protein, vitamin, mineral and carbohydrate, high oil content with medicinal uses and coagulation (Foidl *et al.,* 2001). *Moringa oleifera* has been used for variety of purposes and as therapy for some ailment e.g. hypertension, diabetes, cancer, epilepsy, conception, tuberculosis. Also properties such as anti-inflammatory, antibacterial, antifungal and antiviral have been attributed to *Moringa oleifera* extracts (valia *et al.,* 1993). Furthermore, the administration of *M. oleifera* seed powder has been reported to ameliorate diabetic nephropathy and restore normal histology of both kidney and pancreas when compared with a diabetic positive control group in a study carried out by Al-Malki in 2015. Lead a metal with a symbolic-element of pb. It is a shiny, blue-white soft metal when its surface is fresh. On exposure to the air, it becomes covered by a dull gray layer of basic carbonate that adheres closely and protects it from further alteration (Loghman-Adham, 1997). Lead is commonly used in several industrial fields by people who are unaware of its adverse effects on human health. It is an accumulative poison that builds up until it reaches a toxic level. Lead is usually taken spontaneously from the lead polluted environment via water, air and food (Joshua *et al;* 2013). Acute lead poisoning results from ingesting soluble lead compounds (Loghman-Adham, 1997).Its absorption in the body is enhanced by Calcium and Zinc deficiencies (Duruibe *et al.,* 2007). Acute lead poisoning may result in a dysfunction in the kidney, reproduction system, liver, lung and brain resulting in sickness and death (Jaishankar *et al.,* 2014). Lead poisoning also causes inhibition of the synthesis of haemoglobin, cardiovascular system and acute and chronic damage to the central nervous system (CNS) and peripheral nervous system (PNS) (Wani *et al.,* 2015). Other effects include damage to the gastrointestinal tract (GIT) and urinary tract resulting in bloody urine, neurological disorder and severe or permanent brain damage (Jaishankar *et al.,* 2014). Lead has more detrimental effects on children particularly of age 2-3 years, causing poor development of the grey matter of the brain, thereby resulting in poor intelligence quotient (Duruibe *et al*., 2007). While inorganic forms of lead, typically affect the CNS, PNS, GIT and other biosystems, organic forms predominantly affect the CNS (Duruibe *et al.*, 2007). Although the effects of lead taken orally on gastrointestinal and urinary systems have been well established, it has been suggested that lead inhalation may be more dangerous than any other route of consumption. The damage appears to be mainly to the nervous system and the effects not as acute, as those of mercury poisoning (Wani, *et al.,* 2015).The toxic effects of lead on the lung are the most harmful when compared to other body organs (Valia *et al*., 1993). Also Haslam *et al.*, 1983 reported that consumption or inhalation of lead or other metals for a long time can cause pneumonia or asthma. Furthermore, the study by Valia *et al*., (1993) showed that oral administration or inhalation of lead could lead to fibrosis of lung tissue. Incessant exposure of human to this debilitating metal in our rural area, most especially where rampant or illegal mining are being carried out. The people of such local area are expose to lead poison and lack the awareness of toxic effects caused by lead (Joshua *et al*., 2013).

Most people in this rural area are battling with degrees of health care challenges arising from the menace of lead toxicity. *Moringa oleifera* growing everywhere with many therapeutic constituent may serve as a cheap remedy and play a role in addressing the toxicity effect of lead. Thus this study elucidates the histomorphological changes in Lead-induced lung tissue lesions of mice and treated with *Moringa oleifera* leaves extracts.

MATERIALS AND METHODS

Study design

The study was an experimental study. Ethical approval for the research was soughed and obtained from Research Ethical Committee, College of Health Science, Bayero University Kano, Kano state.

Preparation of Experimental Animal

Twenty (20) Healthy adult mice were purchased from the Animal House unit of the department of Pharmacology, Faculty of Pharmaceutical Sciences, College of Health Sciences, Bayero University Kano. Animals were maintained on standard lab pellet diet and tap water. They were housed in clean plastic cages with wood chip bedding, natural day/light cycle, at ambient temperature. The Mice were acclimatized for 2 weeks prior to experimental regimen. All animals were handled in accordance with guidelines for animal research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (NIH, 2020).

Preparation of *Moringa oleifera* extracts

Fresh mature leaves of *M. oleifera* were collected from Rimi Market, Kano State from early rainy season and taken to the Department of Botany, Faculty of Life Sciences, Bayero University Kano for identification and assignation of herbarium number. The leaves were washed thoroughly to remove debris and dust particles and were dried at room temperature for 15 days. The leaves were homogenized to powder form using mechanical grinder, two hundred (200) grams of the powdered material was macerated in 1Liter of distilled water and left for 24 hours after which it was filtered using Whatman's filter paper. The filtrate was dried in a hot air oven at 40o C to give 24.5g of the aqueous leave extracts. The extracts were collected into an air tight container and kept until use.

Lungs toxicity induction

Lungs toxicity of the mice were induced with 0.3% of lead acetate per kg of body weight by using four equally divided gavages in 30 minutes' intervals orally, after abstaining from normal feed and water for six (6) hours (Ibrahim *et al*., 2012).

Animal grouping and intervention

Twenty (20) were use for this research. The animals were randomly divided into 4 groups; labeled as group A, B, C and D, with each group having five (5) rats. The groups constitute of three test group and one control group. The test group A animals were administered orally with *Moringa oleifera* leaves extract and Lead acetate at a dose of 10mg/ml and 5mg/ml lead /kg of body weight respectively and concurrently. The Group B animals were administered orally with 5mg/ml lead /kg of body weight only, while Group C animals were administered only with 10mg/ml *Moringa* leaves extract /kg of body weight orally. These interventions were carried out every day for a period of three weeks. Group D animals served as control group and did not receive any intervention but were rather administered with distilled water. The animals were weighed just before and after the intervention.

Preparation and removal of the lungs

On the end of the 22nd day, the animals were sacrificed by cervical dislocation and Lung organ were removed, fixed for 48 hours in neutral buffered formalin and manually processed for histological examinations. The Processed tissues were embedded in paraffin wax and tissue sections of three (3) microns were cut using a Leica brand of Microtome. The histological sections floated out in tissue water bath, picked with a glass slide and stained with Hematoxylin-Eosin stain for demonstrations of the tissue architecture and examinations. in the histology laboratory. (Avwioro, 2014).

Data analysis

The results obtained were presented in tables and photomicrographs. All the test groups animals were compared with the control group using one-way analysis of Variance (ANOVA) tool of Statistical Package for Social Sciences(SPSS) IBM version 20.0 where necessary.

RESULTS

Physical property of the *Moringa oleifera* Leave extracts The aqueous extraction procedure yielded 50.8 grams, thus the percentage yield extract is 25.4g. while physical properties of the extracts are green in colour, gel in consistency with a burned smell.

Table: 1 Physical Properties of *Moringa oleifera* Leave Extract

Plant Part	Extract Type	% Yield	Texture	Colour	Smell
Leaves	Aqueous	25.4%	Gelly	Green	Burned smell

Acute Toxicity Studies

The aqueous extract administered to the mice showed no sign of toxicity or behavioral change. After 24 hours observation, no deaths were recorded in both Phase I and Phase II of the experiment. Thus the Lethal Dose (LD_{50}) of *Moringa oleifera* leaves extract was therefore above 5000mg/kg as shown in the table below:

Table:2 Showing the result for LD_{50} of aqueous extract of the leaves of *Moringa oleifera*

DOSE	OBSERVED CHANGES / MORTALITY	
(mg/kg)	PHASE I	PHASE II
10	0/3	-
100	0/3	-
1000	0/3	-
1600	-	0/1
2900	-	0/1
5000	-	0/1

Using the formula by Lorke (1983), $LD_{50} = \sqrt{(D_0 \times D_{100})}$

Where LD_{50} = Lethal Dose

LD_0 = Highest dose that gave no mortality

LD_{100} = Lowest dose that gave mortality

Therefore, the LD_{50} of the aqueous leaves of *Moringa oleifera* is greater than 5000mg/kg body weight. There was no any observable physical change due to consumption of the aqueous extract of *Moringa oleifera* in extract alone treated group (Group C), but retained weights was observed. Lead treated group alone shows a little decrease in weight (Group B), the weight was maintained in the group treated with both lead and the aqueous plant extract (Group A). Also group D (control group) showed mild increase in weight which might be due to the regular feeding.

Effects of *Moringa oleifera* extract on the Gross Morphology of the lungs

There were remarkable morphological changes seen both in size and weight of the lungs of mice from test group B when compare to the lungs of mice in the control group. Histological changes associated with effect of aqueous extract of *Moringa oleifera* Histological changes were evaluated in lungs sections stained with Haematoxylin and eosin. In the test group B treated with lead acetate only. There are Alveoli ill-defined, distorted, thinned alveolar wall and respiratory cells are distorted (Plate 2).

While these features where minimize with an improve conditions, where seen in the group A animal treated with both Lead acetate and *Moringa oleifera* concurrently (Plate 1). No Histological changes observed in test group C treated *Moringa oleifera* leave extract only. Rather a well define Alveoli with normal Alveolar wall and intact respiratory cell were observed (Plate 3), which correlated with that of the control group D administered with distilled water only (Plate 4).

Histological Study

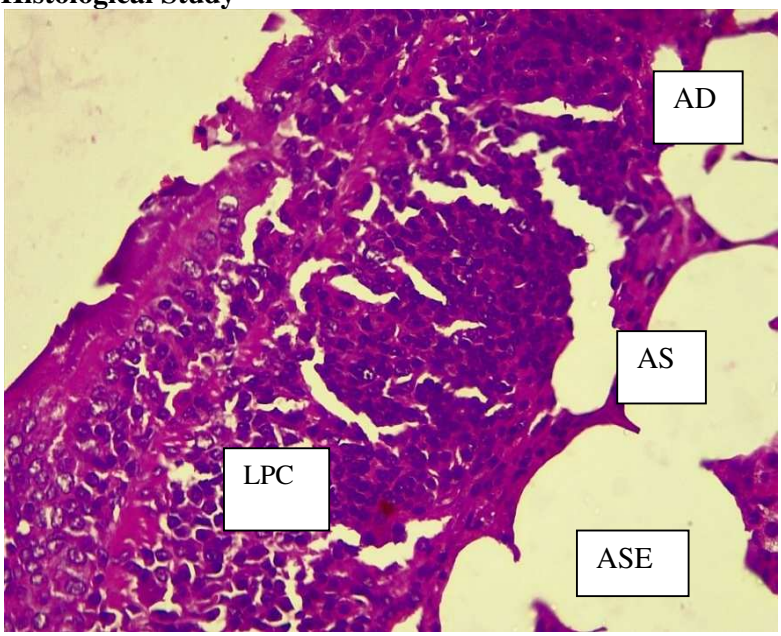

PLATE1: Photomicrograph of lung section of group A animal (5mg/kg lead and 10mg/kg *Moringa oleifera*) showing mild dilatation of respiratory bronchioles and alveolar sac and intact lung parenchyma cells (H & E stain ×400).
KEY: AS, Alveolar sac, AD, Alveolar duct, ASE, Alveolar sac epithelium, LPC, Lung parenchyma cells.

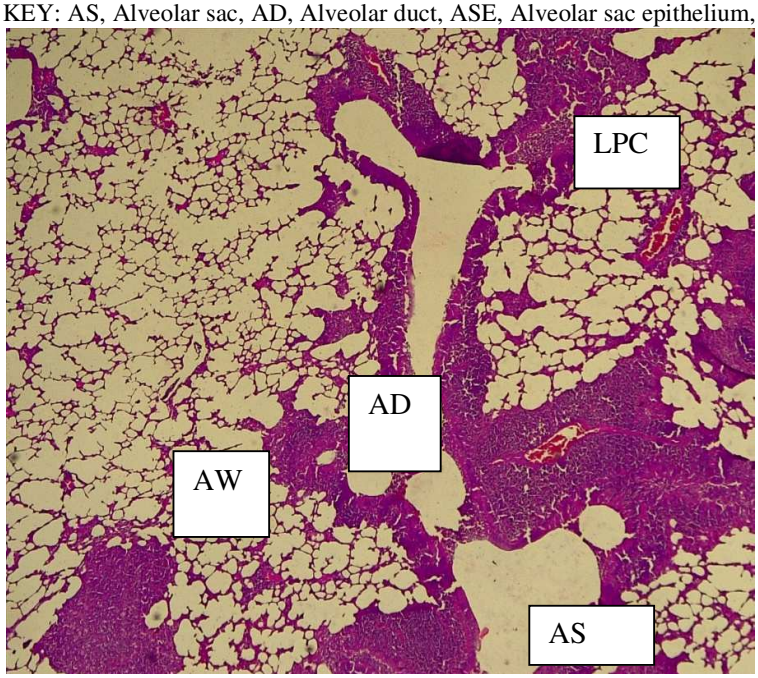

PLATE2: Photomicrograph of lung section of group B animal (5mg/kg lead) showing Alveoli ill-defined and distorted, thinned alveolar wall and respiratory cells are distorted (H & E stain ×400).
KEY: AS, Alveolar sac, AD, Alveolar duct, ASE, Alveolar sac epithelium, LPC, Lung parenchyma cells.

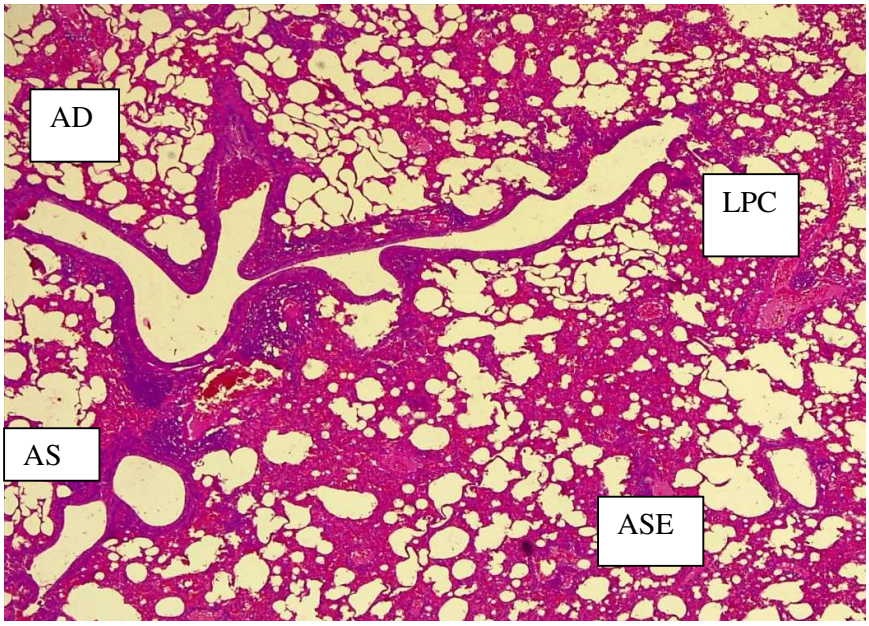

PLATE3: Photomicrograph of lung section of group C (10mg/kg *Moringa oleifera*) showing alveolar wall, many intact respiratory cells (H & E stain ×400).
KEY: AS, Alveolar sac, AD, Alveolar duct, ASE, Alveolar sac epithelium, LPC, Lung parenchyma cells.

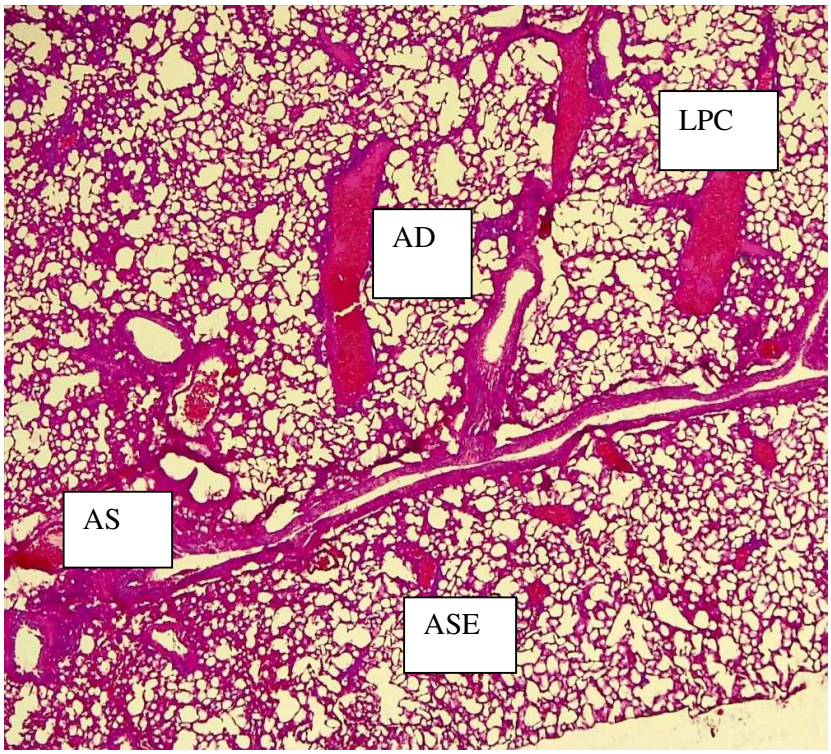

PLATE4: Photomicrograph of lung section of group D (control group) showing the normal histology of the lungs in terms of the general lung histo-architecture and alveoli are visible with normal appearances (H & E stains ×400).
KEY: AS, Alveolar sac, AD, Alveolar duct, ASE, Alveolar sac epithelium, LPC, Lung parenchyma cells.

DISCUSSION

The extraction yield is a measure of the solvent efficiency to extract specific components from an original material. The aqueous extraction method used in this research produced a percentage yield of 25.4%. This is in agreement with the work of Akuodor *et al*. (2013) who obtained a yield of 49.9%. In the acute toxicity study, all the graded doses up to 5000mg/kg showed no sign of toxicity in the animals and no mortality was recorded in the study. The $LD_{50}$of aqueous leaves extract of *Moringa oleifera* was found to be higher than 5000mg/kg body weight which is consistent with the findings of Fiot *et al*.(2006) and Akuodor *et al*.(2013).The measurement of the mice weights before the intervention were within the normal ranges of healthy adult mice (20-30kg) of both the test group A, B, C and control group D. However change in the weight of the animal after the intervention was observed in group B animal. While other groups maintain a relatively similar weight of (18-19kg). This loss in weight is attributed to toxic effect of lead poisoning as it causes loss of appetites, difficulty in breathings. Furthermore, this work also aimed at evaluating and elucidating some anti-fibrotic effects of aqueous *Moringa oleifera* leaves extracts on lead induced lungs lesion of mice. Several researches on *Moringa oleifera* leaves extracts suggested a regenerative property of the leaves extracts on the cyto-architecture of the tissue. This corroborate with our findings, as seen in Group A treated with lead and *Moringa oleifera* leaves extracts. Group B treated with lead only shows deleterious effects on the lungs, Alveoli are ill defined and in other instances distorted. The walls and cells that constitute the lung tissue are distorted, scarring from destroyed tissue was observed with accompanying cellular debris within the alveolar sac. These observations show that lead destroyed lung tissue and finally emphysema may occur. This finding is consistent to work done by Jankeer and El-Nouri, (2009). However, these deleterious effects were ameliorated and the lung histology features show improvement when compared with that of Group A administered with lead and *Moringa Oleifera* simultaneously. Thus the histological findings of the lung tissue in the group A indicate reduced severity of lead toxic and deleterious effects, especially by preserving the lung tubules and alveoli. This also agree with work done by Owolabi *et al*. (2013). On the other hand Group treated with *Moringa Oleifera* extracts only indicated that moringa has no deleterious effects on the lungs, therefore *Moringa* did not produce observable histological distortions to the lung alveolar or damages to the cells of the lung tissue, this is similar to work done by Babita and Anita (2008). Control group showed normal mice lung structure such as normal alveoli, bronchiole, no congestion of pulmonary blood vessels, no hemorrhages, no hypertrophy in the epithelial cells of bronchiole, no fibrosis and emphysema, this is similar to work done by Owolabi *et al.,* (2013).More studies will further inform on the period or duration of the lead exposure and *Moringa oleifera* impact.

CONCLUSION

Lead was observed to cause damages to the lung tissue; the damages revealed in this study include the disruption of the general histological architecture of the lung tissue and abnormal alterations of the lung. *Moringa oleifera* Leave extracts administration reduced the severity of lead's damaging effects to the lungs, with possible anti-fibrotic as well as prophylactic properties for repairing damaged alveoli, thereby preserving and/or regenerating the lung tubules.

CONFLICT OF INTEREST

Authors declare no conflict of interest

REFERENCES

Akuodora G. C., Essien A. D., David-Oku E., Chilaka K. C., Akpan J.L., Ezeokpo B., E zeonwumelu J.O.C. Gastroprotective effect of the aqueous leaf extract of *Guiera senegalensis* in Albino rats. *Asian Pacific Journal of Tropical Medicine* 6:10, 771-775

Al-Malki, A.L. (2015). The antidiabetic effect of low doses of *Moringa oleifera* Lam. seeds on streptozotocin induced diabetes and diabetic nephropathy in male rats. *Biomedical Research Institute*, 381040.

Avwioro, O. G. (2014). Histochemistry and Tissue Pathology in; *Principle and Technique.* Third edition, pp 133-168.

Babita, A. and Anita, M. (2008). Anti-asthmatic activity of *Moringa oleifera* Lam: A clinical study. *Indian Journal Pharmacology*, **40:** 28-31.

Duruibe, J.O., M.O.C. Ogwuegbu and J.N. Egwurugwu, 2007. Heavy metal pollution and human biotoxic effects. *International Journal of Physical Science* **2**(5): 112-118.

Fahey, J.W. (2008).*Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. *Trees for life Journal*.1:5–15

Fahey, J.W., Zalcmann, A.T., and Talalay, P.(2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry.* **56**, 5–51.

Fiot J, Sanon S, Azas N, Mahiou V, Jansen O, Angenot L, Balansard G, Ollivier E (2006). Journal of *Ethnopharmacological*. 106(2): 173-178.

Foidl, N., Harinder, P. S., and Becker, K. (2001) Potentiel du *Moringa oleifera* pour les besoins agricoleset industriels. In: *L'arbre de la vie, Les multiples usages du Moringa*. pp 45-78.

Goyer, R.A., 1996.Toxic effects of metals. In: Casarett and Doull's Toxicology: Basic Science of Poisons. Klaassen, C.D. (Ed), New York. McGraw-Hill, 623-680pp.

Haslam P.L., Turton C.W.G., Lukoszek A., Salsbury A.J., Dewar A., and Collins J.V.(1980) Bronchoalveolar lavage fluid cell counts in cryptogenic fibrosingalveolitis and their relation to therapy. *Thorax*.**35**:328-339.

Haslam, P. L, Davison, A. G, Corrin, B., Dewar A, Riding, W. D and Studdy, PR. (1983). Interstitial lung disease and asthma in hard-metal workers: Bronchoalveolarlavage, ultrastructural and analytical findings and results of bronchialprovocation tests. *Thorax*, **38**: 119-128.

Ibrahim, N. M., Esam, A. E., El-Beltagi, H. S., and Abdel-Mobdy, Y. E. (2012). Effect of lead acetate toxicity on experimental male albino rat. *Asian Pacific Journal Tropical Biomedicine.* 2(1): 41–46.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., and Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology* 7(2): 60–72.

Jankeer, M.H. and A.A. El-Nouri, 2009. Histological study of the liver and kidney of albino mice *Mus musculus* exposed to lead *Rafidain Journal of Science* 20: 42-51.

Joshua, O., Olaide, G., Micheal, D., John, O., Ademola, C., and Felicia, W.(2013). Prophylactic and regenerative effects of alcoholic extract of *Moringa oleifera* on rat lung tissue following lead-induced damage. *Journal of Anatomy.* **17** (2): 115-122

Loghman-Adham, M. (1997). Renal effects of environmental and occupational lead exposure. *Environmental Health Perspectives*. 105(9): 928–939.

NIH (2020). Guide Laboratory Animals For The Care And Use Of Laboratory Animals. Eighth Edition. https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf [Retieved 31st January, 2020].

NLI (2021). Pregnancy and Lead Poisoning Protect Yourself, Protect Your Baby. National Lead Information Center. https://health.mo.gov/living/environment/lead/pdf/PregnancyLeadPoisoningFactsheet.pdf [Retrieved 21st January, 2020].

Owolabi, J. O, Opoola, E and Caxton-Martins E, A (2012). Healing and Prophylactic Effects of *Moringa oleifera* Leaf extract on Lead Induced Damage to Haematological and Bone Marrow Elements in Adult Wistar Rat. *Models.* 1:386.

Skerfving, S. and Bergdahl, I.A. (2007): *Handbook on the Toxicology of Metals*, 3rd Edition, Academic Press, Amsterdam, pp599-643.

Steenland, K. and Boffetta, P. Lead and cancer in humans: Where are we now? (2000). American *Journal of Industrial Medicine*. 38, 295–299.

Valia, R. V, Patil., V. K, Patel, Z. N, Kapadia, and P, K (1993). Physiological responses of drum stick (*Moringa oleifera*) land to varying levels of ESP. *Indian Journal of Plant Physiology*. **36**: 261-266.

Wani, A. L., Anjum A., and Usmani, J. A. (2015). Lead toxicity: a review. *Interdisciplinary Toxicology.* 8(2): 55–64