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Prophylactic and regenerative effects of alcoholic extract of *Moringa oleifera* on rat lung tissue following lead-induced damage

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SUMMARY

The research work was carried out to investigate and evaluate the prophylactic and regenerative effects of alcoholic extract of *Moringa oleifera* leaves on lead induced damage to lung tissues of adult Wistar rat models. A total of 30 adult male Wistar rats (n=30) were used for the investigation and were randomly distributed into groups to give relatively equal average group weights. Animals were distributed into five groups of six animals each and labeled Groups A-E. The Group A served as the control group; Group B was administered moringa extract only; Group C was administered lead acetate solution only; Group D was administered lead acetate solution for the first eight days and *moringa oleifera* for the remaining eight days and the Group E was administered Lead and *Moringa oleifera* concurrently for the whole 16 days of administration. A relative uniform dosage of 50mg/kg body weight of lead was administered to the rats in the treated groups while 100g/kg body weight of *Moringa oleifera* extract was administered. The rats were sacrificed, 24 hours after the last administration using the method of cervical dislocation. The lung tissues were excised. Half of the samples from each group were fixed in formol saline and thereafter processed for histological demonstrations using the Haematoxyline and Eosin staining techniques as well as the Feulgen DNA staining techniques. The remaining parts were homogenized for biochemical assays. Results indicated that lead would cause observable tissue damage to lung when administered at the dosage used in this investigation. The damages observed in this study included the disruption of the general histological architecture of the lung, disruption of the alveoli as well as damage to lung tissue cells as well as disruptions in carbohydrate metabolism enzymes levels. Moringa administration at this dosage reduced the severity of lead damaging effects to the lung by producing mild regenerative effect.

Key words: *Moringa oleifera* extract – lead toxicity – regeneration – prophylaxis– Wistar rat

INTRODUCTION

Moringa oleifera (moringa) and related species are well known for their medicinal value and are largely grown in Asia and in the tropics, with India reportedly being the largest producer of the plant. Several studies have been carried out on the

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plant over the years by different researchers with many encouraging results. However, there are some arguments regarding the authenticity of some of the claims on the capacity of the plant to cure some diseases such as cancer, epilepsy, conception, tuberculosis and others. Plants are popularly used in treating various disease conditions by about 78% of the world population, especially those in the third world countries and the tropics.

This research work was aimed at discovering and evaluating some of the regenerative effects of concentrated *Moringa oleifera* leaves extracts on the lead-induced damage to the lung tissues of adult Wistar rats after 16days of treatment. Several research reports have been published on the *Moringa oleifera*, mostly using ethanolic extracts of the plant especially on pathogens. More investigations need to done on the therapeutic effect and tissue regenerative ability of the plant as well as its antitoxic properties. This research work, thus was aimed at investigating the regenerative effect of the concentrated extract of the plant on the cyto-architecture of the lungs.

MATERIALS AND METHODS

Moringa: a plant with several acclaimed medicinal and nutritional benefits

There has been increased focus on plant research in recent times all over the world, and results have shown immense potentials of some plants (Amaglah and Benang, 2009). *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. *Moringa oleifera* is the most useful tropical tree. The relative ease with which it propagates through both sexual and asexual means and its low demand for soil nutrients and water after being planted makes its management and production easy (Foidl et al., 2001).

Moringa is a fast-growing, drought-resistant, deciduous tree reaching 3m in height, just within 10 months of cultivation (Valia et al., 1993). It can be propagated through both sexual and asexual means of reproduction (Foidl et al., 2001). Moringa is propagated by planting 1-2 m long limb cuttings, preferably from June to August. The plant starts bearing pods 6-8 months after planting, but regular bearing commences after the second year, continuing for several years. It can also be propagated by seeds, which are planted an inch below the surface and can be germinated year-round in well-draining soil. It is a sun- and heat- loving plant, and thus does not tolerate freeze and frost. Ounce-for-ounce, Moringa leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas; also, the protein quality of Moringa is unrivalled by milk and eggs (Fahey, 2008).

Very importantly, components of Moringa preparations that have been reported to have hypotensive, anticancer, and antibacterial activity include: 4-(4'-O-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocy-anate; 4-(α-L-rhamnopyranosyloxy)benzyl isothiocy-anate; Niazimicin;Pterygospermin; Benzyl isothiocyanate and 4-(α-L-rhamnopyranosyloxy).

The plant was found to contain also carotenoids, rhamnose sugar, pterygium and glucosinolates (Caceres et al., 1991; Bharah et al., 2003). Generally, moringa preparations have been cited in the scientific literature as having antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypo-cholesterolemic, and hypoglycemic activities, as well as having considerable efficacy in water purification by flocculation, sedimentation, antibiosis and even reduction of Schistosomecercariae titer (Fahey et al., 2008).

Lead toxicity

Lead is commonly used in several industrial fields by people who are unaware of its adverse effects on human health. Lead is usually taken spontaneously from the lead polluted environment via water, air and food. It is well established that lead intake, such as through lead vapour inhalation, may cause irreversible effects in several organ systems in mammals. Previous studies have demonstrated the effects of lead exposure on gastrointestinal and urinary systems, which were adversely affected by lead intake. 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 the effects mentioned above on lung histology.

The toxic effects of lead on the lung are the most harmful when compared to its effects on the other organs in the body (Valia et al., 1993). The work done by Valia, shows that inhalation of lead could lead to fibrosis of lung tissue. Consumption or inhalation of lead or other heavy metals for a

long time can cause pneumonia or asthma (Haslam et al., 1983). Moringa oleifera has got some positive effects on the lung in certain instances- for instance, it was found to reduce the severity of pulmonary asthma and improved lung parameters such as force vital capacity(FVC) and force expiratory volume(FEC) (Babita et al., 2008). Indian scientists have identified Pterygospermin as the active compound in moringa that causes its antibacterial action.

About the lungs

The lungs are cone-shaped organs that lie in the thoracic cavity, occupying the space between the clavicle and diaphragm, lying above the rib posteriorly and anteriorly. The two lungs are separated by the heart, viscera and great vessels (Moore and Dalley, 2006). Histologically, the lung is covered by the pleura, consisting of a layer of mesothelial cells with underlying connective tissue. This connective tissue extends inwardly between pulmonary lobes and lobules and septa carrying ramification of pleura blood and lymphatic vessels. The respiratory lining undergoes continuous transition from a tall pseudo-stratified columnar ciliated type in the larynx and trachea to a simple cuboidal non-ciliated type in the smallest airways. The respiratory bronchiole is lined with cuboidal epithelium at the proximal part of the respiratory bronchiole. Small branch of the pulmonary artery accompanies the bronchiole and numerous alveoli open into the alveolar duct. Oval alveoli are lined by squamous epithelium. The most important part of the lung is the pulmonary alveolus; it is the thin walled circular compartment at the termination (Singh, 2006).The alveoli are rounded or polygonal with diameter of about 200 micrometers, but their size and shape vary greatly depending on the level of post-mortem collapse of the lungs and the amount of distortion of their thin walls during specimen preparation (Singh, 2006).Components of alveolar wall include the epithelium that provides a continuous lining to each alveolus, consisting of the type 1 and the type 2 pneumocytes as well as supporting tissue that forms attenuated layer beneath the epithelium and surrounding the blood vessels of the alveolar wall. There are blood vessels- main capillaries (7-10um in diameter) that form an extensive plexus around each alveolus. In most alveolar wall, the basement membrane which supports the capillary endothelium is directly applied to the basement membrane supporting the surface epithelium (Young et al., 2006).

Extract preparation

Fresh *Moringa oleifera* leaves were collected in Ilorin metropolis. It was identified and authenticated at the Plant Biology Department of University Of Ilorin. It was air-dried and grounded to powder form using mortar and pestle. Alcoholic extract of the powdered leaves were thereafter prepared.

Animal grouping and treatment

In this study, 30 male adult Wistar rats weighing between 150 and 250 g were procured for the experiment. The rats were kept in animal cages. The acclimatization of the animals was done at the animal house of the Department of Anatomy, University of Ilorin. The rats were fed on growers feed obtained from the Livestock feeds depot, Ilorin and water from the laboratory the tap supply was given.

The experimental animals were randomly divided into five groups of 6 animals (n=6) labeled Groups A-E. The Group A served as the control group; Group B was administered moringa only; Group C was administered lead acetate solution only; Group D was administered lead acetate solution for the first eight days and *Moringa oleifera* for the remaining eight days and the Group E was administered lead and *Moringa oleifera* concurrently for the whole 16 days of administration. A relative uniform dosage of 50 mg/kg body weight of lead was administered to the rats in the treated groups while 100 g/kg body weight of *Moringa oleifera* extract was administered.

Animal sacrifice and tissue processing

The rats were sacrificed in the Anatomy laboratory, University of Ilorin, following the method of cervical dislocation (Owolabi et al., 2011).The lung tissues were excised. Half of the samples from each group were fixed in formol saline and thereafter processed for histological demonstrations using the Haematoxyline and Eosin (H&E) staining techniques as well as the Feulgen DNA (FDNA) staining techniques. Excised lung tissues were first fixed in 10% formol saline and then processed for histological demonstrations. Sectioning was done with the rotary microtome-Shandon Finesse® Manual Rotary Microtome, Model 325, into sections of approximately 30 µm thickness.

The remaining parts were homogenized for biochemical assays. The homogenate was centrifuged using the Corning® LSE mini microcentrifuge working at 6000 rpm and then the supernatant was collected and assayed for glucose-6-phosphate dehydrogenase (G-6-PDH) and the lactate dehydrogenase (LDH) using the Sigma enzyme assay kits.

Data analysis

Photomicrographs were obtained from the tis-

sue slides using the Digital BresserTrino Researcher microscope. Each whole lung was homogenized in the homogenizer, using formol saline as a medium. The mean values of the results obtained from the assays of the enzymes G-6-PDH and LDH across the groups were calculated and compared using the SPSS statistical software, 14.0 versions. Statistical significance was considered for the value of P≤0.5. Mean values were represented for easier comparison using the bar chart.

RESULTS

Results of enzyme assays

Moringa administration reduced the level of G-6 -PDH in the lung of the animals in group B administered with moringa photochemical extracts only. This suggests that moringa significantly reduced carbohydrate metabolism within the lungs of the animals in this group. Lead administration to the animals in group C however increased the level of this enzyme with further increases in groups D and E, administered with lead and moringa. Major changes in enzyme levels across the groups were of statistical significance.

Moringa administration slightly elevated the level of LDH in the group B, possibly as a compensating effect for the reduction it caused in the level of G-6-PDH in the same group. Lead caused significant elevation in the level of LDH making this group have the highest LDH level. Moringa administration appear to effect lowering of LDH level in groups D and E and appear to have greater reductive effect in the group D where moringa was administered after lead. Major changes in enzyme levels across the groups were of statistical significance.

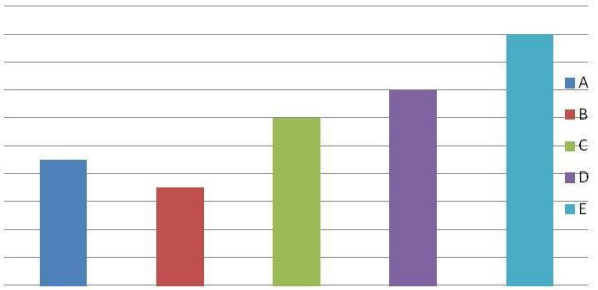


Chart 1. Levels of glucose-6-phosphate dehydrogenase -G-6-PDH-, in the homogenates of lung tissues of the animal groups.

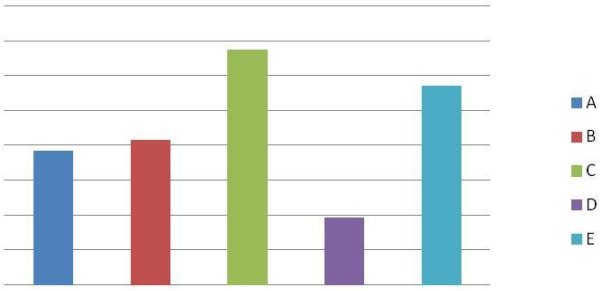


Chart 2. Levels of lactate dehydrogenase –LDH-, in the homogenates of lung tissues of the animal groups.

Histological observations

Figs. 1-2, 11: These are photomicrographs of the lungs of the animals in the control group A indicating that the histology of the lung in this group appears normal in terms of the general lung histo-architecture while the alveoli in particular are visible and with normal appearances.

Figs. 3-4, 12: These are the photomicrographs of the lungs of the animals in the group B administered with moringa phytochemicals only. The lung alveoli are observable and normal in appearance. The cells of the lung tissue upon close examination are also morphologically normal while the alveolar walls are retaining their integrity. These observations show that Moringa phytochemicals did not produce observable morphological distortions to the lung alveolar or damages to the cells of the lung tissue.

Figs. 5-6, 13: These are photomicrographs of the lungs of the animals in the group C treated with lead only. Alveoli are ill defined and in other instances distorted. The walls present thinning not found in the control group in several parts, and the cells that constitute the lung tissue are distorted. Scarring from destroyed tissue is observable with accompanying cellular debris within the alveolar sac. Alveoli appear to have been merged at the higher magnification. These observations show that lead destroyed lung tissue and the alveoli.

Figs. 7-8, 14: These are the photomicrographs of the lung of the animals in the group D, treated first with lead and thereafter with Moringa extracts. Persistence of distorted alveoli as well as signs of cellular damages are indications that Moringa administration within this duration could not totally restore the damaging effects of lead to the lung tissue. Cells of the lung tissue are prominently stained, the alveoli are however large as if merged. Generally speaking, the deleterious, hence cell-damaging and histoarchitecture-disruptive effects in this group were ameliorated when compared with the observation in group D.

Figs. 9-10, 15: These are photomicrographs of the lungs of the animals in the group E treated with lead and moringa simultaneously. Scarring of the lung tissue is observable as well as enlarged, but fewer number of alveoli suggesting merging of alveoli due to lung tissue damage by lead. The prophylactic effect of lead in this group is thus histologically less than adequate. Distortions of the alveoli are observable; tubules however appear intact. Comparing these observations to those in the control- Group A and

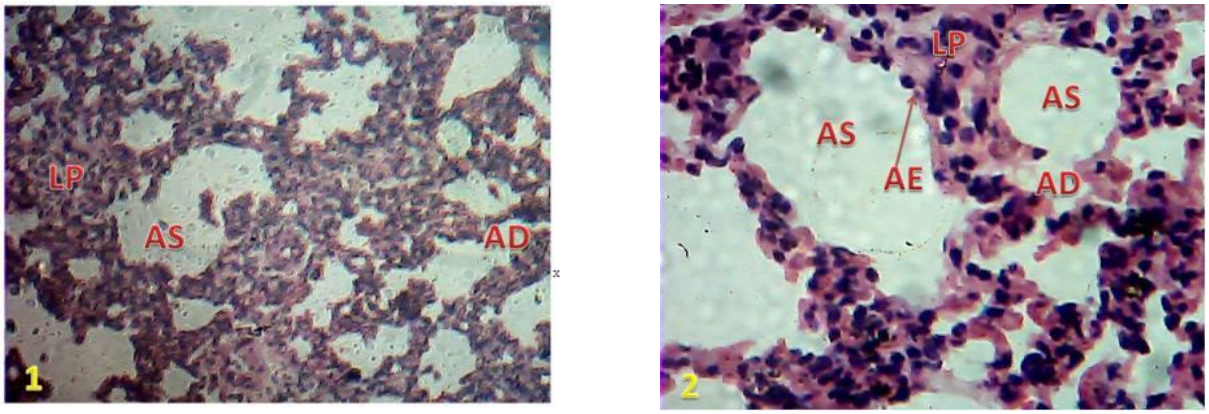
in the group administered with lead alone- Group C, it is reasonable to state that the administration of Moringa in the group E reduced the severity of lead toxic and deleterious effects, especially by preserving the lung tubules.

DISCUSSION

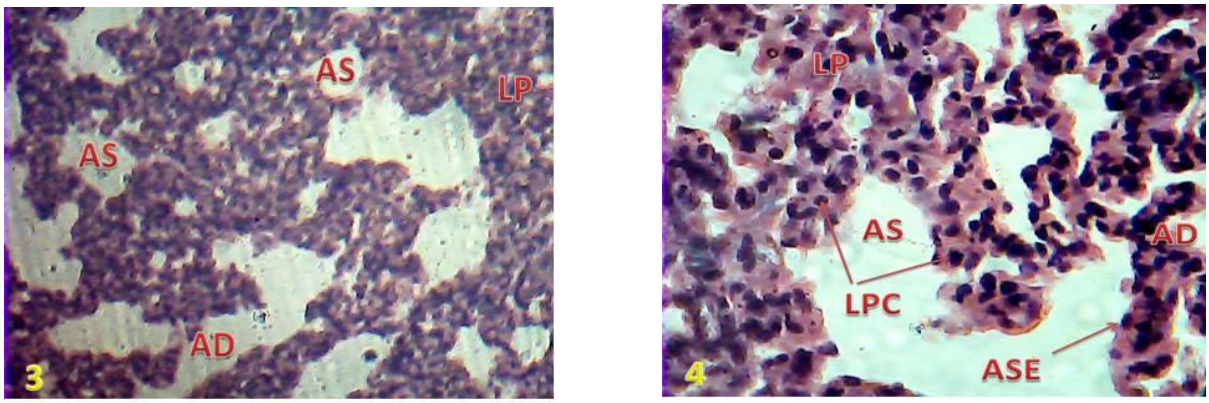
Glucose-6-phsophate dehydrogenase enzyme assay results suggest that moringa influenced further increases in carbohydrate metabolism when administered to lead-treated animals but not when administered alone. Increase in this enzyme of carbohydrate metabolism could be linked to the fact that surviving and regenerating cells would undertake rapid carbohydrate metabolism, especially as rapid differentiation and maturation will be required to provide adequate replacement for damaged and lost cells.

Generally, lead would therefore increase LDH level while moringa would lower it; these observations are also indicating the fact that moringa would reduce the level at which lead would alter the conventional and normal carbohydrate me-

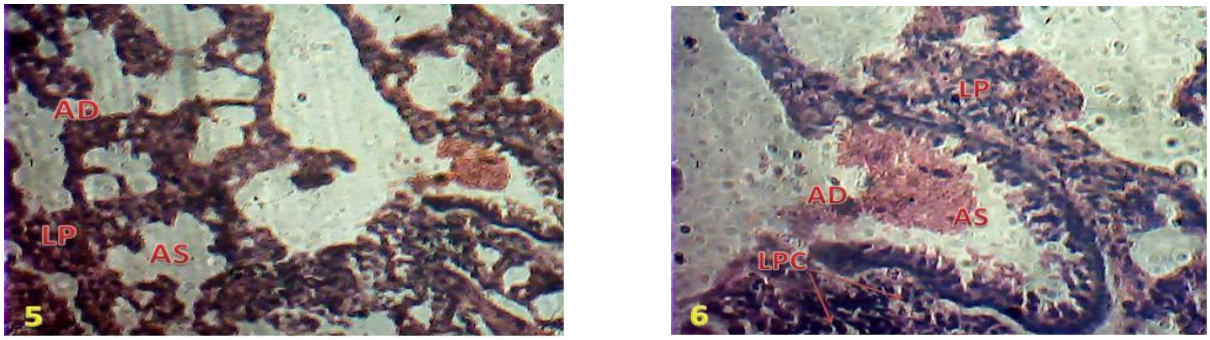
Common abbreviations for all figures: AS, alveolar sac; AD, alveolar duct; ASE, alveolar sac epithelium; H&E, haematoxyline and eosin stain; LP, lung parenchyma; LPC, lung parenchyma cells.



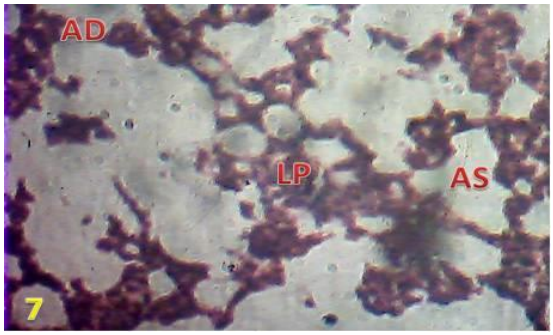
Figs. 1-2. Lung of adult male Wistar rats (control Group A). Haematoxyline and eosin (H&E) staining. 1 = x 100; 2 = x 300.

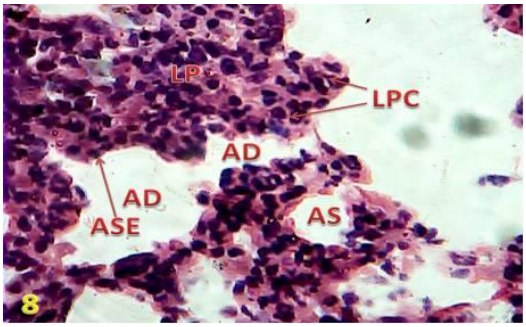


Figs. 3-4. Lung of adult male Wistar rats (Group B) treated with moringa only. H&E. 1 = x 100; 2 = x 300.

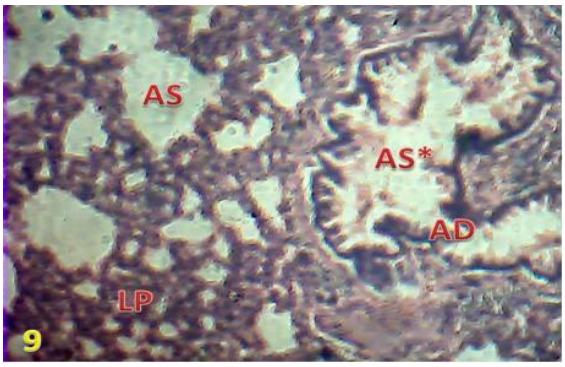


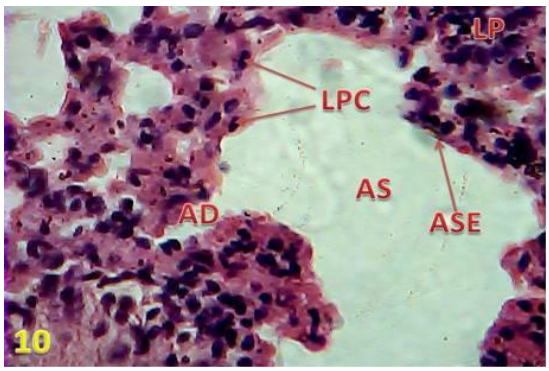
Figs. 5-6. Lung of adult male Wistar rats (group C, administered lead acetate only). H&E. 5 = x 100; 6 = x 300.





Figs. 7-8. Lung of adult male Wistar rats (Group D administered lead acetate for the first 8 days and thereafter Moringa extract for the remaining 8 days). H&E. 7 = x 100; 8 = x 300.





Figs. 9-10. Lung of adult male Wistar rats (Group E administered lead acetate and moringa extract concurrently). H&E. 9 = x 100; 10 = x 300.

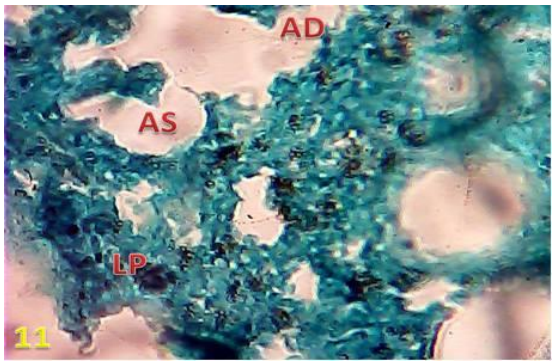


Fig 11. Lung of adult male Wistar rats (control Group A) FDNA staining technique. x 300.

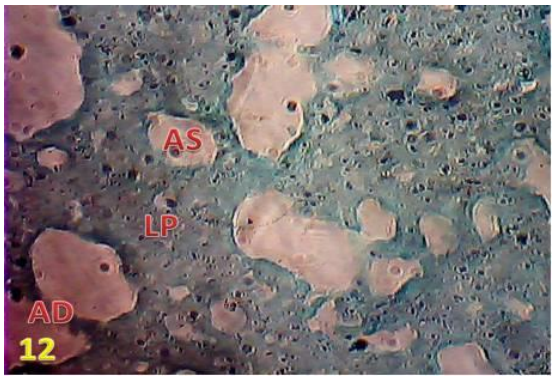


Fig. 12. Lung of adult male Wistar rats (Group B administered moringa only). FDNA staining technique. x 300.

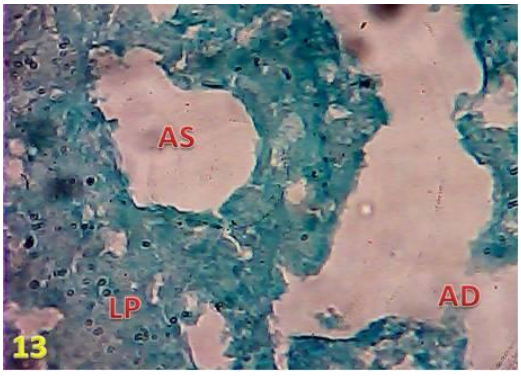


Fig. 13.- Lung of adult male Wistar rats (Group C administered lead acetate only). FDNA staining technique. x 300.

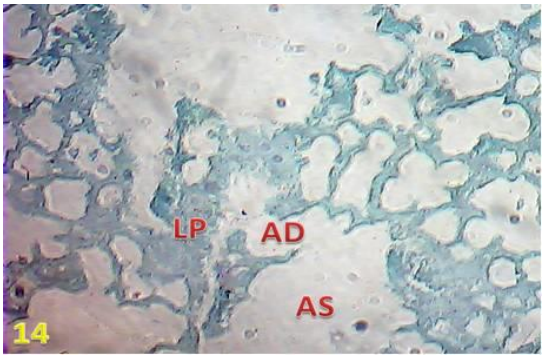


Fig. 14. Lung of adult male Wistar rats (Group D administered lead acetate for the first 8 days and thereafter Moringa extract for the remaining 8 days). FDNA staining technique. x 300.

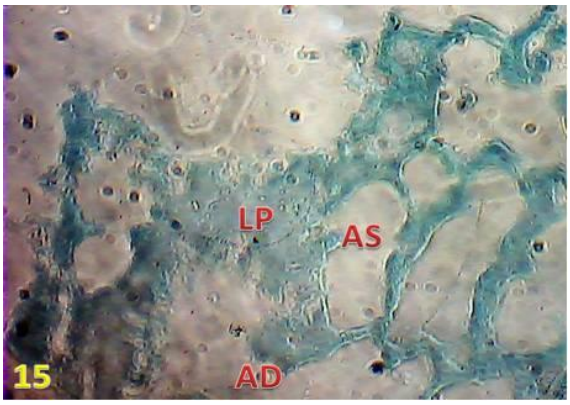


Fig. 15. Lung of adult male Wistar rats (Group E administered lead acetate and moringa extract concurrently). FDNA staining technique. x 300.

tabolism in the lungs of the experimental animals.

These observations in alterations in enzyme levels are of statistical significance for the value of P≤0.05.

Histological preparations of the lung tissue indicate that moringa has no deleterious effects on the lung; observations show that *Moringa oleifera* phytochemicals did not produce observable morphological distortions to the lung alveolar or damages to the cells of the lung tissue.

Lead on the other hand produced deleterious effects on the lung. Alveoli are ill defined and in other instances distorted. The walls present thinning not found in the control group in several parts and the cells that constitute the lung tissue are distorted. Scarring from destroyed tissue is observable with accompanying cellular debris within the alveolar sac. Alveoli appear to have been merged at the higher magnification. These observations show that lead destroyed lung tissue and the alveoli.

When moringa is administered after lead toxicity, the deleterious effects were ameliorated and the lung histological features show improvement when compared with the observation in the group administered with lead only. Observations of the histological preparations of the lung tissue in the group administered lead and moringa simultaneously indicate reduced severity of lead toxic and deleterious effects, especially by preserving the lung tubules and alveoli.

The lungs serve as the main organ of respiration in humans and other mammals including Wistar rats used as models in this investigation; damage to the organ could be life-threatening, hence the need for adequate investigations on the organ. This investigation showed that lead is a poisonous metal and poses threat to the respiratory system and life if inhaled or consumed; this is evident in the results across the animal groups. It evidently caused significant damage to the lung tissue, abnormal alterations in G-6-PDH and LDH levels and distortion in blood vessels in this study. These observations are in line with those of Nolan and Shaikh (1992). The regenerative ability of *Moringa oleifera* was proven in groups D and E, where there was just a mild distortion to the histology of the lung tissue as well as the biochemical parameters when compared to those in the Group B administered lead only.

Conclusion

Lead will cause observable damage to lung tissue when administered at the dosage used in this investigation. The damages observed in this study included the disruption of the general histological architecture of the lung; disruption of the alveoli as well as the lung tubules, and abnormal alterations of carbohydrate metabolism of the lung tissue. Moringa administration at this dosage could reduce the severity of lead's damaging effects to the lung by causing regenerative effects as well as prophylactic effects: in the former by repairing damaged alveoli and in the latter case by preserving and/or regenerating the lung tubules. Moringa at this dosage will however not totally neutralize the effects of lead toxicity on the lung.

REFERENCES

Amaglah FK, Benanng A (2009) Effectiveness of *Moringa oleifera* seed as coagulant for water purification. *Afr J Agric Res*, 4: 119-123.

Babita A, Anita M (2008) Antiasthmatic activity of *Moringa oleifera* Lam: A clinical study. *Indian J Pharmacol*, 40: 28-31.

Bharah R, Tabassum J, Azad MRH (2003) Chemomodulatory effect of*Moringa oleifera* Lam on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papilloma genesis in mice. *Asian Pac J Cancer Prev*, 4: 131-139.

Caceres A, Cabrera O, Morales O, Mollinedo P, Mendia P (1991) Pharmacological properties of *Moringa oleifera*. 1: Preliminary screening for antimicrobial activity. *J Ethnopharmacol,* 33: 213-216.

Fahey JW (2008) *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Trees for Life Journal.

Foidl N, Harinder PS, Becker K (2001) Potentiel du *Moringa oleifera* pour les besoins agricoles et industriels. In: *L'arbre de la vie, Les multiples usages du Moringa*. CTA et CWS, Dakar, pp 45-78.

Haslam PL, Davison AG, Corrin B, Dewar A, Riding WD, 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.

Moore KL, Dalley AF (2006) *Clinical Oriented Anatomy*,

5th edit. Lippincott Williams and Wilkins, United Kingdom.

Nolan CV, Shaikh ZA (1992) Lead nephrotoxicity and associated disorders: biochemical mechanisms. *Toxicology*, 73: 127-146.

Owolabi JO et al. (2011) Introduction to Biomedical Research Methodology, Published by Extension Publications Nigeria Limited, Nigeria.

Singh KK, Kumar K (1999) Ethnotherapeutics of some medicinal plants used as antipyretic agents among the tribes of India. *J Econ Taxon Bot*, 23: 135-141.

Valia RV, Patil VK, Patel ZN, Kapadia PK (1993) Physiological responses of drum stick (*Moringa oleifera*) land to varying levels of ESP. *Indian J Plant Physiol,* 36: 261-266.

Young B, Lowe JS, Stevens A, Heath JW (2006) Wheater's Functional Histology: A Text and Colour Atlas, 5th edit. Churchill Livingstone Elsevier, United Kingdom.