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Adansonia digitata ameliorates lead-induced hepatic, renal, and bone toxicity in mice

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ABSTRACT
Adansonia digitata (*A. digitata*) is used in many African and Asian countries for food, beverage and medicine. Lead is one of the toxic heavy metals whose widespread use has resulted in continuous environmental contamination. The current study evaluated the effect of lead acetate on mice's liver, kidney, and bone tissues and the protective role of *A. digitata* fruit pulp. Twenty-five mice were distributed into five groups, each having five mice. The groups received distilled water, 50 mg/kg lead acetate, *A. digitata* methanolic fruit extract (ADMFE) at 250 mg/kg plus lead acetate, 500 mg/kg ADMFE plus lead acetate, and 50 mg/kg Succimer plus lead acetate, respectively, for four weeks. All the mice were euthanized thereafter, and the right kidneys, liver, and bone were processed for light microscopy. The left kidneys, part of the liver, and bone were dried and assayed for lead concentration using an Atomic Absorption Spectrophotometer. The lead concentration in the kidney of mice pre-treated with 500 mg/kg ADMFE and succimer was significantly lower ($p<0.05$) relative to the mice treated with lead acetate only. Treatment with lead acetate resulted in hepatocyte degeneration, renal tubule damage and hemorrhagic hematopoietic cells with immature and degenerating lymphocytes. Pre-treatment with ADMFE at 250 mg/kg and 500 mg/kg prevented hepatocyte and renal tubule degeneration. It also prevented hemorrhage within hematopoietic cells. Our findings suggest that *A. digitata* could be used as a metal chelate. It could also prevent lead-induced liver and kidney damage.
Keywords: *Adansonia digitata*, hepatocytes, hematopoietic cells, hemorrhage, renal tubules.

INTRODUCTION
Adansonia digitata (*A. digitata*), also known as Baobab, is a large tree with an unusual shape and succulent trunk. It can survive a long period of drought due to its ability to conserve water. The plant is used in the African and Asian regions in food, beverage and medicine for many ailments[1-3]. *A. digitata* leaves were reported to be rich in phytochemicals and minerals and are used to prepare soup in Northern Nigeria[4-5]. A previous study revealed that *A. digitata* represents one of the most natural sources of ascorbic acid, containing more than five times the amount found in citrus fruit[3]. The dry *A. digitata* fruit pulp is very nutritious, with a refreshing taste, and contains high amounts of carbohydrates, calcium, potassium, thiamine, nicotinic acid, and vitamin C[6-7]. It is a dry, acidulous, and mealy,

fruit rich in mucilage, pectins, tartrate, and free tartaric acids. The presence of the tartrate gives it the name 'cream of tartar tree[8]. The gum is used for cleansing sores and as an expectorant, while the bark is used in steam baths for calming shivering and high fever[9]. It also possesses anti-diabetic, anti-inflammatory, analgesic, antipyretic, immunomodulatory, and neuroprotective properties[9-12].

Lead is considered a public health concern globally, with developing countries at higher risk of exposure and toxicity[13]. Cosmetics, cigarette and automobile smoke, paints, lead pipes, fruits, and vegetables grown in contaminated soil are reported to be the common sources of lead exposure[14]. People working in plastic and battery manufacturing industries, construction workers, miners, and smelters are at risk of exposure to the toxicity[15]. The earlier reports suggest that lead exposure results in cognitive impairment, learning challenges, and delayed puberty in children[16-18]. Hence, the impact of lead is more severe in children than adults. Lead is a multi-organ toxicant affecting almost every organ/system of the human body, especially the central nervous system, reproductive, hepatic, renal, and immune systems[19-21]. Most lead is initially mainly stored in the kidneys and the liver[22]. Therefore, it may cause pathological changes to these organs and decrease their function. The current study evaluated the effect of lead acetate on liver, kidney, and bone tissues in mice and the protective role of *Adansonia digitata* fruit pulp.

MATERIALS AND METHODS

Drugs and chemicals

Lead acetate (CAS# 6080-56-4, Carolina Biological Supply Company, Burlington, USA) was used to induce liver, kidney, and bone damage. Methanol (CAS# 67-56-1, Sigma Aldrich, St. Louis, USA) was used to extract *A. digitata* fruit pulp, Succimer (Kremers Urban Pharmaceuticals Inc, USA) was used as a standard agent for acute lead poisoning and ketamine injection (Swiss Parental, India) was used as an anesthetic agent. Succimer and ketamine were purchased from a local Pharmacy in Maiduguri, Nigeria.

Extraction

Adansonia digitata fruit shells were purchased from Gamboru market in Maiduguri and authenticated by a Botanist at the University of Maiduguri (UMM/FPH/AD/007). They were ground and soaked in 80% methanol (1 kg in 2.5 ml) for three days. The mixture was filtered with Whatman filter paper size 1 and evaporated in an oven.

Ethical approval

The research was approved by the Department of Human Anatomy Ethical Committee and conducted following the ARRIVE guidelines and the directive 2010/63/EU of the European Council.

Experimental animals

Twenty-five albino mice (18-21 g) were procured and kept at the Department of Biochemistry Animal House at the University of Maiduguri. They had free access to food and water.

Experimental design

The mice were distributed into five groups with each having five mice. The groups received distilled water (normal control), lead acetate at 50 mg/kg (negative control), *A. digitata* methanolic fruit extract (ADMFE) at 250 mg/kg plus 50 mg/kg lead acetate, 500 mg/kg ADMFE plus 50 mg/kg lead acetate, and 50 mg/kg Succimer plus 50 mg/kg lead acetate respectively for four weeks. The lead acetate was given one hour after ADMFE and Succimer administration. All the mice were euthanized thereafter and the right kidneys, part of the liver, and right thigh bone were removed, fixed in neutral buffered formalin, and processed for light microscopy. The left kidneys, part of the liver, and left thigh bone were dried and assayed for lead concentration using an Atomic Absorption Spectrophotometer (AAS-AA500G, England).

Assessment of lead levels in tissues

A mixture of perchloric acid (4 ml), HNO_3 (24 ml), and concentrated sulphuric acid (2 ml) and about 0.2 g of each tissue were placed in an Erlenmeyer flask in a fume cupboard. The mixture was heated gently until a white and dense fume appeared. It was transferred to a Pyrex flask, and distilled water was added to make 50 ml. The content was then filtered with Whatman filter paper, and lead concentration was measured spectrophotometrically and expressed in µg/g.

Histology

The liver, bone and kidney were dehydrated in graded ethanol, cleared in xylene, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Ten tissues were observed per group and interpreted by two investigators blinded to the groups. Photomicrographs of the liver, bone and kidney were taken with a microscope camera at x200 magnification.

Statistical analysis

Values were analyzed using GraphPad Prism 7 (San Diego, USA). A one-way ANOVA followed by Tukey's multiple comparisons assessed the differences between groups. The results were presented as mean± standard error of the mean (SE), and $p< 0.05$ was considered statistically significant.

RESULTS

Lead concentration

The lead concentration in the liver, kidney, and thigh bone of lead acetate-treated mice was significantly higher ($p<0.05$) than the control. The liver and thigh bone of mice pre-treated with *A. digitata* methanolic extract also had a significantly higher ($p<0.05$) lead concentration compared to the control. Conversely, the lead concentration in the kidney of mice pre-treated with 500 mg/kg *A. digitata* methanolic extract was not significantly changed ($p>0.05$) compared to the control. The lead concentration in the liver of *A. digitata* methanolic extract pre-treated mice was significantly higher ($p<0.05$) than in mice treated with lead acetate. However, no significant change ($p>0.05$) was observed for bone lead concentration in *A. digitata* methanolic extract pre-treated mice compared to the lead acetate-only treated mice. The lead concentration in the kidney of mice pre-treated with 500 mg/kg *A. digitata* methanolic extract and succimer was significantly lower ($p<0.05$) relative to the mice treated with lead acetate only (Figure 1).

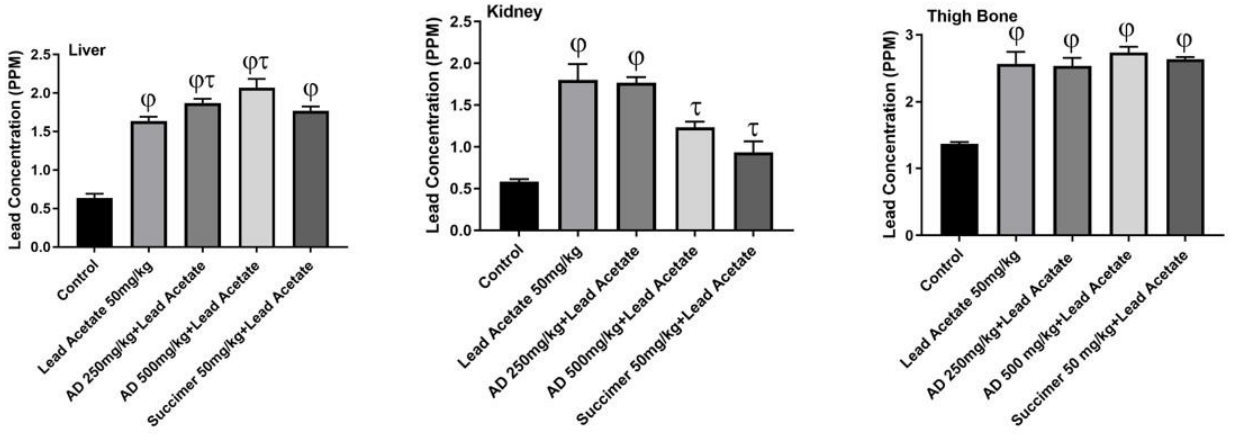

Figure 1. The lead concentration in the liver, kidney and thigh bone in mice treated with lead acetate, *Adansonia digitata* methanolic extract plus lead acetate and succimer plus lead acetate. The bars are presented as Mean±SEM. φ and τ are indications of significant difference ($P<0.05$) with the control and led acetate-treated mice respectively. n= 3. PPM= part per million, SE= standard error.

Histological observations

The liver tissue of the control mice and succimer pre-treated mice revealed normal hepatocytes (Figure 2a & e). Lead acetate-treated mice liver showed degenerating hepatocytes (Figure 2b). In contrast, mild distortion of the hepatocytes was observed in the liver of *A. digitata* methanolic extract pre-treated mice (Figures 2c & d). The kidneys of control mice showed normal glomeruli and renal tubules (Figure 3a). The kidneys of lead acetate-treated mice showed distortion of both the renal tubules and glomerulus (Figure 3b). The mice pre-treated with *A. digitata* methanolic extract at 250 mg/kg and 500 mg/kg showed mild distortion of both the renal tubules and glomerulus (Figures 3c & d). Pre-treatment with succimer showed distorted renal tubules and normal glomerulus (Figure 3e). The bone tissues of control mice, lead acetate-treated mice, *A. digitata* methanolic extract pre-treated mice, and succimer pre-treated mice revealed normal bone matrix containing osteocytes, osteoblast, and osteoclast (Figure 4). An even distribution of hematopoietic cells was found in the bone marrow of the control mice with visible lymphocytes at different stages of development (Figure 4a). Lead acetate-treated mice had marrow containing an uneven distribution of hemorrhagic hematopoietic cells with immature and degenerating lymphocytes (Figure 4b). The marrow of 250 mg/kg *A. digitata* methanolic extract pre-treated mice contains a less dense even distribution of hematopoietic cells without lymphocytes (Figure 4c). Pre-treatment with *A. digitata* at 500 mg/kg revealed hemorrhage within unevenly distributed hematopoietic cells in the marrow with no visible lymphocytes (Figure 4d). The marrow of succimer pre-treated mice revealed hemorrhage within the hematopoietic cells with immature and degenerating lymphocytes (Figure 4e).

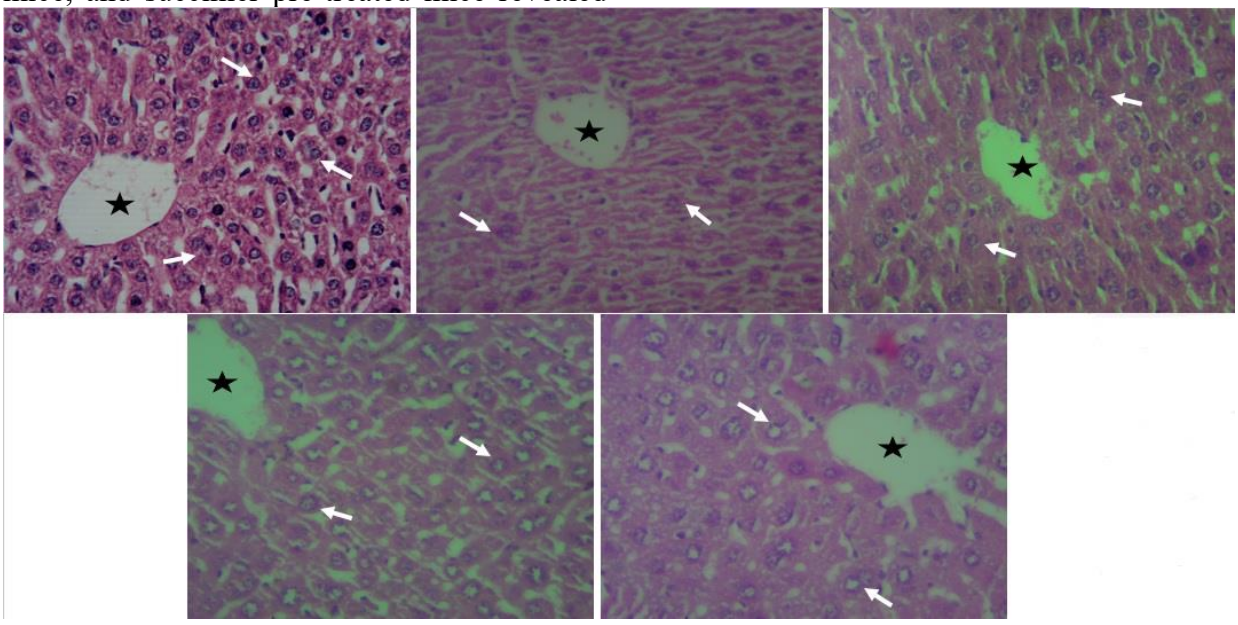

Figure 2. The liver tissue micrograph of mice treated with lead acetate, *Adansonia digitata* methanolic extract plus lead acetate, and succimer plus lead acetate. The white arrows and stars denote hepatocytes and central vein respectively. H&E x200 magnification. a= control, b= lead acetate, c= 250 mg/kg ADMFE plus lead acetate, d= 500mg/kg ADMFE plus lead acetate while e= succimer plus lead acetate, ADMFE= *Adansonia digitata* methanolic extract.

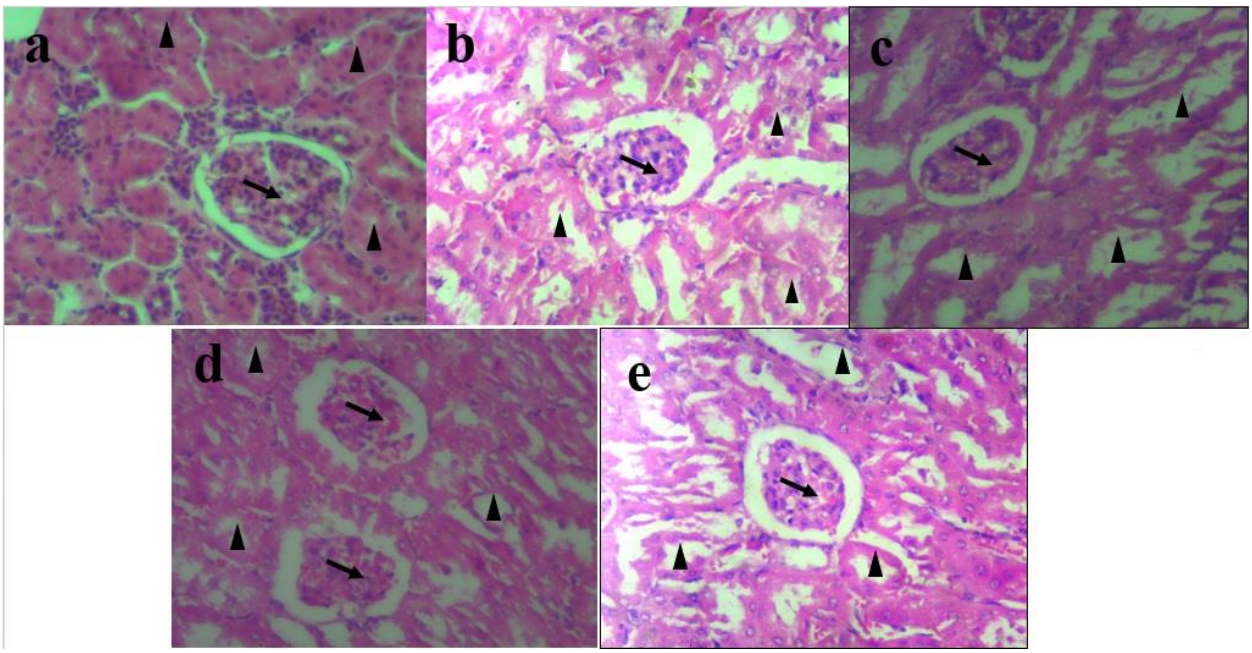

Figure 3. Kidney tissue micrograph of mice treated with lead acetate, *Adansonia digitata* methanolic extract plus lead acetate and succimer plus lead acetate. It shows the severe and mild distortion of the renal tubules and glomerulus in b and c-d, respectively. The arrows and arrowheads denote the glomerulus and renal tubules, respectively. H&E x200 magnification. a= control, b= lead acetate, c= 250 mg/kg ADMFE plus lead acetate, d= 500mg/kg ADMFE plus lead acetate while e= succimer plus lead acetate, ADMFE= *Adansonia digitata* methanolic extract.

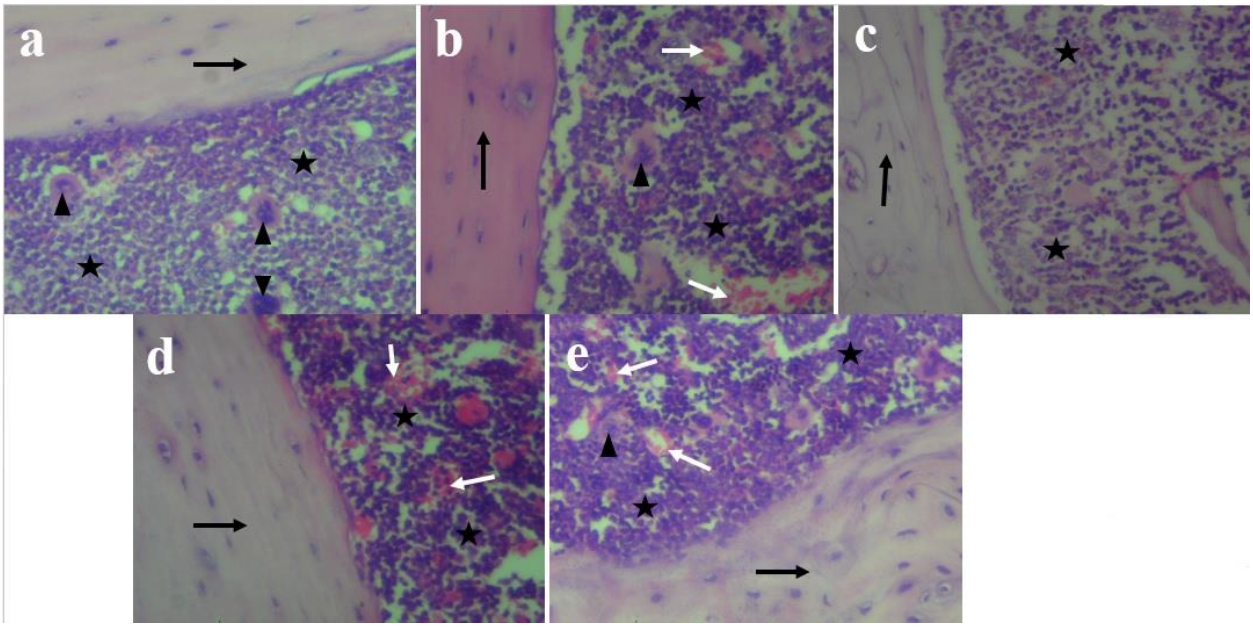

Figure 3. Bone tissue micrograph of mice treated with lead acetate, *Adansonia digitata* methanolic extract plus lead acetate, and succimer plus lead acetate. The black arrows and arrowheads denote the bone matrix and lymphocytes while the white arrows and stars denote hemorrhage and hematopoietic cells respectively. H&E x200 magnification. a= control, b= lead acetate, c= 250

mg/kg ADMFE plus lead acetate, d= 500mg/kg ADMFE plus lead acetate while e= succimer plus lead acetate, ADMFE= *Adansonia digitata* methanolic extract.

DISCUSSION

The study revealed that *A. digitata* fruit pulp prevented lead accumulation in the kidney of mice. It also attenuated kidney and liver damage and hematopoietic cell degeneration in the bone marrow. This suggests that *A. digitata* fruit pulp could serve as a natural remedy for lead-induced toxicity. The study was conducted to ascertain the role of *A. digitata* fruit pulp on lead accumulation in mice. A 28-day repeated treatment of mice with lead acetate resulted in an increased lead concentration (>1.5 PPM) in the liver, kidney, and bone. Dutta and Sengupta[23] provided a connection between mice and humans, suggesting that 9 days of mice is equivalent to 1 human year. Following the estimate, 28 days of exposure to lead in mice is equivalent to 28/9 years in humans. Hence, lead exposure for three years could increase lead concentration in vital organs to a level above 1.5 PPM[23]. The significant reduction of lead concentration in *A. digitata* fruit pulp-pretreated mice relative to the lead acetate-only treated mice suggests that *A. digitata* fruit pulp can protect the kidney against lead toxicity. Different parts of *A. digitata* have been reported to possess medicinal properties in previous studies. A study demonstrated how *A. digitata* fruit prevented lead accumulation in mice brains[21]. *A. digitata* leaf and fruit were reported to prevent inflammation and kidney injury[3,24]. *A. digitata* seedlings have been shown to clean soil contaminated with heavy metals[25]. The mechanism through which *A. digitata* prevents lead accumulation might be metal chelation. The antioxidant and metal chelation role of *A. digitata* was reported in previous studies[9,26].

The histological study was conducted to evaluate the role of *A. digitata* on lead acetate-induced liver, kidney, and bone marrow injury. The result demonstrated that *A. digitata* could prevent liver injury by attenuating hepatocyte damage. It also ameliorates kidney injury by maintaining glomerular integrity and preventing renal tubular damage. The protective and therapeutic role of *A. digitata* on kidney and liver-related diseases has been demonstrated in some previous studies[27-28]. The current study has also shown that *A. digitata* prevented the degeneration of hematopoietic cells by promoting their proliferation and lymphocyte maturation. This suggests that *A. digitata* could enhance blood production and improve immunity. Previous studies have also proven that *A. digitata* could improve immunity and prevent hematologic alteration[11,29-30].

Conclusion

Adansonia digitata fruit pulp was shown to prevent lead accumulation in the kidney and liver of mice. It also ameliorated hepatic and renal injury and promote hematopoiesis in the bone marrow. The findings of this study suggest that *A. digitata* could be used as a metal chelate for the treatment of liver and kidney disorders.

Conflict of Interests: The authors have no conflict of interest to declare.

Acknowledgements: None

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