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Original Research

Effect of aqueous extract of mistletoe leaf (*viscum album*) on the spleen of lead acetate-induced toxicity in adult Wistar rats

***[1]Ekeolu O.K., [2]Oduma-Sandy C.I., [3]Aiyewosa Emmanuel, [3]Friday J.I., [3]Calmday-Ombo D., [3]Onyeije B.P., [2]Olise N.A & [3]Innih S.**

[1]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, [2]Department of Anatomy, School of Basic Medical Sciences, [3]Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria

**Correspondence: Oyetunde.ekeolu@uniben.edu, +2348054063270*

ABSTRACT

This work is aimed at the ameliorative effect of aqueous extract of mistletoe on the spleen of lead acetate-induced toxicity in rat. Twenty-five adults male Wistar rats used for this experiment were divided into five groups. Group A (control) was administered 1ml of distilled water. Low dose, 200mg/kg of mistletoe extract and 10mg/kg of lead acetate were administered to Group B. Intermediate dose, 400mg/kg of mistletoe extract and 10mg/kg of lead acetate were administered to Group C. Group D was administered high dose, 800mg/kg of mistletoe extract and 10mg/kg of lead acetate. 10mg/kg of lead acetate only was administered to Group E. The rats in each group were treated *per os* for 28 days. After the treatment, the animals were anaesthetized using chloroform then sacrificed. The harvested spleens were processed for histopathology. Groups: B, C and D histopathological micrograph showed no visible lesions compared to Group A while Group E splenic micrograph revealed cellular damaged. The initial weights in groups A (110.60 ± 2.29) g, B (131.20 ± 8.10) g, C (125.60 ± 13.70) g, D (124.75 ± 7.72) g showed significant ($p<0.05$) difference with final weights in A (174.00 ± 7.80) g, B (159.80 ± 8.12) g, C (157.60 ± 11.57) g, D (196.00± 15.98) g. Group D had the highest final weight gain. The spleno-somatic indices of treated groups: A-E were not significantly different at $p>0.05$. The *Viscum album* extract had ameliorative and protective effects against the toxic effect of lead acetate on the spleen of Wistar rats.

Keywords: *Viscum album*, aqueous extract, lead acetate, spleen, ameliorative effect.

INTRODUCTION

The use of various plant species in the treatment of many different diseases has attained global recognition and its importance cannot be overemphasized. These medicinal plants have bio-substances that are precursors for synthesizing effective drugs against many diseases. The medicinal plants also have diseases-preventing properties (Baharvand *et al*., 2016). The consumption of medicinal plants in Africa and Asia is on the increase, even as pharmaceutic companies all over the world have intensified the use of these plants in the production of synthetic drugs (Baharvand *et al*., 2016, Bahmani *et al*., 2016). One of such medicinal plants of great importance is *Viscum album*.

This plant is commonly known as mistletoe. It belongs to the family *Santalaceae* (Becker, 1986). Mistletoe has been used to treat several diseases and is now known as "cure all" plant (Adodo, 2004). It is hemi-parasitic plant, growing on other plants with almost no plant toxicity to the animal body (Adesina *et al*., 2013). although because of its lectin content, some species of Mistletoe fruit like the African Mistletoe berries, have been reported to be toxic due to their lectin content (Adodo, 2004).

In Europe mistletoe, *Viscum album spp* has been utilized as a therapeutic drug against cancer (Mansky *et al*., 2013) as well as against human immunodeficiency virus, exhibiting its immune-stimulatory and anti-angiogenic properties (Huber *et al*., 2010). However, there are few reports on the protective and curative effects of *Viscum album* on the organ system, such as the previous report of Innih *et al* (2022) on the prophylactic effect of mistletoe on hematological changes, electrolytes imbalance and liver function enzymes in cadmium chloride intoxicated rats. As a follow up, it becomes imperative to investigate the effect of the mistletoe extracts on other heavy metal intoxicated erythropoietic organs such as the spleen.

Lead (II) acetate, also known as lead acetate, a white crystalline chemical compound with a slightly sweet taste, $Pb(C_2H_30_2)_2$, can cause oxidative stress in erythropoietic

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organs including the spleen (Haleagrahara *et al*., 2011, Samarghandian *et al*., 2013). Lead acetate is soluble in water and in glycerin. The substance is used as a reagent to make other lead compounds and as a fixative for some dyes (Sampathkumar &Yesudas, 2009). In low concentrations, it is the principal active ingredient in hair colouring dyes. Lead (II) acetate is also used as a mordant in textile printing and dyeing, and as a drier in paints and varnishes. Lead acetate was historically used as a sweetener and preservative in wines and in other foods and for cosmetics (Rathee *et al*., 2023). Exposure to lead can result in anorexia, vomiting, malaise, and convulsions due to increased intracranial pressure, mostly seen in children. Sources of child exposures to lead acetate are typically environmental such as to paint chips, pottery, drinking water, and dust. Acute exposure in adults may cause gastrointestinal effects, pain in arms and legs, hypertension and very high level of exposure may lead to coma (Amadi *et al.*, 2017).

The spleen is the largest lymphatic organ in the body and plays a role in the body' s defense system as a site of white blood cells proliferation, immune surveillance and response (Bronte and Pittet, 2013). The spleen is a dark red to blue-black ovoid organ, located in the left upper quadrant of the abdomen. It is 12 cm and 7cm in length and breadth, respectively. It is adjacent to the greater curvature of the stomach and situated within the omentum, with its smooth convex surface facing the diaphragm (Keith *et al.*, 2010). It is somewhat triangular in cross section, externally it is divided by a ridge into the anterior gastric portion and posterior renal portion. The gastric surface is directed anterosuperior. (Ostermann *et al.*, 1987). There is species variation in the gross appearance and size of the spleen. The weights of the spleens are important in their evaluation. The ratio of splenic weight to body weights is usually constant regardless of age (Losco, 1992).

The deleterious effect of continuous human and animal exposure to lead acetate through contaminated water, battery recycling, industrial use of lead as raw materials, paints, fertilizers and body cream and soaps (Rossi, 2008, Wani *et al*., 2015) in traces or large amount need to be remedied. One of such ways to solving this problem of heavy metal toxicity to the spleen because of its role in the clearing of blood from its debris and other foreign bodies, like heavy metal, is through alternative medicine. Therefore, this work is directed towards the investigation of the ameliorative effect of mistletoe, *Viscum album,* on lead acetate toxicity in the spleen.

MATERIALS AND METHOD

COLLECTION OF PLANT AND IDENTIFICATION

The leaves of *Viscum album* used for this work were collected in a plastic bowl and identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria. The collected leaf samples were chopped into fine portions and air-dried (at room temperature) for about a week. It was then oven-dried at a temperature of 40^0C for about 30 minutes and then pulverized into powder using the British Milling Machine. The weight of the powdered sample was then actualized to 100g. Cold maceration was carried out; the powdered material was macerated by soaking the 100g powdered Mistletoe leaves sample in 1.4L of distilled water for 24 hours at room temperature with constant shaking and stirring every six (6) hours using Whatman® filter paper, paper funnel and conical flask. The filtrate was concentrated over hot water bath using crucibles to obtain a paste like extract which was then preserved in a sample bottle inside a refrigerator.

EXPERIMENTAL ANIMALS

Twenty-five (25) male adult Wistar rats of 180-250g weight, procured from the Animal House, Department of Anatomy, University of Benin, were utilized for this study. The rats were acclimatized for 2 weeks before commencement of the experiment. The animals were fed with grower mash (from Premier Feed Mills, Nigeria) and clean water. The weight of each animal in each group was checked weekly (to get the cumulative weight required for experimental use). The procedure was carried out in accordance with approved protocols and recommendations for the proper management and utilization of laboratory animals. The dosage was given through an orogastric tube to ensure accuracy in treatment. Throughout the period of the experiment, the experimental animals had access to grower's mash feed (Premier Feeds limited, Nigeria) and clean water *ad libitum*. They were weighed before commencement and during the period of the experiment.

EXPERIMENTAL DESIGN

Twenty-five (25) experimental adult Wistar rats of either sexes were randomly assigned into five (5) groups; Groups A – E comprising of five rats per group. Group A**:** Rats served as control. Each rat was administered 1ml of distilled water daily**.** Group B: rats were treated daily with 200mg/kg body weight of mistletoe (Low dose) and 10mg/kg body weight of lead acetate. Group C: rats were treated daily with 400mg/kg body weight of mistletoe (intermediate dose) and 10mg/kg body weight of lead acetate. Group D: rats were treated daily with 800mg/kg body weight of mistletoe (High dose) and 10mg/kg body weight of lead acetate. Group E: rats were administered daily 10mg/kg body weight of lead acetate only.

TISSUE SAMPLES COLLECTION

All treatments were humane and followed ethical standard with ethical clearance was approved by ethic committee of

the college of medical sciences, University of Benin, Benin-city, Nigeria; Registration number: CMS/REC/2023/340.

At the end of the series of treatment for 4 weeks (28 days) the rats were weighed and then sacrificed using ketamine HCl (Biotechnica Pharma Global® (BPG), China) at 25mg/kg body weight intramuscularly on the medial side of the thigh muscle. The spleen of each rat was harvested, weighed using weighing balance and then immediately fixed in 10% formal saline for tissue processing and light microscopy. The tissue samples were dehydrated in increasing gradient of 70% to 90% alcohol and absolute alcohol. The alcohol was cleared with xylene. The tissues were infiltrated in molten paraffin wax in an oven at a temperature of 65-70°C, for 30minutes. Embedding was carried out using an embedding mould. Molten paraffin wax was poured into the mould. The infiltrated tissues were placed in it. The molten paraffin wax solidifies and then form tissue blocks. After trimming, sectioning of the tissue blocks was done using the rotary microtome to cut tissue into thin ribbon like sections of 5µm thick. 20% alcohol was applied to the ribbon and then spread in a water bath at a temperature of 30°C. The sectioned tissues were picked with slides and allowed to dry. The tissue sections were placed in xylene for 15 minutes to remove excess paraffin wax from the tissues and were then subjected to hydration by passing them through descending grades of alcohol (100%, 90%, 70%) and then into water for 5 minutes each. Staining of the tissue was done using H&E dyes. The stained tissue sections on the slides were mounted with glass cover slip using xylene (DPX). The sections of the spleen were obtained and examined under Leica DM750 research light microscope with a digital camera (LeicaCC50) attached. Digital photomicrographs of the tissue sections were taken at ×40 and × 100 magnifications (Innih *et al*., 2022).

RESULTS

The body weights of the Wistar rats increased across the groups. The increase in the body weight was statistically significant ($P<0.05$) when the initial weight was compared with the final. The weight gain in the treated groups with lead acetate and low dose of *V. album*, and lead acetate and intermediate dose of *V. album* was poor when compared to the lead acetate group and high dose of *V. album*. There was a lot of improvement in the body weight gain of the group D experimental animals compared to the control group A (Table 1 & Figure 1).

The splenosomatic index across the group showed no statistically significant difference. The high dose V. album and lead acetate group showed a splenosomatic index value close to the splenosomatic index of the control group (Table 2 & Figure 1).

The photomicrograph of the Wistar rat spleen under the light microscope revealed normal microarchitecture for the control group, with apparently normal lymphoid follicles across the treated groups. There were severe red pulp sequestrations in the lead acetate treated group alone, and compromised trabeculae around the sinusoids of both the red and white pulps (Figure I, A-E).

Table 1. The Initial and Final Body Weights of the Control and Treated Groups of Wistar Rats

Groups	Initial body weight	Final body weight	P-value
Control (A)	110.60±2.29	174.00±7.80*	0.001
Pb + Low dose V. album (B)	131.20±8.10	159.80±8.12*	0.012
Pb + Intermediate dose V. album (C)	125.60±13.70	157.60±11.57*	0.004
Pb + High dose V. album (D)	124.75±7.72	196.00±15.98*	0.049
Pb Only (E)	161.00±7.65	190.25±7.59	0.110

Table II. The splenic Weights of the Control and Treated Groups of Wistar Rats and Their Spleno-somatic Indices

	Control	Pb + Low dose V. album	Pb + Intermediate dose V. album	Pb + High dose V. album	Pb Only	P-value
Splenic weight (g)	0.70±0.05	0.66±0.06	0.78±0.09	0.83±0.09	0.90±0.07	0.613
Splenosomatic index (%)	0.41±0.04	0.41±0.03	0.50±0.05	0.42±0.01	0.47±0.04	0.663

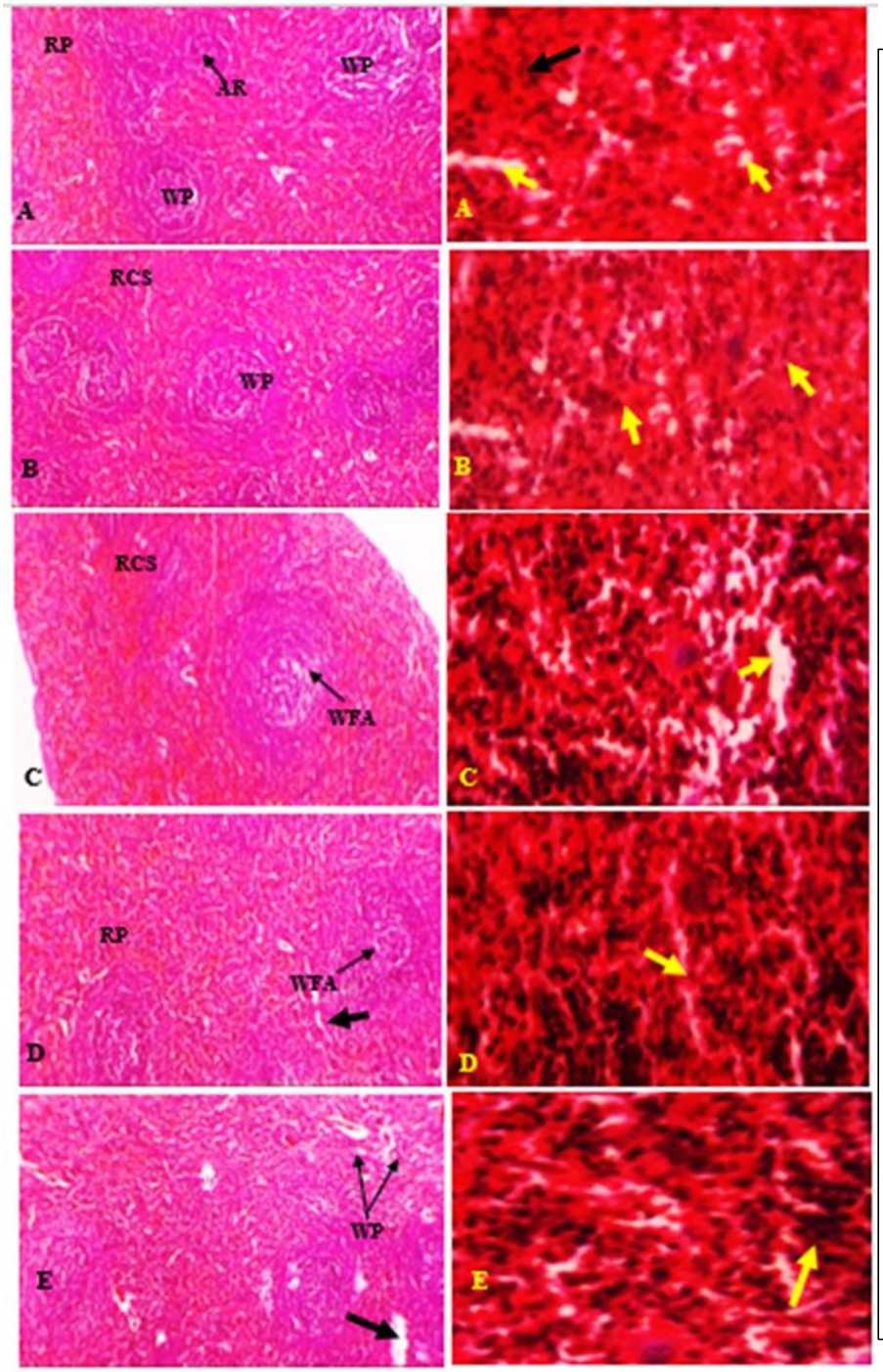

Figure II. The photomicrograph of Wistar rat spleen, in (**A**) the control group with the normal tissue and splenic architecture with the red pulp (**RP**), white pulp (**WP**) arterioles (**AR**), and sinuses (yellow arrow). In (**B**), photomicrograph of the Wistar rat spleen given low dose of *V. album* extract and lead acetate showing increased red cell sequestration (**RCS**), normal white pulp follicles (**WPF**). In (**C**), photomicrograph of the Wistar rat spleen given intermediate dose of *V. album* extract + Lead acetate, showing increased red cell sequestration (**RCS)**. follicular activation (**WFA**). In (**D**) Photomicrograph of Wistar rat spleen given high dose of *V. album* extract + Lead acetate: increased red cell sequestration (**RCS)**, and follicular activation (**WFA**) activation. Black arrow points to the sinusoid. In (**E**) Wistar rat spleen given Lead acetate: increased red cell sequestration, (**RCS)**, follicular activation (**WFA**). Black arrow point to the degenerating sinusoids. Note, on the left side of Figure 1, (A), black arrow points to normal red cells and macrophages of the red pulp. Yellow arrow points to the sinusoids. In (**C**), observe the degenerating trabaculae-yellow arrow, and the regenerating trabaculae in (**D**). Note also, the red cell infiltration in (**E**).

DISCUSSION

This study investigated the effects of *Viscum album* on lead acetate-induced toxicity in the spleen of adult Wistar rats. In this experiment, there were significant increases in the final body weights of the rats in Groups A-D when compared to their initial body weights, but group E which were administered the toxicant only (lead acetate), did not experience a significant increase in the final body weight when compared to the initial body weight. Hence, lead acetate and extract did not prevent the rats from growing in weight, but lead acetate alone did, suggesting that lead acetate had a growth-stunting effect. The adverse effect was reduced by the administration of *V. album* extract in Groups B-D. Our finding was similar to the report of Khamphaya *et al* (2022) that showed that medicinal plant improved the body weight in the male Wistar rat after been induced with lead acetate liver toxicity.

No significant increase was observed in splenic weights and splenosomatic index across the groups unlike in the report of Mylroie *et al* (1986) where they observed that lead acetate

induced rapid splenic inflammations which were largely dose dependent, thus causing increased splenic weight.

Slight hyperplasia or hypertrophy of the spleen and accumulation of degenerated cellular debris and inflammatory fluid may account for the rather constant splenic weights in the treated groups when compared to the control. However, lead acetate toxicity cause growth reduction (Ibrahim *et al*., 2012), suggesting decrease in the organ weight which progresses with increase in lead acetate dosage. This indicates that heavy metal toxicity may cause hypertrophy of organs as well as organ atrophy or, no significant change in organ weight in heavy metal toxicity.

In the group treated with lead acetate only (group E), the splenic architecture was compromised with increased red cell sequestration, suggesting an occlusion of the splenic vessels. The red pulp of the spleen appeared usually red with accumulation red cells. However, the white pulp in the rats treated with lead acetate only, showed intact structural integrity with normal follicles and no follicular activation. Although this finding is unlike in the investigation carried out in the male albino rat where the plasma white blood cells were reduced suggesting immunosuppressive effect of lead acetate (Ibrahim *et al*., 2012), which presence in the system should elicit immunological response, rather than suppress it (Faith *et al*., 1979).

The histology of the lead acetate treated groups showed increased red cell sequestration. This finding suggests that the sinus histiocytes (macrophage of the red pulp) rid the red pulp of unwanted material especially the damaged red blood cells by the toxicant (De Back *et al*., 2014). The introduction of *V. album* was clearly identifiable with its effect on the activation of the autoimmune response of the spleen, suggesting that the follicles within the white pulp released lymphocytes which help fight against the foreign harmful bodies (lead acetate).

In our investigation, the ameliorating effects of *V. album* on lead acetate induced splenic toxicity was dose dependent. Group treated with high dose showed normal red pulp and white pulp architecture when compared to the control group. This effect can be attributed to the polysaccharides and lectin present in *V. album* which stimulates immunological response in the body by its effect on immunological parameters, indirectly improving splenic and hepatic functionality and health, preventing diseases like cancer (Gardin, 2009).

CONCLUSION

In conclusion, aqueous extract of *V. album* had an ameliorative and protective effect in the histopathological damage caused by lead acetate on the spleen. The treatment was dose dependent as the higher dose proved to be more effective.

CONFLICT OF INTEREST

Authours have no conflict of interest to declare

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