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Aqueous Extract of Banana *(Musa Spp)* Blossom and Silymarin Ameliorates Spleen Toxicity Induced by Lead Acetate in Albino Wistar Rats

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ABSTRACT

This study evaluated the protective effects of aqueous extract of banana blossom (Musa spp.) and silymarin against lead acetate-induced spleen toxicity in albino Wistar rats. Thirty male rats were divided into six groups: control, lead acetate only, lead acetate plus banana blossom at 200 mg/kg or 400 mg/kg, lead acetate plus silymarin, and banana blossom only. Treatments were administered for 21 days following 14 days of lead exposure. Hematological parameters, body and organ weights, and spleen histology were assessed. Lead acetate caused weight loss, reduced packed cell volume, decreased hemoglobin, and altered spleen architecture, indicating toxicity. Banana blossom at 400 mg/kg showed marked improvement in body weight and preservation of normal splenic histology, while silymarin also produced protective effects. The findings suggest that banana blossom possesses potential antioxidant and ameliorative properties against lead-induced splenic damage. Further studies are recommended to clarify the mechanism of action and optimize dosage for therapeutic use.

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INTRODUCTION

Lead is a bluish-white lustrous metal which is very soft, highly malleable and ductile and relatively a poor conductor of electricity (Lenntech, 2022). It is very resistant to corrosion but tarnishes upon exposure to air. Lead isotopes are the end products of each of the three series of naturally occurring radioactive elements (Lenntech, 2022).

Lead is a soft heavy metal that has various applications over the years. It has been applied to metal products, cables and pipelines, but also in paints and pesticides since 5000BC (Lenntech, 2022). It is one of the four heavy metals that have the most damaging effects on human health. It can enter the human body through uptake of food (65%), water (20%) and air (15%) (Lenntech, 2022). Lead can enter (drinking) water through corrosion of pipes. This is more likely to happen when the water is slightly acidic. That is why public water treatment systems are now required to carry out pH-adjustments in water that will serve drinking purposes (Lenntech, 2022). Foods such as fruit, vegetables, meats, grains, seafood, soft drinks and wine may contain significant amounts of lead. Cigarette smoke also contains small amounts of lead (Lenntech, 2022). It causes several unwanted effects, such as: Disruption of the biosynthesis of haemoglobin and anaemia, raises blood pressure, causes Kidney damage, miscarriages and subtle abortions, disruption of nervous systems, brain damage, declined fertility of men through sperm damage, diminished learning abilities, behavioural disruptions in children, such as aggression, impulsive behavior and hyperactivity (Lenntech, 2022).

Lead acetate is a white crystalline compound of lead with a sweetish taste. Known as “sugar of lead”, it is water-soluble and one of the most bioavailable forms of lead. Similar to other lead compounds, it is very poisonous and soluble in water. In the presence of water, lead acetate forms the trihydrate, Pb(CH3COO)2 · 3H2O, a colorless or white monoclinic crystalline substance that is commonly known as sugar of lead (Padyot, 2003).

Banana blossom (*Musa acuminate*) is a flower of a banana species known as Musa *Spp* that is believed to be native to Malaysia but has spread to India and Burma. They grow at the tip of the banana clusters and hang at the end of the stem. If the blossoms are left on the tree, part of them will eventually turn into the banana fruit that we know (Novella *et al*., 2023). Like the banana fruit, these teardrop-shaped blossoms are edible and are commonly eaten as a vegetable in India, Sri Lanka and Southeast Asian countries like Malaysia, Thailand and Vietnam. As the banana bunch grows, some farmers may cut off these purple-colored blossoms to decrease the weight on the banana branches and ensure the nutrients go to the banana fruits (Novella *et al*., 2023).

Research suggests that the antioxidants in banana blossoms may have properties that help to prevent certain types of cancer and diabetes. A 2021 computer-simulated study published in the Journal of Bio Molecular Structure & Dynamics also noted that the antioxidants found in banana blossoms, quercetin and catechin, may interfere with an enzyme that assists with carbohydrate absorption, thereby decreasing blood sugar levels after eating. However, with very few research studies of banana blossoms involving human participants, evidence of the potential health benefits of the flower is limited (Novella *et al*., 2023).

What is known is that the flowers are high in fiber. Promoting the feeling of fullness, fiber also helps prevent constipation and improves digestive health. Banana blossoms are particularly high in insoluble fiber, an indigestible fiber that bulks up stools to ensure that waste moves through the colon (Novella *et al*., 2023).

Figure 1. Banana Blossom or Banana Flower
Source: (Wendy, 2023).

The spleen is a dark red to blue-black organ located in the left cranial abdomen. It is adjacent to the greater curvature of the stomach and within the omentum. It is an elongated organ, roughly triangular in cross section. The gross appearance and size of the spleen are variable, depending on the species and the degree of distension; nonetheless, spleen weights can be important in its evaluation. The ratio of splenic weight to body weight remains fairly constant regardless of age and, in rats, is typically around 0.2% (Losco *et al*., 1992).

Silymarin was first isolated in 1968 by German scientists at the University of Munich and then described and patented by the German herbal medicine manufacturer Madaus as a specific treatment “against liver diseases” (Hahn *et al*., 1968). The first commercial preparation of silymarin was developed by Rottapharm/Madaus (Cologne, Germany) and complies with the analytical specifications reported in the European Pharmacopoeia 01/2005 under “Milk Thistle fruit.” It is registered as a drug for liver diseases in many countries in Europe, Asia, America, Africa and Australia. Different forms, including capsules and tablets, are available with different dosages; the recommended daily dosage (depending on the commercial formulation used) is between 420 and 600 mg, and the majority of clinical trials have been conducted with a dosage of 140 mg three times a day (Javed *et al*., 2011).

Figure 2. Silymarine (Silybon-70 Tablet 70mg) Source: (Rastogi, 2008).

MATERIALS AND METHOD

3.1 Plant Collection and Authentication

Banana blossom (flower) was harvested during the dry season (April, 2023) from home- grown garden in Maiduguri, Borno State, Nigeria. The plant was authenticated by a plant taxonomist, Mr. Philip Edward of the Department of Biological Sciences, Faculty of Science University of Maiduguri, Nigeria. A voucher specimen (No.UMM/FPH/MUS/001) was prepared and deposited at the Herbarium Department pharmaceutical sciences, University of Maiduguri. Two kiliograms of the banana blossom were cut into pieces and air dried under the shade for seven days.

3.2 Plant Extraction

The dried banana blossom was pulverized using mortar and pestle. The dried powder (4kg) was subjected to soxhlet extraction using distilled water, as described by Trease and Evans (2002). The percentage yield was determined and the extract was stored at the Human Anatomy Laboratory until use.

3.3 Experimental Animals

The rats were obtained from the animal house of the department of animal science department bayero University Kano and kept in the animal house of the Department of Biochemistry, University of Maiduguri for the experiment. They were housed in rubber cages covered with wire-mesh. The animals were fed with pelletized animal feed (Growers mesh vital feed, Jos) and water *ad libitum*. The rats were allowed to acclimatize to the existing climatic condition for 14 days. Thirty male adult Wistar albino rats weighing between 90g and 180g were used for the study.

3.4 Materials Used

Materials used include; Glucometer, test strips, weighing balance, photomicroscope, light microscope, syringes, needles, feeders, cages, specimen bottles, test tubes, slides, cover slips, dissecting kits, beddings and improved Neubauer ruled counting chamber (haemocytometer).

Laboratory Reagents used include; lead acetate, haematoxylin and eosin (H&E), alcohol, xylene, distilled water, paraffin wax, formalin and EDTA sterilized bottles. Photochemical reagents used include Molisch's reagent, Fehling's solution, tetraoxosulphate VI acid, hydrochloric acid, ammonia solution, chloroform, acetic anhydride, and distilled water.

Determinations of Body Weight, Organ Weight and the Relative Body to Organ Weight Ratio.

Thirty adult male rats were marked with permanent makers of different colors and weighed using the digital balance. The animals were divided into six groups (A, B, C, D, E and F) groups of 5 rats each and the weights of animals were recorded weekly for the period of the experiment. The organ weights were obtained by weighing the organs on sensitive electronic weighting balance after clearing excess fats and fascia. The body to organ weight ratio was determined by dividing the weight of the organ by the body weight of the rat under investigation and multiplying the values by 100.

3.5 Experimental Design

A total of 30 Wister albino rats were used for the study. After acclimatization of the animals for 14 days, the rats were divided into 6 groups of 5 rats each.

The rats were grouped as follows:

Group A was the control group in which the rats received only the vehicle (distilled water) in equivalent dose volume.

Group B contained 5 rats. The rats served as the lead acetate non- treated group; they received 150mg/kg of lead acetate solution for 14 days.

Group C composed 5 rats which received 150mg/kg of lead acetate solution for 14 days followed by 200mg/kg banana blossom aqueous extract for 21 days.

Group D, received 150mg/kg of lead acetate solution for 14 days followed by 400mg/kg banana blossom aqueous extract for 21 days.

Group E, received 150mg/kg of lead acetate solution for 14 days and 100mg/kg for the standard drug silymarin for 21 days.

Group F, consists of 5 rats that received banana blossom only for 21 days. All treatments commenced on 15th day of lead acetate solution and lasted for 21 days.

3.6 Collection of Samples

At the end of the experiment, blood samples were collected by cardiac puncture into sterilized EDTA bottles for biochemical analyses. The animals were then sacrificed by injecting the animals with 150mg/kg ketamine injection single dose. Antero- median incision was made on the abdominal wall of the Wistar rats for the removal of the whole pancreas lying inferior to the stomach and spleen and attached to the center of the curvature of the duodenum.

3.7Hematological Parameters Determination

The following hematological parameters were also determined at the department of hematology laboratory of the University of Maiduguri Teaching Hospital, where the full blood count were determined. The full blood count test conducted are:

Test for Packed Cell Volume (PCV): Fill about three quarter (3/4)* of either a plain capillary tube with well mixed EDTA anti-coagulated blood (tested within 6 hours of collection), or a heparinized capillary tube with capillary blood, seal the unfilled end of the capillary tube using a sealant material (e.g plasticine), carefully locate the filled capillary in one of the numbered slots of the micro haematocrit rotor with the sealed end against the rim gasket, then position the inner lid carefully to avoid dislodging the tubes, centrifuge for 5 minutes (RCF 12 000 – 15 000 xg), complete packing of the red cells and immediately after centrifuging, read the PCV.

Test for White Blood Count (WBC): Measure 380µl (0.38 ml) of diluting fluid and dispense into a small container or tube, add 20µl (0.02 ml) of well-mixed EDTA anticoagulated venous blood or free flowing Capillary blood. Mix well and leave to stand for 5 minutes, assemble the counting chamber and moisten the surface of each side of the grid areas, slide the cover glass into position over the grid areas and press down on each side until rainbow colours (Newton's rings) are seen, re-mix the diluted blood sample. Using a Pasteur pipette held at an angle of about 45 degrees, fill one of the grids of the chamber with the diluted sample, taking care not to overfill the area and read the white blood count.

Test for Hemoglobin Count (HBC): centrifuge the samples at 1200g for 5 minutes, dilute 20µl of the packed red cells with 150µl of the haemolysing reagent. Mix gently and leave for at least 5 minutes, with the power supply disconnected, pour 100 ml of the Tris-EDTA-borate buffer into each of the outer sections of the Zip-Zone electrophoresis chamber, wet two chamber wicks (as supplied) in the buffer and drape one over each support bridge, ensuring each makes contact with the buffer and that there are no air bubbles under the wicks. Cover the chamber to prevent evaporation, transfer 5µl of each haemolysate sample (tests and controls) into the Zip-Zone well plate, clean the applicator tips immediately prior to use by loading with zip-prep solution and then applying them to a blotter, remove the cellulose acetate membrane from the buffer and blot twice between two layers of clean blotting papers. Do not allow the cellulose acetate membrane to dry, load the applicator by depressing the tips into the sample wells twice and apply this first loading onto some clean blotting papers, reload the applicator and apply the samples to the cellulose acetate membrane, immediately place the cellulose acetate membrane (plate) across the bridges in the Electrophoresis chamber, with cellulose acetate side down, connect the chamber to the Power Supply and electrophorese the plate for 25 minutes (or shorter) at 350 volts and 50 mA and read the Hemoglobin count.

3.8 Assessment of Effects on Histology of Spleen

The tissues were trimmed, dehydrated in graded series of alcohol in ascending order of 30%, 50%, 80%, 95% and 100% (appendix I). The tissues were cleared in xylene, embedded in paraffin wax. The tissues were sectioned between 5 to 7µ and stained with hematoxylin and eosin. Photomicrograph of the tissues was taken using photomicroscope (Olympus C-5A, Tokyo Japan 203250) at x100, x200 and x400 magnifications (Das *et al*, 2012).

3.9 Statistical Analysis

Data obtained from this study were analyzed to determine the differences between and within groups. One-way analysis of variance (ANOVA) was conducted using statistical package for social sciences (SPSS) version 21. All the values were presented as Mean ± SD. Values of $p>0.05$ were considered statistically significant.

4.1 Effect of Banana Blossom on Lead Acetate Induced Toxicity on Blood Parameter

The result of the hematological parameters presented in table 1. shows that, the values of the packed cell volume (PCV) decreased in group B which received lead acetate 150mg/kg only, group C which received lead acetate 150mg/kg and banana blossom 200mg/kg and group F which received banana blossom 400mg/kg only and increased in group E which received lead acetate

150mg/kg and sylimarin 400mg/kg which was statistically significant at p≥0.05. While group D which received lead acetate 150mg/kg and banana blossom 400mg/kg values are similar to that of the control group.
The values in the white blood count (WBC) decreased in group C which received lead acetate 150mg/kg and banana blossom 200mg/kg and group E lead acetate 150mg/kg and sylimarin 400mg/kg and increased in group B which received lead acetate 150mg/kg only which was statistically significant at p≥0.0. While group D which received lead acetate 150mg/kg and banana blossom 400mg/kg and F which received banana blossom 400mg/kg only are similar to that of the control group.
The values in the hemoglobin count (HBC) decreased in group B which received lead acetate 150mg/kg only, group C which received lead acetate 150mg/kg and banana blossom 200mg/kg and F which received banana blossom 400mg/kg only and increased in group E which received lead acetate 150mg/kg and sylimarin 400mg/kg which was statistically significant at p≥0.05. While group D which received lead acetate 150mg/kg and banana blossom 400mg/kg is similar to that of the control group.

Table 4.2 Effect of Banana Blossom on Lead Acetate Induced Toxicity on Blood Parameter

(N-5)

Groups	PVC	WBC	HBC
Control (0.00)	40.00 ± 0.02	2.86 ± 0.02	13.66 ± 0.02
LA 150mg/kg	36.00 ± 0.02***	4.16 ± 0.02***	12.26 ± 0.02***
LA 150mg/kg/ BB 200mg/kg	27.40 ± 0.24***	2.04 ± 0.20**	9.04 ± 0.02***
LA 150mg/kg/ BB 400mg/kg	40.40 ± 0.24	2.74 ± 0.02	13.24 ± 0.02
LA 150mg/kg/ SLR 100mg/kg	44.60 ± 0.24***	2.06 ± 0.02*	15.26 ± 0.02***
BB 400mg/kg	35.40 ± 0.25***	2.16 ± 0.02	11.66 ± 0.02***

Values are Presented as Mean ± SEM. LA= Lead acetate, BB = Banana Blossom, SLR= Silymarin, PVC= Park Cell Volume, WBC = White Blood Cells and HB = Hemoglobin, Statistical values of p≥0.05 is considered significant, * = level of significant

4.3 Effect of Banana Blossom on Lead Acetate Induced Toxicity on Histology of the Spleen

The control group showed normal aggregation of lymphoid tissues around eccentric arteriole, white pulp, blood vessel and even distribution of red pulp, the group administered 150mg/kg of lead acetate showed mild wide spread hyperplasia of the white pulp and normal white pulp, the group administered with 150mg/kg and banana blossom 200mg/kg showed wide spread hyperplasia of the white pulp and enlarged vascular channel, the group administered with 150mg/kg and banana blossom 400mg/kg showed mild widespread hyperplasia of white pulp and normal white pulp, the group administered with 150mg/kg and sylimarin 100mg/kg showed wide spread hyperplasia of white pulp and trabeculae and the group administered with banana blossom 400mg/kg only, showed normal distribution of white pulp and red pulp.

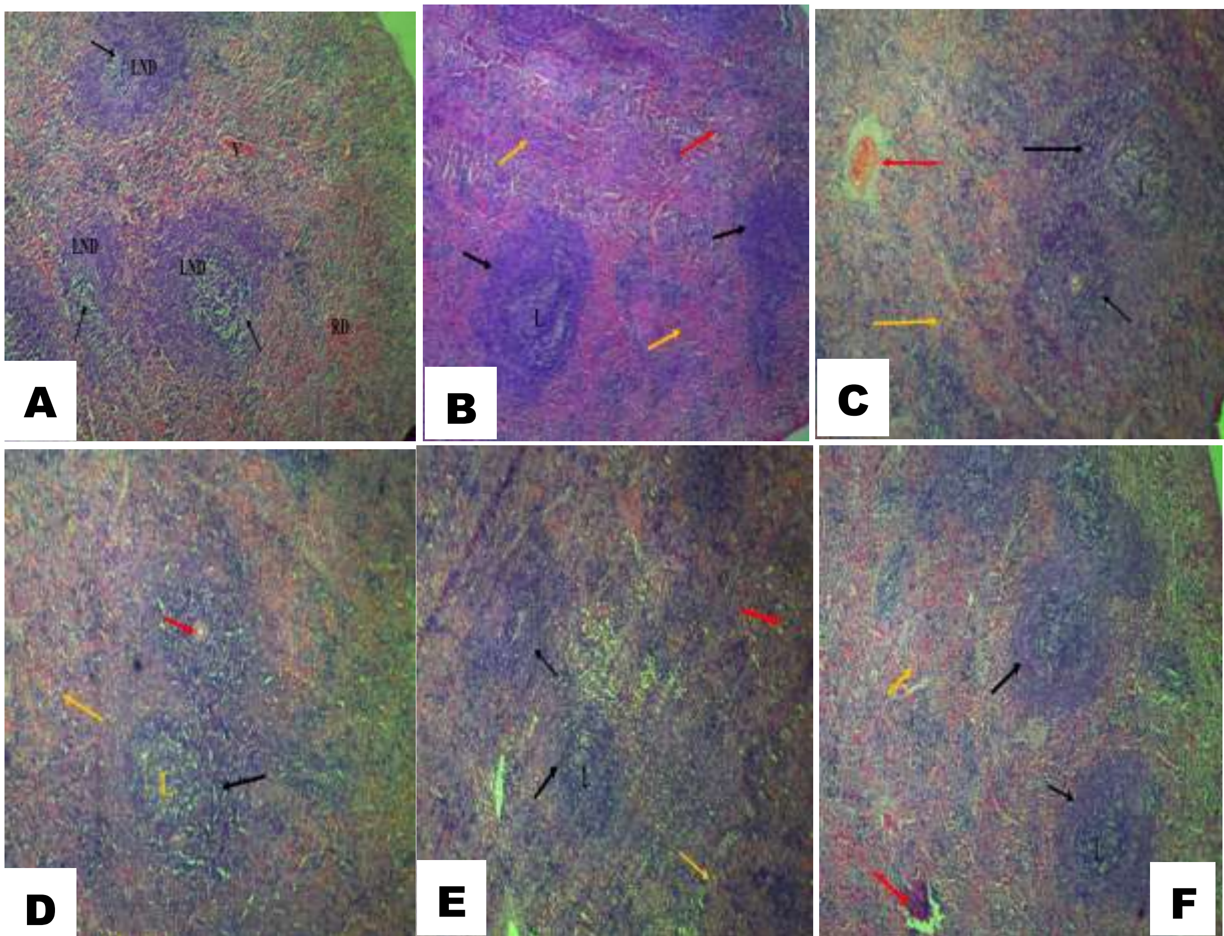

Figure 3. Composite Micrograph of Rats Spleen Showing: A (Control group) normal aggregation of lymphoid tissues (LND) around eccentric arteriole (arrows) white pulp, blood vessel (V) and even distribution of red pulp (RD). B. the group treated with lead acetate solution 150mg/kg showed mild wide spread hyperplasia of the white pulp and normal white pulp (WP). Group C and D. showed mild to moderate wide spread hyperplasia of the white pulp and enlarged vascular channel (VC). Group E Photograph showed wide spread hyperplasia of the white pulp and trabeculae (arrow) and Group F showed Photograph of Rat Spleen showed distribution of white pulp and red pulp similar to normal.

DISCUSSION

The present study digs into the complex interplay between lead-induced spleen toxicity in Albino Wister rats and the potential ameliorative effects offered by banana blossom, and silymarin, unraveling the nuances of this interaction. With regards to the result on the effects of lead acetate on organ and body weight, it is evident that lead acetate causes weight loss, as the final body weight of the group (B) which received Lead Acetate 150mg/kg was lower than the control group (A). This result is in line with a study evaluating the effect of lead acetate toxicity on experimental male albino rat by Nabil *et al.,(* 2012).

Results obtained from Table 1 showed that, the changes observed in the mean body weight of the treatment group when compared with the control group was statistically significant only in the group treated with banana blossom 400mg/kg.

Results obtained from table 2 showed that, the changes observed in the packed cell volume (PCV) was statistically significant in the groups that received; lead acetate 150mg/kg (Group A), lead acetate 150mg/kg and banana blossom 200mg/kg (Group C), lead acetate 150mg/kg and silymarin 100mg/kg (Group E) and group F that received Banana blossom 400mg/kg. The changes observed in white blood cells (WBC) was statistically significant in group B and E. The changes observed in Haemoglobin count (HBC) was statistically significant in groups B, C, E and F.

Under histological sections,the Spleen tissues of the control group showed normal architecture of the two components of the parenchyma of [1]the spleen (splenic pulp) which are the white and red pulps. The white pulp is occupied by the central arteriole. Lymphatic follicles containing lymphocytes were present. The lymphocytes were closely packed. The red pulp is demarcated from the white pulp by the marginal zone which is delimited from the lymphatic follicles by the marginal sinus. Cell cords networks called splenic cords constitute the red pulp and were separated by vascular sinuses with bulged nuclei. RBCs, plasma cells containing eccentric nuclei, small and large lymphocytes were noticed. This result corresponds to the study on the Spleen as a digestive system (Trayhurn *et al*., 2004).

CONCLUSION

The study into the effects of banana blossom and silymarin on the histology of the lead acetate induced toxicity on the spleen of albino wistar rats, unveils promising methods or means to reverse or reduce the impacts lead acetate on splenic architecture, weight and overall function. Through cautious histological analysis, this study clarifies on the curative potential of both banana blossom and silymarin, highlighting their capacity to reduce the adverse alterations induced by lead acetate, thereby preserving the structural integrity of splenic tissues, and also the effect of banana blossom increasing body weight. There were significant decrease in packed cell volume in groups administered lead acetate only, banana blossom and silysmarin was seen to help increase the packed cell volume, there were significant decrease in white blood cells in groups that were administered lead acetate while the Haemoglobin count also decreased in groups administered lead acetate and silymarin.

RECOMMENDATIONS

Based on the compelling findings observed in this study on the effects of banana blossom and silymarin on spleen histology amidst lead acetate-induced toxicity in albino Wister rats, both banana blossom and silymarin were observed to have been effective in mitigating the destructive effects of lead acetate on the testis, and banana blossom was observed to have been effective in increasing body weight.

The duration and dosage should be further looked into, in order to dive deeper into the full blood count analysis of the albino Wister rats.

Further research should be carried out along this line to delve deeper into the mechanistic actions underlying the curative effects of banana blossom and silymarin. Understanding their specific pathways and interactions within the spleen microenvironment would fortify their potential applications. Exploration of varying doses of banana blossom and silymarin to ascertain the most effective concentrations that offer maximal protection against lead acetated-induced testicular toxicity without adverse effects and also assessing the prolonged and cumulative effects of banana blossom and silymarin administration to evaluate their sustained efficacy and safety profiles are also encouraged.

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