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Effects of Banana Blossom (*Musa Acuminate*) and Silymarin on the Histology of the Testis and Testicular Sperm Concentration Following Lead Acetate Induced Toxicity in Albino Wistar Rats

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ABSTRACT

*This study was aimed to determine the Effects of Banana Blossom (Musa Acuminate) and Silymarin on the Histology of the Testis and Testicular Sperm Concentration Following Lead Acetate (LA) Induced Toxicity in Albino Wistar Rats. Thirty male albino Wistar rats were divided into 6 groups. Group **I** serve as the control group. Group **II** was treated with 150mg/kg of LA. Group **III** was administered 150mg/kg LA and 200mg/kg banana blossom (BB) aqueous extract. Group **IV** received 150mg/kg of LA and 400mg/kg BB. Group **V** received 150mg/kg of LA plus 100mg/kg Silymarin. Group **VI** received BB only. Lead acetate was administered for 2 weeks followed by BB and Silymarin treatment for three weeks. Animals were sacrificed and the testes were removed. The result of this study showed that testicular sperm concentration was significantly reduced in the group treated with LA only, while the groups that were treated with BB and Silymarin showed non-significant decreased when compared to the LA group. Lead acetate caused severe degeneration of seminiferous tubules, thickening and disruption of the basement membrane and degeneration of the interstitial connective tissue, while banana blossom and Silymarin showed some degree of amelioration with the micrograph showing moderate to mild degeneration of seminiferous tubules, Spermatogenic Series cells, diminished basement membrane and the interstitial connective tissue. The group that received BB only also showed mild distortion of cyto-architecture of the testicular tissue. Higher doses of 500mg/kg and above as seen in this study may affect the integrity of the testicular tissue.*

Keywords: *Albino Rats; Banana Blossom; Lead Acetate; Silymarin; Testicular Sperm Concentration and Toxicity*

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INTRODUCTION

Banana blossoms are rich sources of various minerals and antioxidants including various bioactive compounds which helps in improving various diseases and disorders. The flower (Figure 1) extract helps in exhibiting cytotoxic effects by regulating the inflammatory response (Liu *et al*., 2018).

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

Due to it being rich in fibre and iron the blossoms helps in reducing blood sugar level and raising blood hemoglobin levels. Using different standard methods it is revealed that the flower is a rich source of fibre (70%), carbohydrates (53.78%) and Protein (19.60%) (Ramu *et al*., 2017). Preliminary phytochemical analysis was performed in order to know different types of compounds present in the banana flowers of two cultivars (Preethi and Balakrisshnamurthy, 2011; Khin, 2012). The results of phytochemical tests are shown in (Table 1) (Khin, 2012).
Lead is a soft metal that has known many applications over the years. It has been used widely since 5000 BC for application in metal products, cables and pipelines, but also in paints and pesticides (Lenntech, 2022). Lead is one out of four 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). 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. 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. For as far as we know, lead fulfils no essential function in the human body, it can merely do harm after uptake from food, air or water (Lenntech, 2022). The deterioration of male fertility, found in numerous epidemiological studies of past decades, can be connected to growing exposure to environmental toxins (Martynowicz, *et al*., 2005). Heavy metals, especially lead is widely spread and extremely toxic. The mechanism by which lead exerts toxic effects on testis is quite complex. It involves spermatogenesis, steroidogenesis, and red-ox system. The chronic lead exposure can induce functional disorder (decrease of testosterone synthesis) or morphological disorder (decrease of testicular weight and seminal vesicle, peritubular fibrosis, seminiferous tubular diameter decrease and decrease in germ cell population related to an apoptotic process). Currently existing environmental and occupational exposure to lead and increasing combined exposure to environmental toxins, results in constantly increasing number of diagnosed fertility impairments. (Martynowicz, *et al*., 2005).

Silymarin, a flavonolignan from the seeds of 'milk thistle' (Silybum marianum), has been widely used from ancient times because of its excellent hepatoprotective action (Dixit *et al*., 2007). It is a mixture of mainly three flavonolignans, viz, silybin, silidianin, and silychristine, with silybin being the most active. Silymarin has been used medicinally to treat liver disorders, including acute and chronic viral hepatitis, toxin/drug-induced hepatitis, and cirrhosis and alcoholic liver diseases. It has also been reported to be effective in certain cancers. Its mechanism of action includes inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane; reduction of glutathione oxidation to enhance its level in the liver and intestine; antioxidant activity; and stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration. It is orally absorbed but has very poor bioavailability due to its poor water solubility (Dixit *et a*l., 2007).

The testis, also called testicle, is the male reproductive glands. They are twin oval-shaped organs located within the scrotum, which is the loose pouch of skin that hangs outside the body behind the penis. The scrotum is often asymmetric, with one testis extending below the other. The testes are connected with the abdomen by spermatic cords, and are attached to the scrotum by testicular ligament (Vasković, 2023). The testes produce sex hormones called androgens (primarily testosterone) in the process of steroidogenesis and are the place of spermatogenesis (production of sperm). While the location of the scrotum makes the testes vulnerable to injury (they have no muscles or bones to shield them), it provides a cooler temperature for the organs. A cooler environment is necessary for healthy sperm production (Vasković, 2023). Testicular disorders can affect a man's sexual functioning, fertility and cause hormonal imbalances. Among the variety of causes of testicular disorder, environmental factors such as drugs and chemical agents seem to be among the most important factors of infertility (Bradley *et al*., 2022).

Table 1. Phytochemical Constituents of Banana Blossom.

Constituents	Extracts	Reagents Used	Observation	Remarks	I	II
Alkaloids	1% HCL	Dragendroff's reagent	Orange ppt	+	+	
Glycosides	Distilled water	10% lead acetate	White ppt	+	+	
Steroids	Pet-ether	Acetic anhydride Conc: H2SO4	Blue colour	+	+	
Saponins	Distilled water	NaHCO3	Frothing	+	+	
Tannins	95% ethanol	10% lead acetate	Yellow ppt	+	+	
Flavanoids	95% ethanol	Conc. HCl, Mg turning	Pink colour	+	+	
Terpenoids	CHCl3	Acetic anhydride, Conc. H2SO4	Brick red color	+	+	
Cyanogenic glycosides	Distilled water	Conc. H2SO4	No brick, red colour	-	-	

Source: (Khin, 2012).

Statement of the problem

Lead has been known to, and used by, humans for many centuries. This easily worked and corrosion-resistant metal has been used for pipes, pewter and paint since Roman times. It has also been used in lead glazes for pottery and, in this century, insecticides, hair dyes and as an anti-knocking additive for petrol. All these uses have now been banned, replaced or discouraged as lead is known to be detrimental to health but it is still widely in used. Lead poisoning also can induce testicular dysfunction. Banana blossoms have many nutritional and medicinal properties as well as function in organs and system of humans. Banana flower/blossoms have been used in traditional medicine across the Americas, Asia, Oceania, India, and Africa to treat various ailments. (Shahzadi *et al.*, 2022). Hence this research was carried out to ascertain the therapeutic effects of banana blossom and silymarin on lead acetate induced testicular toxicity in Albino Wistar rats.

The objective of the study

The objectives of the study are;

i.To assess the effects of banana blossoms and silymarin on sperm count in albino Wistar rats
ii.To determine the effects of banana blossom and silymarin on the histology of the testes in lead acetate induced toxicity in albino Wistar rats.

Research questions

1. What are the effects of aqueous extract of banana blossom and silymarin on on sperm count in lead acetate induced testicular toxicity in albino Wistar rats?
2. What are the effects of aqueous extract of banana blossoms and silymarin on the histology of the testis in lead acetate induced testicular toxicity in albino Wistar rats?

Significance of the study

The study will provide a scientific knowledge on the benefits of banana blossoms against lead acetate induced testis toxicity. This study will bring foreword the health benefits of banana blossoms on the testis.

The scope of the study

This study will cover the effects of banana blossoms and silymarin on testicular sperm concentration and on the histology of the testes in lead acetate induced toxicity in Albino Wister rats.

MATERIALS AND METHODS

Materials

Materials and equipment used include; weighing balance, arm scope, light microscope, syringes, measuring cylinder, needles, feeders, cages, specimen bottles, slides, cover slips, dissecting kits and beddings, microtome, microwave oven, mortar, pestle, hemocytometer. Laboratory Reagents used include; lead acetate, haematoxylin and eosin (H&E), normal saline, alcohol, xylene, distilled water, paraffin wax, formalin and EDTA sterilized bottles.

Plant collection and authentication

Banana blossom was collected in Maiduguri, Borno State, Nigeria, during the dry season (April 2023), from a backyard garden. Mr. Philip Edward, a plant taxonomist with the Department of Biological Sciences in the Faculty of Science at Maiduguri University in Nigeria, verified the plant's authenticity. A voucher specimen number (UMM/FPH/MUS/001) was placed at the University Of Maiduguri Herbarium Department Of Pharmaceutical Sciences. Banana blossom was divided into pieces and allowed to air dry for seven days under the shade.

Plant extraction

The dried banana blossom was crushed with a mortar and pestle after drying. According to Trease and Evans (2002), Three hundred and sixty grams of the dried powder were subjected to soxhlet extraction using distilled water. The extraction was held in the Human Anatomy Laboratory.

Experimental animals

Albino Wistar Rats were used in the experiment, where they were kept in the animal house of the Department of Biochemistry at the University of Maiduguri after being received from the department of animal science's animal house at Bayero University Kano. They were kept in plastic cages covered with wire-mesh. Water was available at all times, and the animals were given pelletized animal feed (Growers mesh vital feed, Jos). The rats were given two weeks to adjust to the current climatic conditions. For the investigation, 30 mature male Wistar albino rats weighing 90–180g were used.

Experimental design

Thirty Wistar albino rats were used for the study. The animals were divided into six groups after acclimatized for 14 days. They were grouped as follows;

Group I: control group which received distilled water in equivalent dose volume.

Group II: served as the lead acetate non- treated group; was administered 150mg/kg of LA solution for 14 days.

Group III: received 150mg/kg of LA for 14 days followed by 200mg/kg banana BB extract for 21 days.

Group IV: received 150mg/kg of LA solution for 14 and 400mg/kg BB aqueous extract.

Group V: received 150mg/kg of LA followed by 100mg/kg for the standard drug silymarin.

Group VI: received BB only for 21 days.
Treatments with BB and silymarin commenced on the 15th day after LA administration and lasted for 21 days.

Collection of samples

At the end of the experiment, the animals were sacrificed by injecting them with 120mg/kg ketamine injection single dose. Median incision was made on the scrotal sac and the testes were removed by using fingers to push it out of the scrotal sac. One of the testes was used for sperm analysis and the other was processed for routine histology.

Method of determining testicular sperm concentration

One of the testes that was removed was crushed in 10ml of normal saline solution. The crushed mixture was pipetted and used to feed the hemocytometer, which was placed under a light microscope (Seed *et a*l., 1999; Ohtani *et al*., 2003; Pant and Srivastava, 2003). The number of sperm head was counted per each quadrant by view through the eye piece of the microscope at x40 magnification. The average number of sperm head was obtained, by adding the number of heads per each quadrant which is five (Seed *et al*., 1999; Ohtani *et al*., 2003; Pant and Srivastava, 2003). The average number of sperm head was divided by the dilution factor which is 10ml and multiplied a standard factor of 10^6 to obtain the total sperm volume microscope (Seed *et al*., 1999; Ohtani *et al*., 2003; Pant and Srivastava, 2003).

Method of tissue preparation

The tissues were trimmed, dehydrated in graded series of alcohol in ascending order of 30%, 50%, 80%, 95% and 100%. 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).

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.

Effect of banana blossom and silymarin on lead acetate induced testicular toxicity on sperm concentration in albino wistar rats

The result of this present study shows that the group that received LA 150mg/kg (5.68×10^6) only for two weeks showed significant reduction in testicular sperm concentration when compared to the control group (9.93×10^6) at $p<0.05$. The group that received LA 150mg/kg followed by BB 200mg/kg, 400mg/kg and silymarin 100mg/kg showed values of 7.92×10^6, 8.07×10^6 and 8.97 93 $\times 10^6$ respectively. This showed an increased when compared to LA only group which was not significant. The result for the group administered banana blossom (500mg/kg) only was 11.46×10^6, which was also statistically significant when compared to the group that received LA only at $p<0.05$. Mean testis to body weight ratio was also statistically significant in the group that was administered BB only.

Effect of banana blossom and silymarin on lead acetate induced testicular toxicity on the histology of the testis in albino wistar rats

Photomicrograph of rat testis of the control group showed normal seminiferous tubules, basement membrane,

Table 2. Effect of Banana Blossom and Silymarin on Lead Acetate Induced Testicular Toxicity on Testicular Sperm Concentration in Albino Wistar Rats.

N-5 Groups	MBWT (g)	MWBT (g)	MOBWTR	MTSC (10^6)
Control (0.00)	199.40 ± 4.40	1.93 ± 0.20	0.01	9.93 ± 2.20
LA 150mg/kg	108.20 ± 13.73	1.78 ± 0.07	0.01	5.68 ± 5.74*
LA 150mg/kg/	187.40 ± 12.43	2.55 ± 0.21	0.01	7.92 ± 3.74
BB 200mg/kg LA 150mg/kg/	174.20 ± 13.20	2.0 ± 0.09	0.01	8.07 ± 3.86
BB 400mg/kg LA 150mg/kg/	182.50 ± 12.16	1.51 ± 0.45	0.01	8.97 ± 7.54
SLR 400mg/kg BB 400mg/kg	151.20 ± 5.51	2.39 ± 0.11	0.01	11.46 ± 7.10*

Values are Presented as Mean ± SEM. N=Number of Rats per Group, LA= Lead acetate, BB = Banana Blossom, SLR= Silymarin, MBWT = Mean Final Body Weight, MBWT= Organ to Body Weight, MOBWTR=Mean Organ to Body Weight Ratio, MTSC =Mean Testicular Sperm Concentration. Statistical values of p≥0.05 is Considered Significant, * = Level of Significant.

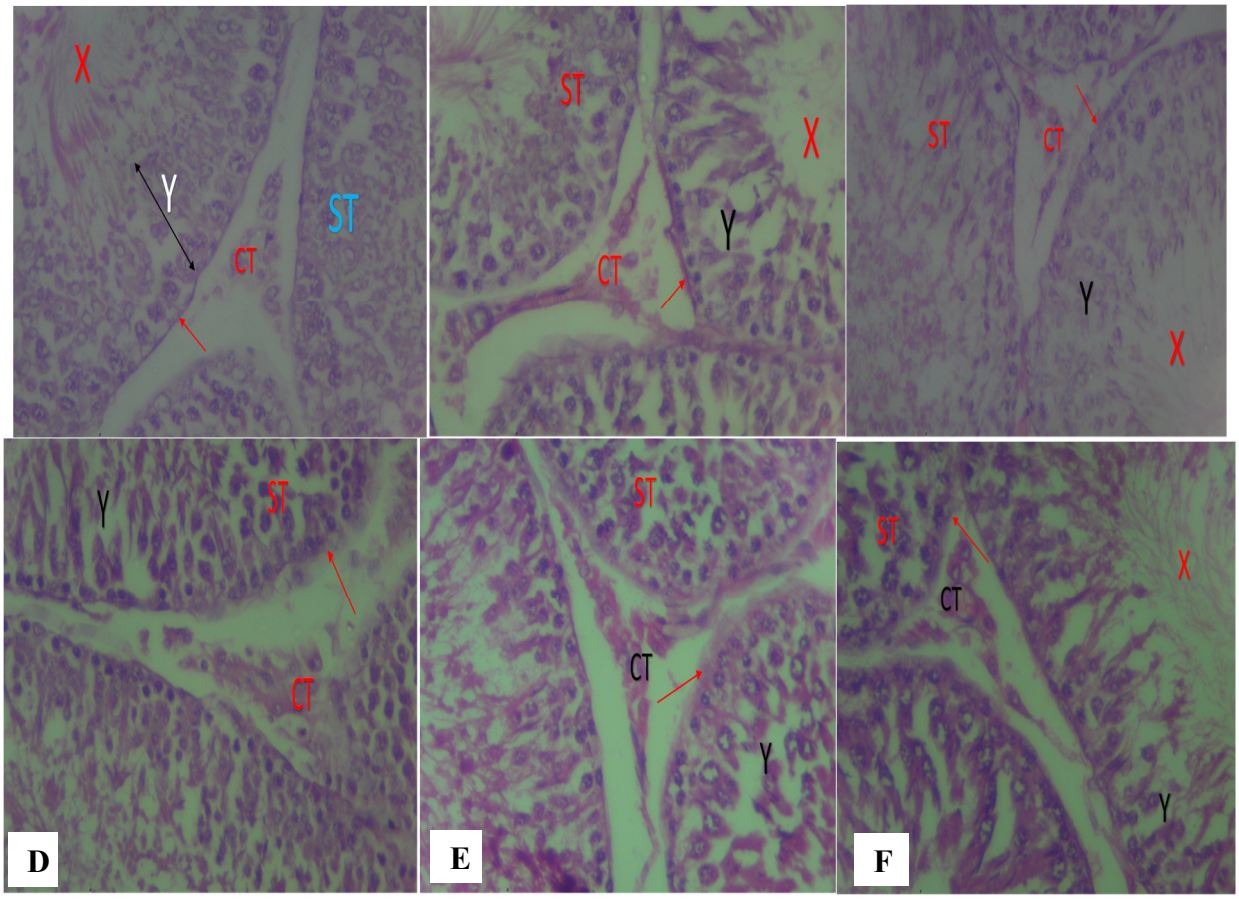

Figure 2: Showing the Composite Photomicrographs of the Testis from the Control group (A) showing normal seminiferous tubules(ST), Spermatogenic Series Cells (Y), Basement Membrane (Red Arrow), Lumen Containing Tuft of spermatid (X) and Leydig cells in Interstitial Connective Tissue (CT); the group treated with LA150mg/kg (B) showing Severe Degeneration of Seminiferous Tubules (ST and Y), Thickening and Disruption of the Basement Membrane (Red Arrow) and Degeneration of the Interstitial Connective Tissue (CT); the group treated with LA 150mg/kg and BB 200mg/kg (C) shows Moderate Degeneration of Seminiferous and Leydig Cells (CT); the group treated with LA 150mg/kg and BB (D) showing Diminished Basement Membrane (Red Arrow), and Degeneration of Spermatogentic Series (Y); the group Treated with Lead Acetate Solution 150mg/kg and Silymarin 400mg/kg (E) Showing Mild Degeneration of Seminiferous Tubule (ST), and Diminished Basement Membrane (Red Arrow); The group that received BB only (F) shown, Mild to Moderate

interstitial connective tissues, tuff of spermatid and cells of the spermatogenic series (Figure 2A-F). The group treated with lead acetate 150mg/kg only for 14 days showed Severe Degeneration of Seminiferous Tubules (ST and Y),

Thickening and Disruption of the Basement Membrane (Red Arrow) and Degeneration of the Interstitial Connective Tissue (CT) The group that received lead acetate 150mg/kg followed by banana blossom 200mg/kg, 400mg/kg and silymarin 100mg/kg showed severe degeneration of seminiferous tubules (ST), basement membrane (CT) (Figure 2).

DISCUSSION

The result of this study showed that testicular sperm concentration decreased significantly in the group that received lead acetate only as shown in (Table 1). This is probably due to the free radicals released due to lead acetate or due to the direct effect of lead acetate on the testicular tissue. This study was in line with the studies conducted by other scholars who also observe significant decreased in testicular sperm count and sperm concentration when lead acetate was administered to rats (Rania *et al*., 2014; Ekeh *et al*., 2015; Ayuba *et al*., 2017; Yeti *et al*., 2020)). An increased in testicular sperm concentration was observed in the group that received lead acetate followed by banana blossom. But the increased observed were not statistically significant when compared to lead acetate only group. The group administered banana blossom only showed a significant increase in testicular sperm concentration when compared to the group that received LA only at $p<0.05$.

Our studies also showed that photomicrograph of rat testis of the control group showed normal seminiferous tubules, basement membrane, interstitial connective tissues, tuff of spermatid and cells of the spermatogenic series. The group treated with lead acetate only showed severe degeneration of seminiferous tubules, thickening and disruption of the basement membrane and degeneration of the interstitial connective tissue. This results obtained was supported by the works of Rania *et al*., (2014), Ekeh *et al*., (2015), Ayuba *et al*., (2017) and Yeti *et al*., (2020) who observed changes in the cyto-architecture of the testis such as degeneration, disruption, necrosis of the seminiferous tubules and the interstitial connective tissue, thickening and detachment of the basement membrane, vacuolation, scanty Leydig cells and congested blood vessels when lead acetate was administered. The group that received lead acetate and then banana blossoms and silymarin showed mild to moderate degeneration of seminiferous tubules, basement membrane (Figure 2).

The group that received BB only showed mild to moderate degeneration of seminiferous tubule, distorted cells of spermatogenic series and normal tails of spermatids. This showed that banana blossom has potential of ameliorating the effect of lead acetate induced testicular toxicity but at lower doses as high doses might also induced testicular toxicity.

Conclusion

This study concludes that there was degeneration of the normal histological cyto-architecture of the testes caused by lead acetate solution .Banana blossom has shown to be helpful in mitigating the negative effects of lead acetate solution on the histology of the testis and testicular sperm concentration. But higher doses seem to compromise the integrity of the testicular tissue.

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