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Original Research Article

Monodora Myristica Seed Extract Mitigates Lead Acetate Induced Hepatic and Testicular Injury in Male Wistar Rats

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ABSTRACT

Monodora myristica (MMS), a tropical botanical species classified within the *Annonaceae* family, is extensively utilized as a condiment in the culinary preparation of various African cuisines to amplify flavour. The current study aims to investigate the mechanism by which MMS protect the liver and testicular tissues from the damage caused by lead acetate (PbAc). For this study, 36 male Wistar rats weighing 180–200 g each were employed and were grouped into six groups of 6 rats each. Group 1; was given distilled water, Group 2; PbAc (15 mg/kg) was given, Group 3 and 4; MMS (200 and 400 mg/kg) were administered; Group 5; PbAc (15 mg/kg) and MMS (200 mg/kg) were given; Group 6; PbAc (15 mg/kg) and MMS (400 mg/kg) were administered. While the right testis was obtained for antioxidant tests, the liver and left testis were removed for histopathological analysis. In the group treated with varying doses of MMS alongside lead acetate, liver enzyme levels—specifically ALT (alanine aminotransferase), AST (aspartate aminotransferase), and ALP (alkaline phosphatase)—were significantly lower compared to those in the group exposed only to lead acetate ($P < 0.05$). This combined treatment resulted in a significant reduction in malondialdehyde levels ($P < 0.05$) while enhancing the activity of antioxidant enzymes like catalase and SOD (superoxide dismutase). These findings indicate that MMS offers protective and restorative function against damage caused by lead acetate exposure to both the liver and testicles through elevation of antioxidant enzymes.

Keywords: Testis, Hepatic, *Monodora myristica* seed, Lead acetate.

Introduction

There is substantial evidence that exposure to environmental chemicals can lead to infertility, reproductive disorders, and sexual dysfunction.[1] Given the liver's crucial role in chemical transformation, detoxification, and elimination, these substances also contribute significantly to hepatic damage. Common examples of such chemicals include lead and dichlorvos, which individuals encounter through industrial and agricultural activities, workplace exposures, and household environments.[2] Lead, a naturally occurring heavy metal, is prevalent in the environment and numerous manufactured products. It can originate from various sources such as contaminated food, lead-containing plumbing systems, improper food preservation methods, industrial waste, vehicle emissions, coatings, personal care products, and drinking water.[3] One of the top ten environmental chemicals that have been found to present serious risks to public health is lead. Similarly, it has been shown that exposure to lead causes more than 1.06 million deaths worldwide, with developing countries having the highest rate.[2] Numerous studies have shown that exposure to lead impairs spermatogenesis by reducing antioxidant reserves and interfering with signaling in the hypothalamic-pituitary-testicular axis.[4,5]

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Research indicates that lead exposure in Wistar rats is associated with liver damage, as evidenced by increased levels of the serum enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT).[6,7]*Monodora myristica*, also known as calabash nutmeg or African nutmeg, is a tropical tree in the Annonaceae family, which includes custard apples. This species is indigenous to numerous African countries such as the Guinea-Bissau, Tanzania, Togo, Republic of the Congo, Sudan, Ghana, Cameroon, Kenya, Liberia, Ivory Coast, Uganda, Democratic Republic of the Congo, Benin, Gabon, Sierra Leone, Central African Republic, Equatorial Guinea, and Nigeria. Locally, it goes by different names like Iwor among Itsekiris, Ikposa in Benin, Ehiri by the Ibo, Gujiya dan miya in Hausa and Ariwo by the Yoruba.[9]

Several extracts of *Monodora myristica* seeds have demonstrated antioxidant qualities in vitro in earlier studies.[10-12] Phytochemical analyses reveal that these seeds are abundant in tannins, saponins, alkaloids, glycosides, flavonoids, and steroids.[13-14] Additionally, *Monodora myristica* seeds have been found to enhance fertility by improving sperm motility, count, and morphology.[15-18] This study aims to investigate the mechanism by which *Monodora myristica* seeds (MMS) protect the liver and testicular tissues from injury.

Materials and Methods

Collection and Identification of Plant Material:

The dried seeds of *Monodora myristica* (Gaertn.) Dunal were sourced from Okada Market, situated at latitude 6.7331405 and longitude 5.3913375 in Edo State, Nigeria. In May 2024, these seeds were botanically identified and authenticated (UBH-M350) by Dr. Henry Akinnibosun, Department of plant Biology and Biotechnology, Faculty of Life Science University of Benin, Benin city Edo State, Nigeria.

Preparation of Plant Material:

The seeds were dried under the sun, sorted, peeled, and ground. The seeds were weighed and air-dried until they reached a constant weight.

Extraction of Plant Material:
The seeds of *Monodora myristica* plant was air-dried and pulverized. The crushed *Monodora myristica* seeds (3 Kg) was subjected to maceration extraction using absolute methanol (5 L) for total period of 24 hours. The extract was concentrated by using Thermostatic digital water bath (Model HH-S4), China to obtain methanol extract of *Monodora myristica.*

Animals:

Thirty-six male Wistar rats weighing 180 and 200 grams were obtained from the Department of Anatomy's Animal House at the University of Benin. These rats were kept in polypropylene cages lined with paddy husk bedding and had constant access to water and standard rat chow. Before starting experiments, they underwent a period of acclimatization to the laboratory environment. All procedures adhered to the guidelines set by the National Institutes of Health for laboratory animal care and use.[19]

Experimental design:

Thirty-Six male Wistar rats were randomly assigned into six groups, with 6 rats in each group. Group 1 received 0.5 mL distilled water orally which served as the control group, groups 2 was induced with 15 mg/kg,[19] of PbAc (JHD-China), group 3 and 4 received 200 and 400 mg/kg of MMS respectively, group 5 was given both PbAc (15 mg/kg) and MMS (200 mg/kg), while group 6 received both PbAc (15 mg/kg) and MMS (400 mg/kg). The administration was done orally for 56 days except for lead acetate that was given intraperitoneally once in a week. All animals were euthanised on day 57 by administering 50 mg/kg of sodium thiopental intraperitoneally. To separate the serum from clotted blood, 3.0 mL of blood samples were obtained by cardiac puncture and centrifuged right away for five minutes at 11,180 × g. The testes and liver were removed for analysis; the left testis was kept in Bouin's fluid for histopathological analysis, and a 2-gram sample of the liver was preserved in 10% formalin. To obtain a supernatant enriched with testicular extract, one gramme of the right testis was homogenised in four millilitres of ice-cold phosphate-buffered saline solution and then centrifuged at 10,000 × g for ten minutes. Malondialdehyde levels were measured along with catalase and superoxide dismutase activities in this supernatant. Furthermore, aspartate transaminase (AST) and alanine transaminase (ALT) activities were assessed using serum samples.
Serum biochemical assay:
Commercial enzymatic assay kits (Randox Laboratories Ltd.) were employed to quantify the enzymatic activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST). For each enzyme, a combination of 0.5 mL of phosphate buffer (L-alanine) and 0.1 mL of serum was prepared. Subsequent to the addition of 0.5 mL of 2,4-dinitrophenylhydrazine to this solution, the resultant mixture was subjected to vortexing and was incubated for a duration of 30 minutes at a temperature of 37°C. Following a 20-minute period of standing at ambient temperature, 5 mL of sodium hydroxide (0.4 mol/L) was incorporated into the mixture. After a further interval of five minutes, the absorbance of the mixture was assessed at a wavelength of 546 nm.[20]

Liver and Testicular histology:

The testis was fixed in Bouin's fluid, while the liver samples were preserved using 10% buffered neutral formalin and prepared for paraffin embedding. Sections with a thickness of 5–6 µm were stained with hematoxylin and eosin. Examination of the slides was performed using an Olympus light microscope from Tokyo, Japan, at a magnification of ×400. For capturing micrographs of the liver tissue, an Omax 10.0MP digital camera was used alongside the microscope.[20]
Testicular malondialdehyde (MDA):
MDA concentration was determined using a modified method[20]. Two milliliters of trichloroacetic acid-thiobarbituric acid hydrochloric acid reagent with one milliliter of testicular supernatant. The mixture was then heated in a boiling water bath for 20 minutes before being cooled and centrifuged. Absorbance at 535 nm was measured from the resulting supernatant to assess colour intensity. For calculating MDA concentration, using an extinction coefficient for the MDA-thiobarbituric acid complex valued at $1.56 \times 10^5\ M^{-1}\ cm^{-1}$. Results were expressed as nM/mg protein.

Testicular catalase activity:

The Alam *et al.* method was used to measure the catalase activity.[21] This process involved mixing 0.5 mL of a 30 mM hydrogen peroxide solution with 0.5 mL of testicular supernatant. Subsequently, 7 mL of a 0.01 M potassium permanganate solution and 1 mL of a 6 M sulphuric acid (H_2SO_4) solution were added. A UV-VIS spectrophotometer (Model UV-1700, Pradesh, India) was used to measure the mixture's absorbance at 480 nm at 30- to 60-second intervals, using distilled water as a blank reference. µM/mg protein units were used to express the results.

Superoxide dismutase (SOD) activity:

A UV-VIS spectrophotometer (Model UV-1700, Pradesh, India) was employed to assess testicular SOD activity. This instrument operated by inhibiting the autoxidation of epinephrine in an alkaline environment at a wavelength of 480 nm. The extent of inhibition provided data for calculating SOD activity, which was expressed in arbitrary units; one unit equating to 1 mU/mg tissue.

Statistical Analysis:

The data were analysed using a one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test in GraphPad Prism. Results are presented with bar charts, and values are expressed as mean ± standard deviation (SD). Group differences were considered statistically significant if $P < 0.05$.

Results And Discussion

This study found that lead acetate increases alanine aminotransferase (ALT) levels, which aligns with previous research showing that lead exposure leads to elevated ALT levels.[22,23] Other studies have shown that exposure to lead compromises the integrity of hepatocyte membranes, leading to the release of liver damage biomarkers like ALT, aspartate aminotransferase (AST), and alkaline phosphatase (ALP) into the bloodstream.[24,25] Furthermore, this study demonstrates that MMS, when combined with lead acetate, reduces ALT levels in a dose-dependent manner. This finding is in line with other studies that report MMS as a suppressor of ALT expression.[26] The ability of MMS extract to decrease alanine aminotransferase is due to the presence of its bioactive components.[26] The extract from this plant contains flavonoids, phenols, alkaloids, saponins, tannins, and cardiac glycosides.[26] The lead-treated group had higher levels of aspartate aminotransferase, which is consistent with previous research that found exposure to lead increases aspartate aminotransferase levels.[22,23]

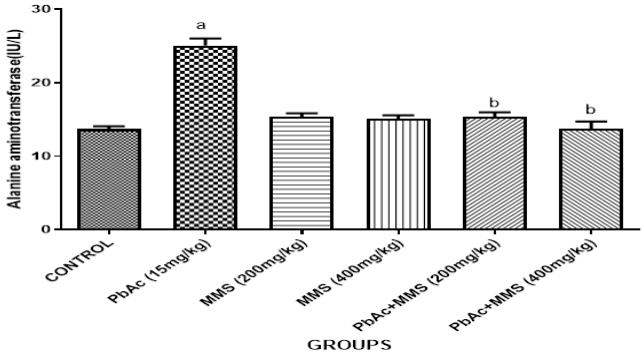

Figure 1: Effect of 56 days oral administration of *Monodora myristica* seed (MMS) extract on alanine aminotransferase (ALT) in lead acetate-induced testicular damage in male Wistar rats
Bars represent Mean ± SEM, [a, b] $p<0.05$ were considered significant relative to control and PbAc groups respectively.
PbAc = Lead acetate; MMS = *Monodora myristica* seed

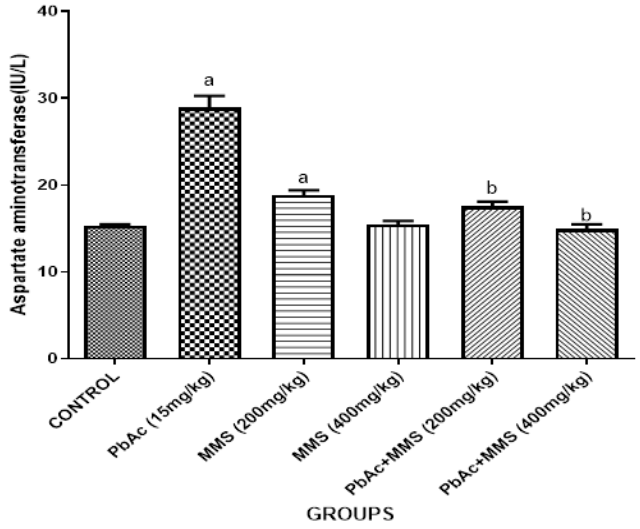

Figure 2: Effect of 56 days oral administration of *Monodora myristica* seed (MMS) extract on aspartate aminotransferase (AST) in lead acetate-induced testicular damage in male Wistar rats
Bars represent Mean ± SEM, [a, b] p<0.05 were considered significant relative to control and PbAc groups respectively.
PbAc = Lead acetate; MMS = *Monodora myristica* seed

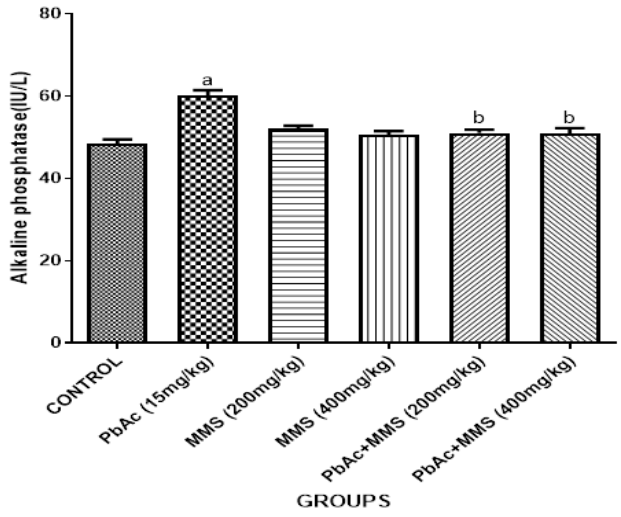

Figure 3: Effect of 56 days oral administration of *Monodora myristica* seed (MMS) extract on alkaline phosphatase (ALP) in lead acetate-induced testicular damage in male Wistar rats
Bars represent Mean ± SEM, [a, b] p<0.05 were considered significant relative to control and PbAc groups respectively.
PbAc = Lead acetate; MMS = *Monodora myristica* seed

The reduction in Aspartate Aminotransferase levels following MMS administration in this study is in line with other studies that have discovered that MMS lowers Aspartate Aminotransferase levels.[26] The bioactive components of aspartate aminotransferase are responsible for the observed drop in its level.[26] Flavonoids, phenols, alkaloids, saponins, tannins, and cardiac glycosides have all been found in this plant's extract.[10,12,26] In line with earlier studies that discovered lead exposure raises alkaline phosphatase, this study demonstrates that lead acetate raises alkaline phosphatase when compared to control.[22,23] In comparison to the group that received treatment with lead acetate alone, the study demonstrates that MMS reduces alkaline phosphatase (ALP) levels. It also shows that alkaline phosphatase levels are lower in the group that receives MMS in addition to lead acetate than in the group that receives lead acetate alone. The results are in line with related studies that discovered MMS lowers alkaline phosphatase.[26] The presence of MMS extract's bioactive components is responsible for its capacity to reduce alkaline phosphotase.[26] Flavonoids, phenols, alkaloids, saponins, tannins, and cardiac glycosides have all been found in this plant's extract.[10,12,25] MMS's anti-inflammatory and antioxidant properties may be the cause of its protective action against lead-induced hepatotoxicity. The group that received both lead acetate and MMS treatment showed dose-dependent Kupffer cell activation, bile duct activation, mild periportal infiltration of inflammatory cells, and normal hepatocytes. These results imply that MMS has a protective effect against lead acetate-induced liver damage.

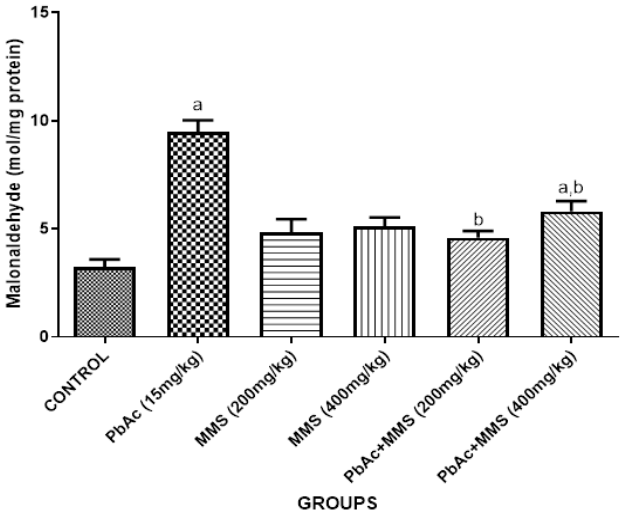

Figure 4: Effect of 56 days oral administration of *Monodora myristica* seed (MMS) extract on malonaldehyde (MDA) in lead acetate-induced testicular damage in male Wistar rats
Bars represent Mean ± SEM, [a, b] p<0.05 were considered significant relative to control and PbAc groups respectively.
PbAc = Lead acetate; MMS = *Monodora myristica* seed

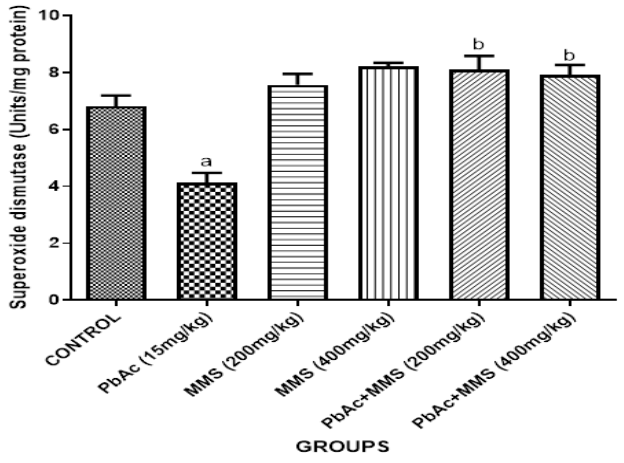

Figure 5: Effect of 56 days oral administration of *Monodora myristica* seed (MMS) extract on superoxide dismutase (SOD) in lead acetate-induced testicular damage in male Wistar rats
Bars represent Mean ± SEM, [a, b] p<0.05 were considered significant relative to control and PbAc groups respectively.
PbAc = Lead acetate; MMS = *Monodora myristica* seed

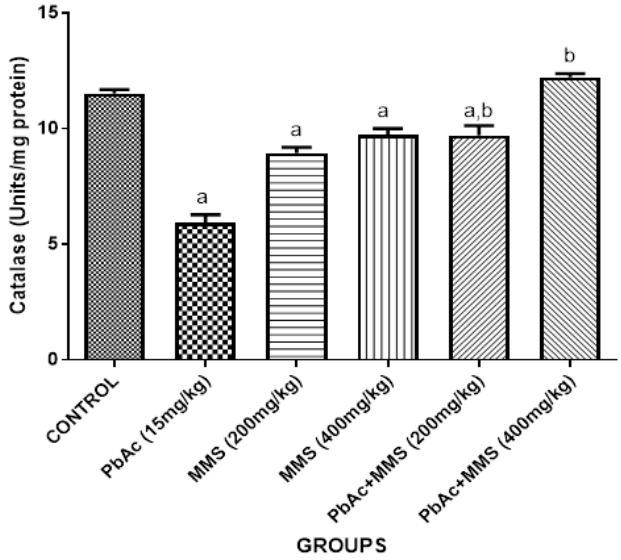

Figure 6: Effect of 56 days oral administration of *Monodora myristica* seed (MMS) extract on catalase in lead acetate-induced testicular damage in male Wistar rats
Bars represent Mean ± SEM, [a, b] $p<0.05$ were considered significant relative to control and PbAc groups respectively.
PbAc = Lead acetate; MMS = *Monodora myristica* seed

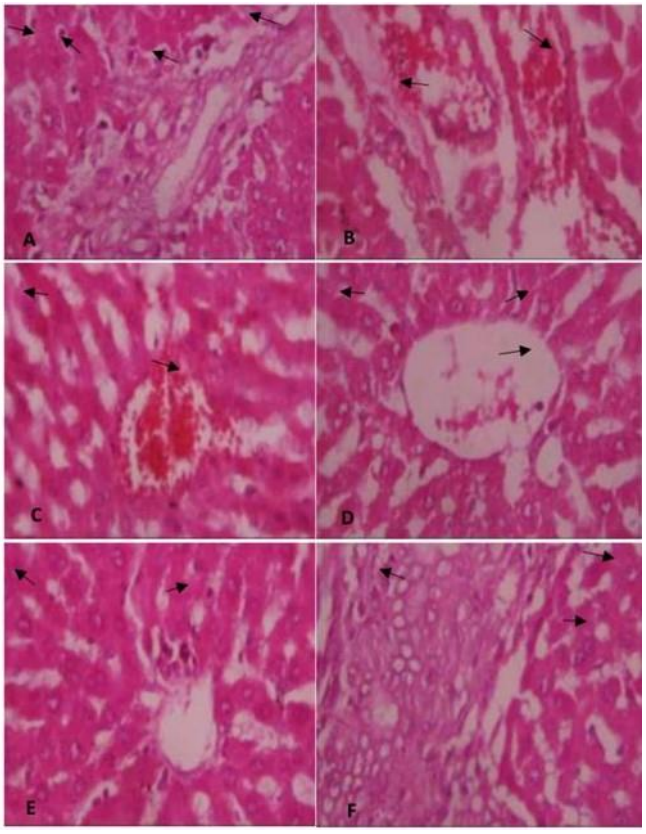

Figure 7: Effect of 56 days oral administration of methanol extract of *Monodora myristica* seed on liver histology in lead acetate-induced male testicular damage (H&E stained) × 400 (A): Control group showed normal hepatocyte, sinusoids, bile ducts, portal vein. (B): PbAc (15 mg/kg) group showed portal congestion, and severe vascular ulceration. (C): MMS (200 mg/kg) group showed normal hepatocyte and an active vascular congestion. (D): MMS (400 mg/kg) group showed normal hepatocytes, portal vein and bile duct. (E): PbAc + MMS (200 mg/kg) group showed normal hepatocytes and mild periportal infiltrates of inflammatory cells. (F) PbAc + MMS (400 mg/kg) group showed normal hepatocytes, bile ducts and Kupffer cell activation.

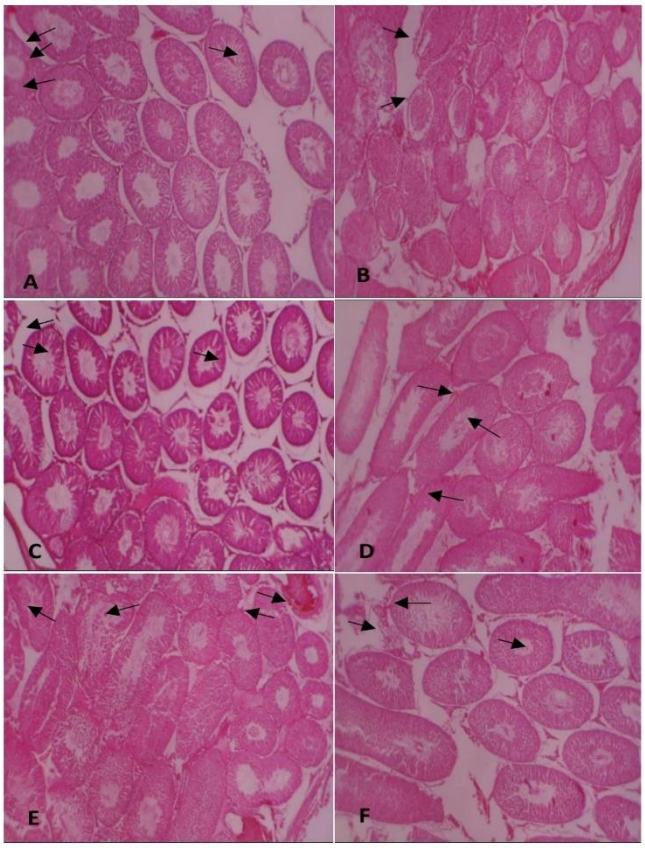

Figure 8: Effect of 56 days oral administration of methanol extract of *Monodora myristica* seed on the testis histology in lead acetate-induced male testicular damage (H&E stained) × 40 (A): Control group showed normal architecture, seminiferous tubules lined by spermatogenic series, Sertoli cells, interstitial cells of Leydig, testicular artery (B): PbAc (15 mg/kg) group showed testicular necrosis, vascular ulceration. (C): MMS (200 mg/kg) group showed tubules lined by spermatogenic series, Sertoli cells and Leydig cells (D): MMS (400 mg/kg) group showed tubules lined by spermatogenic series, Sertoli cells, Leydig cells. (E): PbAc + MMS (200 mg/kg) group showed spermatogenic series, Sertoli cells, Leydig cells, active interstitial congestion. (F) PbAc + MMS (400 mg/kg) group showed spermatogenic series, Sertoli cells, Leydig cells

This study found that lead acetate increases malondialdehyde (MDA) levels, with this effect being closely associated with lead-induced oxidative stress. The increase in MDA levels is a result of elevated reactive oxygen species generated by lead exposure. This observation is supported by the fact that a notable increase in lipid peroxide (MDA) levels occurred at the same time. Similarly, lead acetate significantly increased semen MDA.[27,28] Since SOD and catalase form a mutually beneficial defence mechanism against free radicals, the increase in MDA suggests that exposure to lead acetate caused oxidative stress by inhibiting their activity. Additionally, an increase in MDA or lipid peroxidation has been linked to the various toxic effects of lead exposure on biological systems as an early and sensitive effect.[29] When MMS was administered to rats treated with lead acetate, the observed rise in their testicular MDA levels was decreased.
Two well-known endogenous antioxidant enzymes that remove ROS indirectly are catalase and superoxide dismutase (SOD).[30] Studies have demonstrated the ability of antioxidants to guard against oxidation reactions brought on by reactive species.[31,32] Numerous studies have demonstrated that lead inhibits the production of lipid peroxidation in

the testes[20,33] and endogenous antioxidant enzymes. These two antioxidant enzymes' activities were decreased in this study by the exposure to lead acetate. In the current study, co-treating lead acetate with MMS enhanced the liver and testes' catalase and SOD activities. The ability of MMS to increase catalase and SOD activities in lead acetate treated rat could be ascribed to the extract antioxidant properties. MMS action may be attributed to their antioxidant property which may enhance endogenous antioxidants and prevent the generation of reactive oxygen species.

After 56 days oral administration, testicular histology reveals testicular necrosis and vascular ulceration in the lead acetate group. Groups administered with MMS only and also when it was co-administered with lead acetate gave a positive response (Tubules lined by spermatogenic series, Sertoli cells and Leydig cells) as shown above (Figure 8).

Conclusion

This study shows that *Monodora myristica* seed elevate antioxidant enzymes (catalase and SOD) which may reduce liver and testicular damage. Incorporating it into diet may provide significant health benefits for both the liver and testis which can be ascribe to their protective and restorative properties. However, further studies will be necessary to fully elucidate the mechanism and assess the efficacy of MMS in mitigating lead-induced hepatic and testicular damage.

Conflict Of Interest

The authors declare that there is no conflict of interest.

Author's Declaration

The authors assert that the work described in this article is original and accept complete responsibility for any claims concerning its content.

Authors' contributions

Maureen Isoken Ebomoyi and Ikponmwosa Aikpitanyi both contributed to the conceptualisation, design, and final approval of the version for publication. Ikponmwosa Aikpitanyi conducted the experiment, handled data analysis and interpretation, and prepared the manuscript draft. Maureen Isoken Ebomoyi reviewed and proofread the manuscript.

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