# Effects of Ethanolic Extract of Monodora Myristica (Fresh and Roasted) on Lead Acetate Induced Infertility on Oxidative Stress Markers (SOD, MDA, And CAT) of Male Wistar Rats

**[1]Okwuonu, Ifeoma Frances; [2]Nwaefulu, Kester Eluemunor; [3]Ezeokafor, Emmanuel Nonso; [4] Ezika, Chinedu Anthony; [5] Chidinma, Ifeyinwa Mmaju; [6]Egbunike, Chijioke Geoffery,[7] Umezulike, Anulika Jacinta; [8]Anyiam, Kennedy Ekenedirichukwu***

**[1]Department of Human Physiology, Nnamdi Azikiwe University Nnewi Campus.**

**[2]Department of Human Physiology, Nnamdi Azikiwe University Nnewi Campus.**

**[3]Department of Human Physiology, Nnamdi Azikiwe University Nnewi Campus.**

**[4]Department of Human Physiology, Dennis Osadebay University, Asaba, Delta State.**

**[5]Department of Human Physiology, Nnamdi Azikiwe University Nnewi Campus.**

**[6]Physiology Department, University on the Niger, Faculty of Basic Medicine.**

**[7]Department of Human Physiology, Nnamdi Azikiwe University Nnewi Campus.**

**[8]Faculty of Basic Medical Sciences, Department of Anatomy, Chukwuemeka Odumegwu Ojukwu University, Uli Campus.**

***Corresponding Author**

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## ABSTRACT

Lead toxicity is a major environmental and public health concern associated with oxidative stress and male reproductive dysfunction. Accumulation of lead in testicular tissues has been reported to impair spermatogenesis through excessive generation of reactive oxygen species and depletion of endogenous antioxidant defenses. *Monodora myristica*, a medicinal plant widely used as a spice in Africa, possesses several pharmacological properties including potent antioxidant, free radical scavenging, metal chelating, and lipid peroxidation inhibitory activities attributed to its rich phytochemical composition.

This study evaluated the effects of ethanolic extracts of fresh and roasted *Monodora myristica* seeds on oxidative stress markers in the testes of adult male Wistar rats exposed to lead-induced toxicity. A total of thirty adult male Wistar rats weighing between 120 g and 188 g were used for the study. The animals were divided into experimental groups comprising a normal control group, a lead acetate-treated group, groups administered lead acetate alongside fresh or roasted ethanolic seed extracts, groups pretreated with fresh or roasted extracts prior to lead exposure, and groups administered fresh or roasted extracts only. Lead acetate and the plant extracts were administered orally according to the experimental design.

Biochemical analysis revealed significant alterations in oxidative stress markers following treatment. Administration of fresh and roasted *Monodora myristica* extracts produced significant effects on catalase (CAT) and malondialdehyde (MDA) levels, indicating modulation of antioxidant defense mechanisms and lipid peroxidation. However, no significant effect was observed on superoxide dismutase (SOD) activity. The observed antioxidant effects may be associated with the bioactive phytochemical constituents present in the extracts.

The findings of this study suggest that ethanolic extracts of fresh and roasted *Monodora myristica* seeds possess antioxidant properties capable of ameliorating oxidative stress associated with lead-induced testicular toxicity in male Wistar rats. Therefore, *Monodora myristica* may have potential therapeutic value in the management of oxidative stress-related male reproductive disorders.

**Keywords: Oxidative stress, Lead toxicity, *Monodora myristica*, antioxidant activity, wistar rats**

# INTRODUCTION

Infertility has become a major public health concern globally, affecting millions of couples of reproductive age. Male infertility contributes significantly to the overall burden of infertility, accounting for approximately 40–50% of infertility cases worldwide. Agarwal et al. (2021) reported that oxidative stress, environmental toxicants, and heavy metals contribute significantly to male infertility through mechanisms involving reactive oxygen species generation, lipid peroxidation, DNA damage, and impaired spermatogenesis. Several environmental, occupational, nutritional, and lifestyle factors have been implicated in the increasing prevalence of male reproductive dysfunction. Among these factors, oxidative stress and exposure to heavy metals such as lead have gained significant scientific attention because of their profound effects on reproductive physiology and testicular integrity. Oxidative stress results from an imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms, leading to cellular damage, lipid peroxidation, DNA fragmentation, mitochondrial dysfunction, and impaired spermatogenesis.

Lead is a highly toxic environmental pollutant widely distributed through industrial emissions, contaminated water, food substances, batteries, paints, mining activities, and automobile exhaust. Chronic exposure to lead has been associated with several pathological conditions involving the nervous system, cardiovascular system, hepatic tissues, kidneys, endocrine organs, and reproductive organs. In male reproductive physiology, lead toxicity has been reported to induce testicular degeneration, hormonal imbalance, impaired spermatogenesis, reduced sperm motility, decreased sperm count, and oxidative damage within the testes. The toxic effects of lead are largely mediated through the generation of reactive oxygen species and depletion of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase, with a corresponding increase in malondialdehyde (MDA), a major biomarker of lipid peroxidation.

According to Flora, Gupta, and Tiwari (2012), lead exposure promotes oxidative stress by generating free radicals and interfering with antioxidant defense systems, thereby causing cellular injury in multiple organs. Similarly, Patrick (2006) reported that lead toxicity affects male fertility through endocrine disruption, oxidative stress induction, and direct testicular damage. Akinloye et al. (2006) further demonstrated that heavy metals including lead significantly impair sperm quality and reproductive hormone balance in exposed individuals.

The testes are particularly vulnerable to oxidative damage because spermatozoa possess high concentrations of polyunsaturated fatty acids in their plasma membranes and relatively low antioxidant defense capacity. Excessive reactive oxygen species generation causes lipid peroxidation of sperm membranes, resulting in reduced sperm viability, impaired motility, and defective fertilization capacity. Sharma and Agarwal (1996) explained that oxidative stress is a major contributor to male infertility due to its destructive effects on sperm DNA, proteins, and membrane lipids. Consequently, antioxidant therapy has emerged as an important therapeutic strategy in the management of oxidative stress-induced infertility.

Natural products and medicinal plants have continued to attract scientific attention because of their phytochemical constituents and pharmacological properties. Numerous medicinal plants possess antioxidant, anti-inflammatory, anti-apoptotic, and cytoprotective activities capable of ameliorating heavy metal-induced toxicity. Among such medicinal plants is Monodora myristica, commonly known as African nutmeg. Monodora myristica belongs to the family Annonaceae and is widely distributed in tropical Africa, especially in Nigeria, Ghana, Cameroon, and other West African countries. The seeds are commonly used as spices and flavoring agents in traditional African cuisine and ethnomedicine.

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Modern scientific investigations have shown that Monodora myristica possesses significant medicinal properties attributable to its rich phytochemical composition, including flavonoids, alkaloids, phenolic compounds, tannins, terpenoids, saponins, and essential oils. These bioactive compounds are known to exhibit antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and anti-diabetic activities. Ekeanyanwu and Etienajirhevwe (2012) reported that extracts of Monodora myristica exhibit potent free radical scavenging activity and antioxidant potential. Similarly, Oboh et al. (2012) observed that Monodora myristica inhibited lipid peroxidation and demonstrated strong reducing properties in experimental studies.

The antioxidant properties of Monodora myristica are particularly important in combating oxidative stress-induced tissue injury. Antioxidants function by neutralizing reactive oxygen species, inhibiting lipid peroxidation, enhancing endogenous antioxidant enzyme activities, and protecting cellular structures from oxidative damage. Ethanolic extracts of Monodora myristica have been shown to possess substantial antioxidant activity due to the extraction efficiency of ethanol in isolating phenolic and flavonoid compounds. Roasting, however, may alter the phytochemical composition and antioxidant potential of the seeds through thermal modification of bioactive compounds. Comparative evaluation of fresh and roasted Monodora myristica extracts is therefore essential in understanding the influence of processing on its biological activity.

Several experimental studies have investigated the protective effects of plant extracts against heavy metal-induced toxicity. Recent studies by Anyiam and colleagues have provided valuable scientific evidence regarding the protective effects of medicinal plants against oxidative stress and tissue damage induced by toxic substances. Anyiam et al. (2025) demonstrated that ethanolic extract of Aloe vera exhibited neuroprotective effects against mercury-induced damage in the hippocampus and amygdala of Wistar rats through histological preservation and antioxidant mechanisms. Their findings revealed that phytochemical-rich extracts possess substantial protective activities against oxidative stress-mediated neuronal degeneration.

Similarly, Ezeani et al. (2025) reported that ethanolic leaf extract of Aloe barbadensis ameliorated mercury-induced Alzheimer-like changes in the basal ganglia of albino Wistar rats. The study further established the role of medicinal plants in reducing oxidative injury and neurotoxicity induced by heavy metals. In another related study, Molokwu et al. (2025) evaluated the biochemical and histological protective effects of ethanolic extract of Solanum torvum on mercury-induced kidney and testes toxicity in adult Wistar rats. Their findings demonstrated significant improvements in tissue architecture and biochemical parameters following administration of the plant extract.

Furthermore, Maduanusi et al. (2025) investigated the protective effects of ethanolic extract of Solanum torvum on mercury-induced liver and stomach toxicity. The study showed that phytochemical-rich extracts can attenuate oxidative stress and tissue degeneration caused by toxic heavy metals. Ogbuokiri et al. (2025) also demonstrated the protective effects of Mentha piperita leaf extract against mercury chloride-induced cardiorenal toxicity in adult male Wistar rats, highlighting the antioxidant and cytoprotective potential of medicinal plants.

Additional studies by Ogbuokiri et al. (2025) on the spleen of adult male Wistar rats exposed to mercury chloride revealed that ethanolic leaf extract of Mentha piperita preserved splenic histoarchitecture and reduced toxic injury associated with oxidative stress. Similarly, Ezeokafor et al. (2025) reported that ethanolic extract of Azanza garckeana improved semen quality and hormonal profiles in adult Wistar rats, suggesting possible fertility-enhancing and antioxidant effects of medicinal plants.

Research involving Jatropha tanjorensis has also contributed significantly to understanding the biological effects of phytochemical-rich medicinal plants. Dim et al. (2025) investigated the effects of aqueous extract of dose-dependent Jatropha tanjorensis leaf on hematological parameters of male Wistar rats and observed significant biological activities associated with the extract. Another study by Dim et al. (2025) assessing the dose-dependent impact of Jatropha tanjorensis on reproductive health of adult male Wistar rats further established the relevance of plant-derived antioxidants in reproductive physiology.

Moreover, Dim et al. (2025) evaluated the dose-dependent effects of aqueous extract of Jatropha tanjorensis leaf on biochemical markers in male Wistar rats and reported alterations suggestive of antioxidant and metabolic activities. Collectively, these studies provide strong scientific evidence supporting the therapeutic potential of medicinal plants in mitigating oxidative stress and heavy metal-induced toxicity.

Oxidative stress biomarkers including superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) are important biochemical indicators for evaluating tissue oxidative status. Superoxide dismutase is an enzymatic antioxidant responsible for catalyzing the dismutation of superoxide radicals into hydrogen peroxide and oxygen. Catalase further converts hydrogen peroxide into water and oxygen, thereby preventing oxidative cellular injury. Malondialdehyde, on the other hand, is a major end product of lipid peroxidation and serves as an important indicator of oxidative membrane damage. Increased MDA levels together with reduced SOD and CAT activities are characteristic findings in oxidative stress-mediated disorders.

Lead-induced oxidative stress in testicular tissues has been widely documented. Oyewopo et al. (2014) reported that lead acetate administration caused significant oxidative stress and reproductive toxicity in experimental animals. Similarly, Wadi and Ahmad (1999) demonstrated that lead exposure reduced antioxidant enzyme activities while increasing lipid peroxidation in reproductive tissues. The administration of antioxidants and medicinal plant extracts has therefore been explored as a therapeutic strategy for mitigating lead-induced infertility and oxidative damage.

Despite numerous studies investigating medicinal plants and heavy metal toxicity, there remains limited information regarding the comparative effects of fresh and roasted ethanolic extracts of Monodora myristica on oxidative stress markers in lead-induced testicular toxicity. Roasting is a common traditional processing method that may influence the phytochemical composition and antioxidant potential of plant materials. Understanding whether roasting enhances or diminishes the biological activity of Monodora myristica is important for its nutritional, medicinal, and therapeutic applications.

Therefore, this study was designed to evaluate the effects of ethanolic extract of Monodora myristica (fresh and roasted) on oxidative stress markers including superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT) in male Wistar rats exposed to lead toxicity. The study is expected to contribute to the growing body of scientific literature on medicinal plants, oxidative stress, reproductive toxicology, and the therapeutic management of heavy metal-induced infertility.

## MATERIALS AND METHODS/ EXPERIMENTAL DETAILS / METHODOLOGY

### 2.1 Location Of Study

This study was carried out in the Animal House of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, NnamdiAzikiwe University, Nnewi Campus, Anambra State.

### 2.2 Materials

- 30 Adult Male Wistar Rats
- Ethanolic seed extract of *Monodora Myristica*
- Electronic weighing balance ( NAPCO Precision Instruments JA-410)
- Oral canula
- Distilled water
- Standard plastic cages
- Metal cages
- Cotton wool (KENS LINT, Benin City, Nigeria)
- Lead acetate
- Latex Medical Hand gloves (Supermax Gloves, Selangor, Malaysia)
- Animal Weighing balance (CAMRY LB11)
- Diethyl ether

- Vital top feed (Jos, Nigeria)
- EDTA container and Plain container
- Dissecting kits
- Micro-hematocrit centrifuge SH120
- Thermostat Oven (DHG-9023A, PEC MEDICAL USA)
- Capillary Tube
- Ethanol
- Measuring Cylinder (MINGHE)

**2.3 Plant Collection And Identification**

Samples of *Monodora Myristica* Seed were purchased from Nkwo Nnewi Market Nnewi, Anambra state. The botanical identification and authentication was confirmed in the herbarium of Department of Botany, Nnamdi Azikiwe University Awka, Anambra State.

**2.4 Preparation Of Seed Extract**

*Some seeds were roasted and the Monodora Myristica* seeds were removed manually by breaking them using stones. The pulp was ground into powdered form using sterile electric blender. 500 grams (500g) of the powered sample was macerated in 5 liters of distilled water and allowed for 72 hours with intermittent manual shaking. The mixture was sieved using standard sieve. The filtrate was allowed to settle down for 24 hours, the water was decanted. The extract was dried using extract run dry bag (99 % Sodium oxide) made in England and the extract was air dried to powder form and stored in the refrigerator to be used for the studies.

**Experimental Animals**

The experimental animals were obtained from Oko in Anambra state. The rats were housed in the animal house, College of Health Sciences, Nnewi Campus, Nnamdi Azikiwe University, Anambra State. Animals were kept in standard plastic and metal cages in a temperature controlled room at 25±2ºC, under a 12/12h light/dark cycle, and was maintained on normal laboratory chow (Vital top feed, Nnewi) and water *ad libitum.* Animals were acclimatized for a time frame of four weeks before administration of ethanolic seed extract of *Monodora Myristica*. Rats handling and treatments conformed to the guidelines of the faculty of Basic medical Science, College of Health Sciences, Nnamdi Azikiwe University, Nigeria for laboratory animal care and use.

**2.5 Experimental Design**

A total of thirty (30) male albino wistar rats weighing 120g to 188g (0.12kg to 0.188kg) were randomly divided into five (A, B, C, D & E) groups of six (6) rats per group. Group C, D & E were further divided into two groups of 3 rats each for the administration of the fresh and roasted Monodora myristica extract. Animals were acclimatized for a period of four weeks before the commencement of the administration of ethanolic seed extract of Monodora myristica and lead acetate respectively. The seed extract and lead acetate was administered via oral garvage.

According to (Akinwunmi et al, 2014) It was reported that the LD50 of ethanolic extract of *monodora myristica* seed was Observed to be non- toxic with LD50 greater than 5000mg/ kg body weight when administered orally. Based on the reported oral LD50 of Lead acetate for Wistar rats by (Sujatha et al., 2011)we administered 1/4 of LD50 (600 mg of lead acetate/kg body Weight) in this study.

Group A served as control that was fed normal laboratory chow and water.

Group B were also housed under the same condition as the control group but were administered 150mg/kg of lead acetate for 7 consecutive days.

Group C were divided into two groups of 3 rats each:

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The group C1 was administered 150mg/kg of lead acetate for 7 consecutive days and then treated with 1000mg of fresh M.M/kg body weight via oral garvage for another 7 days.

C2 was administered 150mg/kg of lead acetate for 7 consecutive days and then treated with 1000mg of roasted M.M/kg body weight via oral garvage for another 7 days

Group D were divided into two groups of 3 rats each

First D1 treated with 1000mg of fresh M.M/kg body weight via oral garvage for 7 days and then administered 150mg/kg of lead acetate for another 7 days.

D2 treated with 1000mg of roasted M.M/kg body weight via oral garvage for 7 days and then administered 150mg/kg of lead acetate for another 7 days.

Group E were divided into two groups of 3 rats each:

E1 were housed under the same condition as the control group but were treated with only 1000mg of fresh M.M/kg body weight.

E2 were housed under the same condition as the control group but were treated with only 1000mg of roasted M.M/kg body weight.

The administration of the extract lasted for a period of 14 days. The administration of the extract was within the period of 8am to 10am everyday for 14days.

Three rats from each group were anesthetized and sacrificed following the final lead acetate administration and *Monodora Myristica* administration.

**2.6 Sample Collection**

At the end of the experimental period of 28days, the animals were deprived of food overnight by 24 hours and the animals were anesthetized with chloroform and were sacrificed by cervical dislocation. The semen samples were collected from each group of three animals and the semen was used to test for Superoxide Dismutase (SOD), Catalase (CAT) and Malondiadehyde (MDA) levels. Afterwards, each semen samples were placed in an EDTA tube, labeled according to the group they were collected from. The samples were then centrifuged for 5minutes at 3000 rpm using a centrifuge. The analysis of the parameters was conducted at the Chemical Pathology Laboratory unit of NAUTH and Springboard Research Laboratory, Awka.

**2.6.1 Determination Of Plasma Malondialdehyde (Mda)**

Malondialdehyde(MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, it forms a pink coloured product, which has absorption maximum at 532 nm. The intensity of colour generated is directly proportional to the concentration of MDA in the sample (Gutteridge and Wilkins, 1982).

MDA level was determined by the colourimetric method of Gutteridge and Wilkins, (1982). The reaction mixture contains Glacial acetic acid, 0.05M NaOH (0.2g of NaOH (w/v) (dissolve 1g of TBA in 100ml of 0.05M NaOH). The solution was heated in a hot water bath for 15minutes at 100°C. It was allowed to cool and the turbidity removed by centrifugation at 3000rpm for 10minutes. The clear supernatant was read at 532nm. The same volume of TBA and glacial acetic acid was added to the blank instead of plasma. The level of MDA in the serum is expressed as nmol/ml using the molar extinction coefficient for MDA (1.56×105 M-1cm- 1).

Calculation:

$$\text{MDA (nmol/ml)} = (\text{OD} \times 1000000)/E_{532}$$

Where $E_{532}$= Molar extinction coefficient for MDA ($1.56 \times 10^5$ $M^{-1}$ $cm^{-1}$).

### 2.6.2 Determination Of Superoxide Dismutase (Sod)

Superoxide Dismutase was determined using Misra and Fredovichmethod (1972). The ability of the Superoxide dismutase to inhibit the auto oxidant of the adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. The mixture contains 0.3M Epinephrine: 0.01g f epinephrine was dissolved in 17ml of distilled water, 0.05M Carbonate buffer (pH 10.2): 0.53g of Na2CO3 and 0.42g of NaHCO3 was dissolved in 100ml of distilled water. The % inhibition of auto oxidation of epinephrine by SOD was calculated and the plasma SOD activity was expressed as U/ml. One unit of SOD activity was equivalent to the amount of SOD that can cause 50% inhibition of epinephrine.

Calculation:

% inhibition = $(\Delta OD_{blank} - \Delta OD_{test} / \Delta OD_{blank} \times 100$

Enzyme Unit (U/ml) = (% inhibition/50) × dilution factor.

### 2.6.3 Determination Of Catalase (Cat)

Catalase activity was determined by the method of Luck and Bergmeyer, 1965. Both the blank and the system cuvettes contained 20 to 40 L homogenate and 3 mL 1/15 mol/L phosphate buffer. The system cuvette contained additional 1 mmol/L H2O2. The time required for the optical density at 240 nM of a mixture of homogenate, H2O2, and buffer to change from 0.45 to 0.4 seconds was used as a measure of catalase activity; 1 unit of catalase activity is equal to 17 seconds. Thus, the activity could be calculated from the rate of the reaction. Protein concentration in the aforementioned experiments was determined by the method of Lowry *et al.,* 1951

### 2.7 Declaration of Interest

None

### 2.8 Funding Source

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

### 2.9 Statistical Analysis

All the data that was collected from semen test were analyzed statistically using SPSS software (version 26) and the results were expressed as mean ± SD. Comparison of data for Superoxide dismutase (SOD), Catalase (CAT) and Malondiadehyde (MDA) was analyzed using one-way analysis of variance (ANOVA) followed by Post HOC Fisher's LSD Comparison. P values ≤0.05 were considered statistically significant, while P values ≥0.05 were considered statistically not significant.

## RESULTS

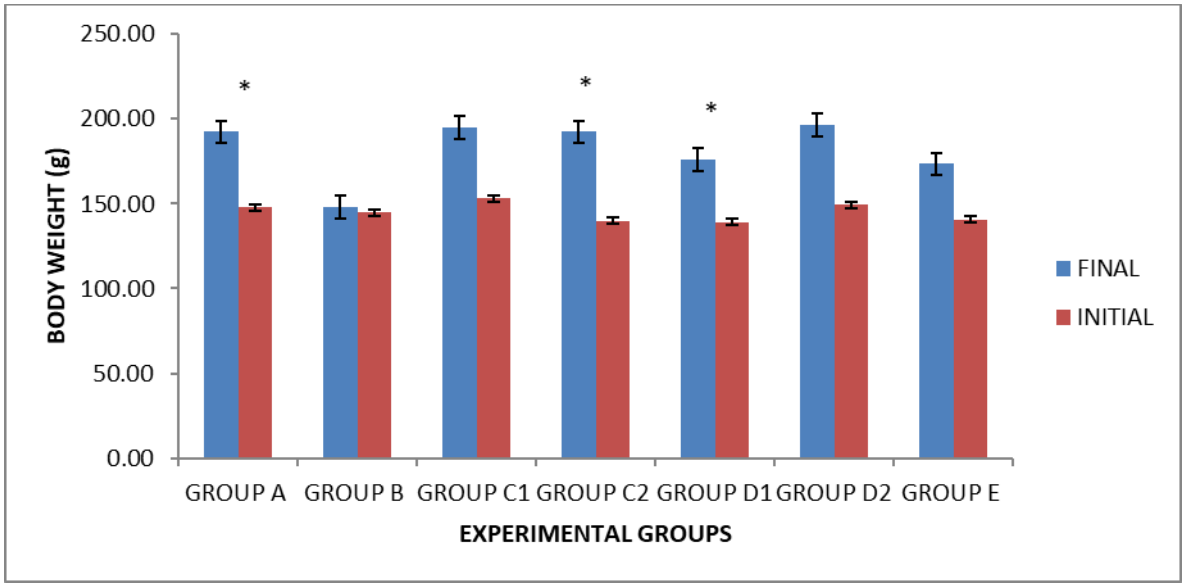
### Observation

Physical and behavioral changes observed during the course of the experiment are as follows:

- Animals in positive control group and test groups showed normal physical appearance
- Animals in negative control group showed different abnormal appearance such as slow movement than normal as time went by.

- During the period of the experiment, there were changes in their movement, they became dull and slow to move
- There was no sign of aggression in the control groups and experimental groups
- Water intake was at minimal
- Food intake was normal

Figure 1: **Comparison of the Initial and final body weights of the experimental animals.**



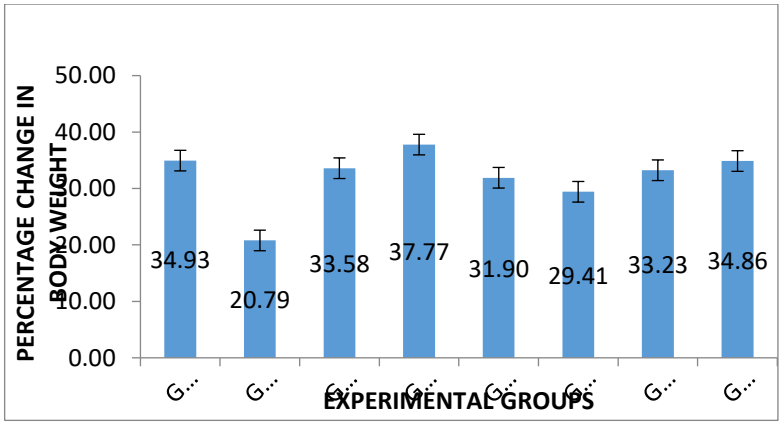

* Significant at P ≤ 0.05

Data were analyzed using Student dependent T-test and values were considered significant at P<0.05.

*P<0.05 means significant. P>0.05 Not Significant.

There was a significant increase in the body weight of groups A, C2 and D1 when the final body weight was compared to the initial body weight.

Figure 2: **Comparison of the percentage change in body weight**




* Significant at P ≤ 0.05

Data were analyzed using ANOVA and values were considered significant at P<0.05.

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There was no significant change in the percentage change in body weight of the test groups when compared to control group.

Table 1: Comparison of the effect of the treatment on the oxidative stress bio markers of the animals between the control group and the other test groups.

| EXPERIMENTAL GROUPS | MDA (nmol/ml) Mean±SEM | F-VALUE | P-VALUE | CAT (ku/l) Mean±SEM | F-VALUE | P-VALUE | SOD(U/L) Mean±SEM | F-VALUE | P-VALUE |
|---|---|---|---|---|---|---|---|---|---|
| GROUP A | 2.61±0.20 | 4.71 | | 19.35±1.24 | 16.68 | | 23.21±4.89 | 1.19 | |
| GROUP B | 3.15±0.32 | | 0.32 | 41.52±3.08 | | 0.00* | 19.05±2.83 | | 0.38 |
| GROUP C1 | 3.78±0.08 | | 0.05* | 26.82±5.87 | | 0.19 | 24.45±1.00 | | 0.79 |
| GROUP C2 | 2.22±0.08 | | 0.47 | 53.93±4.25 | | 0.00* | 24.07±0.95 | | 0.85 |
| GROUP D1 | 2.13±0.05 | | 0.37 | 59.74±0.56 | | 0.00* | 20.60±5.61 | | 0.58 |
| GROUP D2 | 3.37±0.25 | | 0.18 | 57.32±3.28 | | 0.00* | 25.10±3.11 | | 0.68 |
| GROUP E1 | 2.80±0.22 | | 0.72 | 50.88±2.87 | | 0.00* | 30.50±1.50 | | 0.14 |
| GROUP E2 | 4.44±0.66 | | 0.00* | 31.52±5.32 | | 0.05* | 25.56±2.21 | | 0.61 |

*Significant at P≤0.05

Data were analyzed using One-way ANOVA followed by Post HOC Fisher's LSD multiple comparison, and data were considered significant at P≤0.05 and P>0.05 means not significant.

There was significant increase in the MDA levels groups C1 and E2 when compared to control group.

There was significant increase in the CAT levels of all the test groups except C1 when compared to the control group

There was no significant difference in the SOD levels of all the test groups when compared to the control group.

Table 2: Comparison of the effect of the treatment on the oxidative stress bio markers of the animals between the negative control group and the other test groups.

| | Mean MDA (nmol/ml) | F-VALUE | P-VALUE | CAT (ku/l) | F-VALUE | P-VALUE | SOD(U/L) | F-VALUE | P-VALUE |
|---|---|---|---|---|---|---|---|---|---|
| GROUP B | 3.15±0.32 | 4.71 | | 41.52±3.08 | 16.68 | | 19.05±2.83 | 1.19 | |
| GROUP C1 | 3.78±0.08 | | 0.11 | 26.82±5.87 | | 0.02* | 24.45±1.00 | | 0.26 |
| GROUP C2 | 2.22±0.08 | | 0.08 | 53.93±4.25 | | 0.05* | 24.07±0.95 | | 0.29 |
| GROUP D1 | 2.13±0.05 | | 0.68 | 59.74±0.56 | | 0.01* | 20.60±5.61 | | 0.73 |
| GROUP D2 | 3.37±0.25 | | 0.52 | 57.32±3.28 | | 0.02* | 25.10±3.11 | | 0.21 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| GROUP E1 | 2.80±0.22 | | 0.04* | 50.88±2.87 | | 0.11 | 30.50±1.50 | | 0.03* |
| GROUP E2 | 4.44±0.66 | | 0.51 | 31.52±5.32 | | 0.09 | 25.56±2.21 | | 0.18 |

*Significant at P≤0.05

Data were analyzed using One-way ANOVA followed by Post HOC Fisher's LSD multiple comparison, and data were considered significant at P≤0.05 and P>0.05 means not significant.

There was significant decrease in the MDA levels group E2 when compared to negative control group.

There was significant decrease in the CAT levels of groups C1 when compared to the negative control group

There was significant increase in the CAT levels of groups C2, D1 and D2 when compared to the negative control group.

There was significant increase in the SOD levels of group E1 when compared to the negative control group

## DISCUSSION

Oxidative stress remains one of the major mechanisms implicated in lead-induced reproductive toxicity. Reactive oxygen species (ROS) generated during oxidative stress attack cellular macromolecules including lipids, proteins, and nucleic acids, resulting in lipid peroxidation, mitochondrial dysfunction, DNA damage, impaired spermatogenesis, and cellular degeneration. Antioxidant defense enzymes such as superoxide dismutase (SOD) and catalase (CAT) play important roles in neutralizing free radicals and protecting tissues from oxidative injury, while malondialdehyde (MDA) serves as a major biomarker of lipid peroxidation and oxidative membrane damage (Aitken & Roman, 2008; Flora, Gupta, & Tiwari, 2012). Increased oxidative stress has been strongly associated with male infertility and testicular dysfunction because spermatozoa possess high concentrations of polyunsaturated fatty acids and limited endogenous antioxidant defense capacity (Agarwal et al., 2014).

The present study evaluated the effects of ethanolic extract of fresh and roasted Monodora myristica on oxidative stress markers in male Wistar rats exposed to lead toxicity. The findings obtained from this study suggest that Monodora myristica possesses antioxidant activities capable of modulating oxidative stress parameters induced by lead acetate administration. The antioxidant effects observed may be attributed to the phytochemical constituents of the plant including flavonoids, phenolic compounds, tannins, alkaloids, and essential oils, which are known to exhibit free radical scavenging and cytoprotective activities.

The result obtained from Figure 1 revealed a significant increase in body weight in groups A, C2, and D1 when the final body weights were compared with the initial body weights. However, there was a significant reduction in body weight in the lead acetate-treated group when compared with the control group. This observation is consistent with previous reports indicating that lead exposure alters metabolic activities, suppresses appetite, interferes with nutrient utilization, and induces systemic toxicity resulting in weight loss (Flora et al., 2012).

**FINDINGS** from Table 1 demonstrated a significant increase in malondialdehyde (MDA) levels in group C1 and group E2 when compared with the control group. Elevated MDA levels are indicative of increased lipid peroxidation and oxidative membrane damage resulting from excessive reactive oxygen species generation. Lead toxicity has been widely documented to increase lipid peroxidation through depletion of endogenous antioxidants and enhanced free radical production (Patrick, 2006). However, despite the observed increase in MDA in some groups, reductions observed in other treated groups suggest partial antioxidant protection by Monodora myristica extract. The antioxidant effects of medicinal plants have been attributed to their ability to inhibit lipid peroxidation and neutralize free radicals.

The present findings are supported by previous studies demonstrating the protective effects of phytochemical-rich plant extracts against oxidative stress and heavy metal toxicity. Anyiam, Nwakanma, Elemuo, Ezeani, and Osiagor (2025) reported that ethanolic extract of Aloe vera exerted neuroprotective effects against mercury-induced oxidative damage in the hippocampus and amygdala of Wistar rats through antioxidant mechanisms. Similarly, Ezeani, Nwakanma, Elemuo, and Anyiam (2025) demonstrated that Aloe barbadensis mitigated mercury-induced Alzheimer-like changes in the basal ganglia by reducing oxidative injury and preserving neuronal architecture. These studies support the role of medicinal plants as potential therapeutic agents against oxidative stress-mediated tissue damage.

Furthermore, Molokwu, Nweke, Ezeokafor, and Anyiam (2025) reported that ethanolic extract of Solanum torvum improved biochemical and histological parameters in mercury-induced kidney and testes toxicity through antioxidant and cytoprotective mechanisms. Similar protective findings were also reported by Maduanusi, Nweke, Ezeokafor, and Anyiam (2025), who observed attenuation of mercury-induced liver and stomach toxicity following administration of Solanum torvum extract. Ogbuokiri et al. (2025) further demonstrated that Mentha piperita leaf extract significantly ameliorated mercury-induced cardiorenal toxicity through reduction of oxidative stress and preservation of tissue integrity. These studies collectively reinforce the current findings suggesting that Monodora myristica possesses protective antioxidant properties.

The significant increase in catalase (CAT) activity observed in most treatment groups compared with the control group may indicate stimulation of endogenous antioxidant defense mechanisms by Monodora myristica extract. Catalase plays an important role in detoxifying hydrogen peroxide generated during oxidative stress, thereby protecting cells from oxidative injury. The increase in catalase activity observed in this study may therefore represent an adaptive protective response against lead-induced oxidative stress. The phytochemical constituents of Monodora myristica, particularly flavonoids and phenolic compounds, may have contributed to enhanced antioxidant enzyme activity.

This observation agrees with studies demonstrating antioxidant-enhancing properties of medicinal plants. Dim et al. (2025) reported that aqueous extract of Jatropha tanjorensis produced significant biochemical effects associated with antioxidant activity in male Wistar rats. Another study by Dim et al. (2025) assessing the reproductive effects of Jatropha tanjorensis also demonstrated beneficial biological activities attributable to phytochemical constituents. Ezeokafor et al. (2025) similarly reported improvements in semen quality and hormonal profiles following administration of Azanza garckeana extract, suggesting antioxidant-mediated reproductive protection.

The present study, however, observed no significant effect on superoxide dismutase (SOD) levels in most treatment groups when compared with the control group. This finding may suggest that the extract exerted more influence on catalase activity and lipid peroxidation pathways than on superoxide dismutase modulation. Variations in extraction methods, dosage, duration of administration, roasting processes, and experimental conditions may account for differences between the present findings and previous studies. Moukette et al. (2015) demonstrated that *Monodora myristica* possesses significant free radical scavenging and antioxidant activities, including the enhancement of endogenous antioxidant defense mechanisms and inhibition of lipid peroxidation. Variations in extraction methods, dosage, duration of administration, and tissue specificity may account for differences between their findings and the observations of the present study.

**Results** from Table 2 further showed significant increases in catalase levels in groups C2, D1, and D2, as well as increased superoxide dismutase activity in group E1 when compared with the negative control group. These findings suggest that both fresh and roasted Monodora myristica extracts possess antioxidant potentials capable of modulating oxidative stress induced by lead acetate toxicity. The antioxidant activity may be linked to the synergistic actions of phytochemicals present within the extract. Plant-derived antioxidants are known to donate electrons to unstable free radicals, thereby interrupting oxidative chain reactions and reducing cellular damage (Agarwal et al., 2014).

The significant reduction in malondialdehyde levels observed in group E2 when compared with the negative control group further supports the anti-lipid peroxidative effect of roasted Monodora myristica extract. Reduced

MDA levels suggest decreased membrane lipid damage and improved antioxidant defense. However, the reduction in catalase activity observed in group C1 compared with the negative control group may indicate variations in antioxidant responses depending on the treatment regimen, roasting process, or bioavailability of phytochemical constituents.

Overall, findings from this study suggest that ethanolic extracts of fresh and roasted Monodora myristica possess appreciable antioxidant properties capable of modulating oxidative stress markers in lead-induced toxicity. The protective effects observed may be associated with the phytochemical constituents of the plant, which enhance antioxidant defense mechanisms and reduce oxidative membrane damage. These findings further support the growing body of evidence demonstrating the therapeutic potential of medicinal plants in the management of oxidative stress-associated reproductive toxicity and heavy metal-induced infertility.

## CONCLUSION

In conclusion, the ethanolic seed extract of *Monodora myristica* exhibited ameliorative and protective effect on acute lead acetate toxicity on oxidative stress markers of the adult male wistar rat and this is due to its phytochemical constituents such as flavanoid, tannin, saponin, alkaloids, phenolic etc. *Monodora Myristica* improved these antioxidants (SOD, CAT and MDA). Lead has an adverse effect on the Oxidative stress markers.

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## COMPETING INTERESTS

The authors declare that they have no known financial interests that influenced the work reported in this paper. The research was conducted independently, without any financial support, commercial sponsorship, or affiliations that might present a conflict of interest.

## AUTHORS' CONTRIBUTIONS

**"OKWUONU, IFEOMA FRANCES AND NWAEFULU KESTER ELUEMUNOR"** designed the study and managed the literature searches, '**EZEOKAFOR, EMMANUEL NONSO**' wrote the protocol, '**EZIKA, CHINEDU ANTHONY AND CHIDINMA, IFEYINWA MMAJU; EGBUNIKE, CHIJIOKE GEOFFERY**' performed the statistical analysis, and '**ANYIAM, KENNEDY EKENEDIRICHUKWU**' and '**UMEZULIKE, ANULIKA JACINTA**' wrote the first draft of the manuscript…All authors read and approved the final manuscript.

## ETHICAL APPROVAL

This was obtained from the ethical committee, faculty of basic medical sciences Chukwuemeka Odumegwu Ojukwu University, Uli campus in compliance with the relevant laws and institution's guidelines.

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee**.**

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