



## Effect of n-hexane extract of *oryctes rhinoceros* on sperm parameters and oxidative stress markers in male wistar rats following administration of lead

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### Abstract

**Context:** Environmental contamination with heavy metals such as lead remains a major public health concern, especially in developing countries where environmental regulations are weak. Lead exposure has been widely associated with oxidative stress, reproductive dysfunction, and systemic toxicity.

**Objectives:** This study aims at the protective effect of n-hexane extract of *Oryctes rhinoceros* (white) on sperm parameters and oxidative stress markers in male Wistar rats exposed to lead-induced toxicity.

**Materials and Methods:** Twenty-five (25) adult male Wistar rats were randomly divided into five groups of five animals each. Group 1 served as the lead control; Group 2 received olive oil only (vehicle control); Group 3 received a low dose of the extract (0.5 ml/kg) plus lead; Group 4 received a high dose of the extract (0.7 ml/kg) plus lead; and Group 5 received a high dose of the extract co-administered with olive oil and lead. Lead toxicity was induced using 70 mg/kg of lead acetate. Treatments were administered orally for 28 days. At the end of the experiment, animals were sacrificed, and blood samples were collected for biochemical analysis, while testes were harvested for histological studies.

**Results:** The results revealed that lead exposure significantly reduced sperm motility, count, morphology, and volume while increasing oxidative stress and significantly decreases serum electrolyte balance. Treatment with *Oryctes rhinoceros* extract markedly improved sperm quality, reduced malondialdehyde (MDA) levels, and enhanced antioxidant enzyme activities (SOD, CAT, Gpx). The extract also restored electrolyte concentrations toward normal values, indicating improved physiological stability.

**Conclusion:** Hence, n-hexane extract of *Oryctes rhinoceros* demonstrated a strong dose-dependent

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protective effect against lead-induced reproductive and biochemical toxicity in male Wistar rats. These findings suggest that the *Oryctes rhinoceros* possesses potent antioxidant and fertility-enhancing properties, offering potential as a natural therapeutic agent for mitigating heavy

metal-induced infertility.

Key words: Effect, n-Hexane, Extract, Sperm Parameters, Oxidative Stress Markers, Lead

## Introduction

Male reproductive health is a vital component of overall well-being, as it determines fertility and plays crucial role in sustaining the human population. Sperm parameters are the cornerstone of evaluating male reproductive health, as they provide insight into both the quantity and quality of spermatozoa. According to the World Health Organization<sup>1</sup>, key parameters include sperm concentration, motility, morphology, viability, and semen volume. Sperm count reflects the efficiency of spermatogenesis within the seminiferous tubules, while motility is crucial for the sperm's ability to navigate through the female reproductive tract to reach the oocyte. Morphology, assessed by the proportion of normally shaped spermatozoa, is essential for successful fertilization and embryo development<sup>2</sup>. Beyond simple counts, sperm function also depends on the integrity of the plasma membrane, acrosome reaction capacity, and mitochondrial activity. These functions are highly sensitive to the biochemical environment, particularly oxidative stress. Spermatozoa are uniquely vulnerable because they contain abundant polyunsaturated fatty acids in their membranes, which are highly prone to peroxidation, and possess minimal cytoplasmic antioxidant enzymes<sup>3</sup>. Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) generation and the body's antioxidant defense systems. oxygen species (ROS) attack sperm membrane lipids, leading to malondialdehyde (MDA) formation, a biomarker of lipid peroxidation. In addition, oxygen species (ROS) damage mitochondrial DNA and nuclear chromatin, reducing sperm motility and fertilizing ability<sup>4</sup>. To counter oxygen species (ROS), sperm and seminal plasma rely on both enzymatic and non-enzymatic antioxidants. Key enzymatic antioxidants include superoxide dismutase (SOD), which converts superoxide radicals into hydrogen peroxide; catalase (CAT), which decomposes hydrogen peroxide into water and oxygen; and glutathione peroxidase (GPx), which reduces lipid hydroperoxides<sup>3</sup>. Lead can

generate excessive oxygen species (ROS), overwhelm antioxidant defenses, and damage the lipid-rich sperm membrane, leading to impaired motility and viability<sup>5</sup>. The larvae of *Oryctes rhinoceros*<sup>7</sup>, commonly referred to as the large white grub worm or African oil palm larvae, are rich in essential nutrients and have been used in traditional diets and medicine across various African and Asian communities. The larva contains antioxidants, including tocopherols, selenium, and unsaturated fatty acids, which can scavenge free radicals and reduce oxidative stress, a key mechanism in heavy metal toxicity such as lead poisoning<sup>6</sup>. These components may protect reproductive organs by preventing lipid peroxidation and maintaining cellular membrane integrity. In reproductive physiology, *Oryctes rhinoceros*' larva has been studied for its potential to enhance sperm parameters, such as motility, viability, and morphology, due to its essential amino acids and fatty acids that support spermatogenesis. However, there is limited scientific literature on the specific effects of *Oryctes rhinoceros*' larva extract on male reproductive function, oxidative stress markers, electrolytes, and sperm parameters, particularly in the context of lead-induced reproductive toxicity. Given the growing concern over male infertility linked to environmental toxins, and the increasing interest in natural remedies, it is important to investigate the potential protective effects of *Oryctes rhinoceros*' larva extract against lead-induced damage. Such studies could provide valuable insights into novel, affordable, and accessible interventions for improving male reproductive health, especially in populations at high risk of heavy metal exposure.

## Materials and Method

### Animal collection, identification, and preparation

Fresh *Oryctes rhinoceros*' larvae (large white larva) were obtained from Bayelsa Forest and brought to Port Harcourt where the research was conducted

### Animal Extraction

Fresh *Oryctes rhinoceros*' larvae (large white larva) were collected, washed thoroughly with

clean water to remove debris, and then air-dried. The dried larvae were milled into a fine powder using a laboratory grinder.

### Moisture Content Determination

To determine the moisture content of the sample, clean Petri dishes were washed, dried in an oven, and cooled in a desiccator. Exactly 10 g of the milled sample was weighed into a pre-weighed Petri dish. The initial weight of the dish and sample was recorded. The petri dish containing the sample was placed in a hot-air oven at 100 °C for one hour, removed, cooled in a desiccator, and reweighed. This drying and weighing process was repeated at one-hour intervals until a constant weight was obtained.

The moisture content was calculated using the formula:

$$\text{Moisture Content (\%)} = \frac{W1 - W2}{W1} \times 100$$

Where:

- W1 = initial weight of sample before drying
- W2 = final constant weight after drying

### Extraction Procedure

After moisture analysis, 1000 g of the dried larvae powder was soaked in 2.5 L of n-hexane for 48 hours with intermittent shaking to enhance extraction. At the end of the soaking period, the mixture was filtered, and the filtrate was concentrated using a Soxhlet evaporator to remove the solvent. The n-hexane was completely evaporated, and the recovered extract (oil) was stored in a desiccator bottle under refrigeration until required for administration.

### Acute Test Toxicity

The acute toxicity of the n-hexane extract of *Oryctes rhinoceros*' larvae was assessed using Lorke's method (1983)<sup>10</sup>. This procedure was carried out in two phases.

**Phase I:** A total of nine (9) healthy male Wistar rats were randomly divided into three groups (n = 3 per group). The animals received graded oral doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg of the extract respectively. The animals were observed continuously for the first 24 hours and subsequently

for up to 72 hours for behavioral changes, signs of toxicity (e.g., piloerection, tremors, diarrhea, respiratory distress), and mortality.

**Phase II:** Based on the absence of mortality in Phase I, three (3) animals were further assigned into three groups (n = 1 per group) and administered higher oral doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg of the extract respectively. The animals were similarly observed for 72 hours for behavioural or toxicological manifestations and mortality.

**Observation:** Across both phases, no mortality or significant signs of toxicity were recorded in any of the animals within the 72-hour observation period, even at the highest administered dose of 5000 mg/kg body weight.

**Conclusion:** Since no death was observed up to the dose of 5000 mg/kg, the median lethal dose (LD50) of the n-hexane extract of *Oryctes rhinoceros*' larvae in Wistar rats was considered to be 5000 mg/kg body weight. This indicates that the extract is relatively safe and falls into the practically non-toxic category according to Lorke's classification. Lorke, D. (1983).

### Experimental Animal and Management

A total of 25 healthy adult male Wistar rats, weighing between 150 g and 180 g, were used for this study. The rats were obtained from the animal house of Faculty of Basic medical sciences, University of Port Harcourt, Nigeria. The animals were housed in well-ventilated cages lined with wood shavings and maintained under standard laboratory conditions of temperature (25 ± 2 °C), relative humidity (50–60%), and a 12-hour light/dark cycle. The rats were fed with standard commercial rodent pellets and had free access to clean drinking water ad libitum. They were allowed to acclimatize to the laboratory environment for two weeks before the commencement of the experiment. During this period, the animals were observed daily to ensure they were healthy and free from signs of stress or disease. Strict hygienic conditions were maintained by cleaning and changing the bedding of the cages regularly to prevent contamination and infection. Hand gloves were used when handling the

animals to ensure both animal welfare and researcher safety. All experimental procedures involving the animals were carried out in strict compliance with internationally accepted guidelines for the care and use of laboratory animals (National Research Council, 2011).

### Research Design

The present study was designed as an experimental animal study aimed at evaluating the effect of n-hexane extract of *Oryctes rhinoceros*' oil on lead-induced reproductive toxicity in male Wistar rats. A total of twenty-five (25) healthy male Wistar rats were used, and they were randomly selected into five (5) experimental groups of five rats each ( $n = 5$ ). The experiment was conducted for a period of 28 days, animals were weighed before and after they were sacrificed on the 29th day and samples collected for biochemical and the left testes were collected for histological analysis.

Group 1 (Lead Control): Received 70mg/bwt of lead + feed + water for 28days, administered orally.

Group 2 (Olive Oil Control): Received 0.5 ml of olive oil + feed + water orally for 28 days and served as the vehicle control.

Group 3 (Low Dose Extract + Lead): Received a low dose (10% of  $LD_{50}$ ) of n-hexane extract co-administered with lead acetate (70 mg/kg), feed and water for 28 days. The calculated low dose was 0.5 ml/kg based on the average body weight of the animals.

Group 4 (High Dose Extract + Lead): Rats received a high dose (40% of  $LD_{50}$ ) of n-hexane extract co-administered with lead acetate (70 mg/kg), feed and water for 28 days. The calculated high dose was 0.7 ml/kg based on body weight.

Group 5 (High Dose Extract + Olive Oil + Lead): Rats received a combination of high dose n-hexane extract (0.7 ml/kg), olive oil (0.5 ml), lead acetate (70 mg/kg), feed and water for 28 days.

Lead acetate was prepared by dissolving the salt in normal saline to obtain a stock concentration of 10 mg/ml. From this stock, the required dose of 70 mg/kg body weight was calculated and administered orally in a volume of 0.5 ml using an oral gavage. For the larvae oil extract, serial dilution of 8:2 ratios (8 parts extract oil to 2 parts olive oil) was employed to standardize the dosing. Doses of 40% and 10% of the  $LD_{50}$  were determined and

adjusted according to the body weights of the animals to obtain 0.7 ml/kg (high dose) and 0.5 ml/kg (low dose), respectively. All treatments were administered orally once daily throughout the 28-day study period.

### Ethical Approval

The ethical approval for this research was granted for this study by the ethics committee of the Faculty of Basic Medical Sciences, Rivers State University.

### Statistical Analysis

Data obtained from biochemical assays, electrolyte measurements, sperm parameters, and histological studies were expressed as mean  $\pm$  standard error of mean (SEM). Statistical analysis was carried out using one-way Analysis of Variance (ANOVA) to compare differences between groups, followed by post-hoc tests where necessary. A p-value of less than 0.05 was considered statistically significant.

### Results

The result on table 1, presented the result for sperm parameter, after twenty eight days of administration, the outcome for progressive motility showed that the low and high dose group of the oil extract and the co administered group increased significantly in a dose depended manner when compared to the two controls lead and olive oil. The olive oil group increase significantly when compared to the group administered with lead. Also, for the non-progressive motility, the reverse was the case still in a dose depended manner. For sperm count, there was significant increase in a dose dependent manner for the olive oil treated group. Again, in the co-administered groups (low and high dose of extract plus lead) there is significant increased sperm count as well as in the co administered group of high dose, when compared to the lead and olive oil group. Also, the olive oil group increased significantly when compared to the treated with lead. The result for normal sperm morphology showed a significant increase ( $p < 0.05$ ) in all the treated groups with the high and low dose of the extract only having greater percentage when compared to the two control (lead and olive oil.). The result for abnormal morphology showed a significant decrease ( $p < 0.05$ ) for all the treated groups when compared to the control groups. Also, the olive oil group decreased

significantly ( $p < 0.05$ ) when compared to the lead treated group. The result for sperm volume showed a significant increase ( $p < 0.05$ ) for the group treated with olive oil, low and high dose of the oil extract, and the co administered groups of high dose of oil extract when compared to the lead group. Also, the olive oil group significantly decreased ( $p < 0.05$ ) when compared to the low and high dose of the oil extract only and the co administered group of high dose.

The result above for table 2 illustrated the effects of twenty –eight days administration of N-hexane extract of *Oryctes Rhinoceros* on testicular tissue homogenate of oxidative markers Following lead -Induced fertility toxicity in male Wistar Rat. The out come of our findings showed that testicular homogenate for glutathione for, low and high dose of OR oil extract and the co administered group of the high dose group significantly  $p < 0.05$  increased when compared to the lead group. While the low and high dose groups significantly  $p < 0.05$  increased when compared to the olive oil group.

The testicular tissue homogenate of glutathione peroxidase for the group administered high dose and low dose of the oil extract showed significant increase  $p < 0.05$  when compared to both lead and the olive oil group. The testicular tissue homogenate of catalase for olive oil, low and high dose of the oil extract, co administered group of high dose significantly  $p < 0.05$  increased when compared to the lead olive oil control group, also the olive oil group significantly  $p < 0.05$  increased when compared to the lead group. The result for super

oxide dismutase showed a significant  $p < 0.05$  increase in all the treated groups when compared to the lead group in a dose dependent manner also, the olive oil group increased significantly when compared to lead group.

Malondialdehyde testicular tissue homogenate, concentration, significantly  $p < 0.05$  decreased for low and high dose of the oil extract, and the co administered group of the high dose when compared to the lead and olive oil group Also, the olive oil group decrease significantly  $p < 0.05$  when compared to the lead group.

Table 2 Effects of n-hexane extract of *Oryctes Rhinoceros* on of testicular tissue homogenate on oxidative markers Following lead -Induced Toxicity In Male Wistar Rat

Group	GSH(u/dl)	GPX(u/dl)	CAT( u/g)	SOD( u/ml)	MDA(umol/l)
Lead only	1.99 ±0.15	0.04 ±0.00	2.29 ±0.00	0.21 ±0.01	0.546 ±0.00
Olive only	2.05 ±0.01	0.04 ±0.00	3.05 ±0.01 <sup>a</sup>	0.27 ±0.01 <sup>a</sup>	0.48 ±0.00 <sup>a</sup>
Low dose of OR oil only	2.39±0.01 <sup>ab</sup>	0.05±0.00 <sup>ab</sup>	4.33 ±0.01 <sup>ab</sup>	0.36 ±0.01 <sup>ab</sup>	0.40 ±0.00 <sup>ab</sup>
high dose of OR oil	2.59 ±0.01 <sup>ab</sup>	0.05±0.00 <sup>ab</sup>	4.40 ±0.01 <sup>ab</sup>	0.41 ±0.01 <sup>ab</sup>	0.38 ±0.01 <sup>ab</sup>
Co administration of high dose of OR oil and lead	2.17 ±0.01 <sup>a</sup>	0.04±0.00	3.68 ±0.01 <sup>ab</sup>	0.37 ±0.01 <sup>ab</sup>	0.33 ±0.01 <sup>ab</sup>

Data are expressed as Mean ± SEM, n= 5. <sup>a</sup> Values are statistically significant at  $p < 0.05$ . When compared to the lead <sup>b</sup> significant difference relative to olive oil, at  $p < 0.05$

**Discussion**

Sperm parameters include sperm count, motility, morphology, and semen volume. They are important indicators of male fertility because they show the quality and functional ability of sperm cells. Any reduction in these parameters suggests testicular damage, hormonal imbalance, or oxidative stress caused by toxic substances such as lead. Lead exposure markedly reduced sperm motility, count, and morphology in the lead control group compared with the other treatment groups. This outcome aligns with previous studies indicating that lead impairs spermatogenesis, damages seminiferous tubules, and decreases sperm production through oxidative stress and hormonal disruption<sup>5,7</sup>. Lead interferes with the hypothalamic-pituitary-gonadal axis, reducing testosterone levels necessary for sperm maturation and motility. It also increases reactive oxygen species (ROS) generation, which attacks the polyunsaturated fatty acids of sperm membranes, leading to loss of membrane integrity and impaired motility<sup>3</sup>.

Table 1 Effects of n-hexane extract of *Oryctes Rhinoceros* on sperm parameters Following lead -Induced Toxicity In Male Wistar Rat

Group	Progressive mortality (%)	Non progressive mortality (%)	Sperm count (million/ml)	Normal morphology (%)	Abnormal morphology (%)	Volume (ml)
Lead only	10.0±0.70	43.2 ±0.37	10.0 ±0.70	16.8±0.00	83.1 ±0.24	0.40±0.03
Olive only	53.2±0.86 <sup>a</sup>	43.0 ±0.44	38.3±0.46 <sup>a</sup>	55.0±0.37 <sup>a</sup>	38.0±0.44 <sup>a</sup>	1.06±0.02 <sup>a</sup>
Low dose of OR oil only	57.0±0.51 <sup>ab</sup>	33.0 ±0.31 <sup>ab</sup>	41.6±0.05 <sup>ab</sup>	62.0±0.70 <sup>ab</sup>	37.0±0.70 <sup>ab</sup>	1.24±0.02 <sup>ab</sup>
high dose of OR oil	66.6±0.51 <sup>ab</sup>	23.2 ±0.20 <sup>ab</sup>	42.9±0.58 <sup>ab</sup>	62.0±0.44 <sup>ab</sup>	37.0±0.44 <sup>ab</sup>	1.30±0.00 <sup>ab</sup>
Co administration of high dose of OR oil and lead	56.6±0.67 <sup>ab</sup>	43.0 ±0.44 <sup>a</sup>	42.9±0.58 <sup>ab</sup>	57.0±0.44 <sup>ab</sup>	38.0±0.44 <sup>a</sup>	1.10±0.00 <sup>ab</sup>

Data are expressed as Mean ± SEM, n = 5. <sup>a</sup> Values are statistically significant at  $p < 0.05$ . When compared to the lead <sup>b</sup> significant difference relative to olive oil, at  $p < 0.05$

Conversely, rats treated with *Oryctes rhinoceros* extract (low and high doses) showed a dose-dependent improvement in sperm motility, count, and morphology. The high-dose and co-administration groups exhibited the highest recovery, with sperm motility and morphology approaching near-normal levels. This enhancement could be attributed to the rich protein, lipid, and micronutrient composition of *O. rhinoceros*, which supplies essential amino acids, vitamins, and fatty acids required for sperm membrane synthesis and testicular repair<sup>8</sup>. Similar findings have been reported by<sup>9</sup>, who observed that edible insect extracts improved sperm count and viability by modulating antioxidant enzymes and lipid metabolism. Therefore, the improvement in sperm parameters in the treated groups suggests that *O. rhinoceros* extract helps restore spermatogenic function, possibly by reducing oxidative injury and enhancing testosterone-dependent sperm maturation.

These are biochemical indicators used to measure the balance between free radicals and antioxidants in the body. Markers such as MDA, SOD, CAT, and GSH help to assess the level of oxidative damage and the body's ability to neutralize harmful reactive oxygen species. Oxidative stress can damage sperm cells, leading to poor sperm quality and infertility. Oxidative stress plays a central role in lead-induced testicular toxicity. In the present study, the lead control group showed a significant elevation in malondialdehyde (MDA) levels, indicating increased lipid peroxidation. This agrees with the findings of Pandey et al. (2019), who reported that lead exposure elevates MDA due to excessive ROS generation. High MDA levels suggest that the antioxidant defence system of the testes was overwhelmed, leading to oxidative damage to lipids, proteins, and DNA.

However, treatment with *Oryctes rhinoceros* extract resulted in a dose-dependent reduction in MDA and a significant increase in the activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH). This observation suggests that the extract possesses strong antioxidant properties capable of neutralising ROS and enhancing endogenous antioxidant defence. Moreover, the enhanced glutathione

system observed in the treated groups supports the idea that lipids and peptides act as redox-active molecules, preventing oxidative damage to sperm and testicular tissue<sup>4</sup>.

Again, the high-dose group and the co-administration group (high dose + lead + olive oil) demonstrated the greatest antioxidant improvement, implying that the combination of *O. rhinoceros* extract and olive oil might have synergistic protective effects. The findings therefore confirm that *Oryctes rhinoceros* extract effectively counteracts oxidative stress, stabilising cell membranes and restoring redox balance disrupted by lead toxicity.

Overall, the findings demonstrate that *Oryctes rhinoceros* extract mitigated lead-induced reproductive toxicity through antioxidant and cytoprotective mechanisms. The extract improved sperm parameters, enhanced antioxidant enzyme activities, reduced lipid peroxidation, and normalized serum electrolytes. These effects are likely due to the extract's high nutritional content (proteins, fatty acids, vitamins, and minerals) and the presence of bioactive compounds with antioxidant properties. Similar protective effects have been reported in studies involving other edible insect extracts and natural oils<sup>9</sup>.

The observed dose-dependent improvement further suggests that higher concentrations of *O. rhinoceros* extract provide more substantial protection, and its combination with olive oil may yield synergistic benefits. Histological findings confirmed the biochemical and reproductive results. The lead control group showed degeneration of seminiferous tubules, vacuolation of germinal epithelium, and loss of spermatogenic cells. Conversely, the groups treated with *Oryctes rhinoceros* extract, especially at higher doses and in combination with olive oil, exhibited nearly normal testicular architecture with restored spermatogenic activity. Therefore, *O. rhinoceros* extract may represent a novel, natural, and cost-effective approach for managing heavy-metal-induced reproductive toxicity, offering potential for further pharmacological and nutraceutical applications.

## Conclusion

Lead exposure induces significant reproductive toxicity in male Wistar rats by impairing sperm

quality, promoting oxidative stress, and disrupting electrolyte balance. The n-hexane extract of *Oryctes rhinoceros* effectively protects against these adverse effects in a dose-dependent manner. The extract enhances sperm motility, count, and morphology, reduces lipid peroxidation, boosts antioxidant defense mechanisms, and restores ionic homeostasis. The protective effects of the extract may be attributed to its bioactive and nutritional components, which act synergistically to combat oxidative stress and support reproductive function. Therefore, *Oryctes rhinoceros* extract holds strong potential as a natural therapeutic agent for mitigating heavy-metal-induced reproductive toxicity and improving male fertility.

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