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Determination of the Oral Median Lethal Dose (LD_{50}) of Lead Acetate Trihydrate in Wistar Rats Using Probit Analysis

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ABSTRACT

Lead compounds remain significant environmental and occupational toxicants, necessitating accurate characterization of their acute toxicity profiles. This study aimed to determine the median lethal dose (LD_{50}) of lead acetate trihydrate in Wistar rats using probit analysis. Acute toxicity was evaluated by administering graded doses of lead acetate trihydrate (0–40 mg/kg) to experimental groups of rats, and mortality was recorded after 24 hours. Percentage mortality at each dose level was converted to probit units and plotted against $\log_{10}$-transformed doses to establish a dose–response relationship. Linear regression analysis was performed to derive the LD_{50} and its 95% confidence interval. A clear dose-dependent increase in mortality was observed across treatment groups. Regression analysis yielded the equation $y = 10.081x - 9.4686$ with a high coefficient of determination ($R^2 = 0.986$), indicating an excellent fit of the model. The LD_{50} of lead acetate trihydrate was estimated to be 27.2 mg/kg body weight, corresponding to approximately 14.9 mg/kg of elemental lead (Pb), with a 95% confidence interval of 24.9–29.8 mg/kg (13.6–16.3 mg/kg Pb), demonstrating good precision. These findings confirm that lead acetate trihydrate exhibits significant acute toxicity in Wistar rats. The relatively low LD_{50} value underscores the potential health risks associated with acute exposure to lead compounds. This study provides reliable toxicological data that may contribute to risk assessment and the development of safety guidelines for lead exposure.

Keywords: Lead acetate; Acute toxicity; LD_{50}; Probit analysis; Dose-response; Wistar rats

INTRODUCTION

Lead (Pb) is a toxic heavy metal and a significant environmental contaminant introduced into ecosystems through anthropogenic activities, including mining, metal processing, paint manufacturing, and improper waste disposal [1], [2]. Due to its non-biodegradable nature, lead accumulates in soils, water, and biological systems over time, posing a major public health concern, particularly in regions with limited regulatory control [3], [4], [5]. Exposure to lead occurs primarily through ingestion of contaminated food and water, making the oral route a principal pathway for toxicity in both humans and animals [6], [7].

Following absorption, lead is transported via the bloodstream and accumulates in various organs, notably the liver, kidneys, and bones, where it exerts toxic effects and exhibits a prolonged biological half-life [8], [9]. The mechanisms of lead toxicity are complex and involve disruption of cellular processes, including the generation of reactive oxygen species, interference with antioxidant defense systems, and binding to critical biomolecules such as proteins and enzymes. These interactions result in oxidative damage, altered enzymatic activity, and impairment of normal physiological functions [10],[11],[12].

Lead-induced toxicity varies depending on the dose and duration of exposure. Acute exposure is typically associated with rapid onset of toxic effects, whereas chronic exposure leads to gradual accumulation and progressive damage to vital organs [5], [13], [14], [15], [16]. In addition, variations in susceptibility among experimental animals have been reported, highlighting the importance of controlled conditions in toxicity assessment [17], [18].

The median lethal dose (LD_{50}) is a fundamental parameter in toxicological studies used to estimate the dose of a substance required to cause death in 50% of a test population. It serves as an essential index for assessing acute toxicity and provides a basis for comparing the relative hazards of chemical compounds [19], [20]. Determination

of LD_{50} is particularly important for establishing baseline safety data in experimental and regulatory toxicology [21].

Lead acetate trihydrate is commonly used in experimental studies due to its high solubility and bioavailability, making it suitable for oral toxicity assessment. However, the toxic effects of lead may vary depending on its chemical form, necessitating compound-specific evaluation [19].

Probit analysis is a widely accepted statistical method for analyzing dose–response relationships in toxicity studies [22]. By transforming non-linear mortality data into a linear model, it enables accurate estimation of LD_{50} values and their associated confidence limits [23].

Therefore, this study was designed to determine the oral median lethal dose (LD_{50}) of lead acetate trihydrate in Wistar rats using probit analysis.

MATERIALS AND METHODS

Chemicals

Lead acetate trihydrate [$Pb(CH_3COO)_2 \cdot 3H_2O$] was procured from Loba Chemie Pvt. Ltd. (India). All other items used in this study were of analytical grade.

Experimental Animals

Female albino Wistar rats (180 ± 20 g) were obtained from Department of Medical Biochemistry Faculty of Basic Medical Sciences, Animal House, University of Cross River State, Nigeria. Animals were housed under standard laboratory conditions (12 h light–dark cycle) with free access to feed and water. Animals were acclimatized for seven days before experimentation. All procedures were approved by the Faculty of Basic Medical Sciences Ethical Committee, University of Cross River State (UNICROSS/FBMSEC/2026-MB002), in accordance with international guidelines for laboratory animal care.

Determination of Acute Toxicity (LD_{50}) of Lead Acetate Trihydrate

The median lethal dose (LD_{50}) of lead acetate trihydrate was determined in Wistar rats using a modified method of [21]. A total of forty (40) adult Wistar rats, weighing 180–200 g, were used for the study and randomly assigned into five groups (n = 8), including a control group.

Lead acetate trihydrate was dissolved in distilled water and administered orally via gavage at graded dose levels of 25, 30, 35, and 40 mg/kg body weight. Each dose was administered in a constant volume adjusted according to body weight, while the control group received distilled water only. Following administration, the animals were observed intermittently and periodically for 24 hours for signs of toxicity and mortality. Clinical signs of toxicity included reduced locomotor activity, anorexia, piloerection, tremors, diarrhea, lethargy, and impaired coordination. Mortality within 24 hours was recorded for each group. The percentage mortality at each dose level was calculated and converted into probit units. The logarithm of the administered doses was plotted against the corresponding probit values to generate a dose–response curve. The LD_{50} and its 95% confidence limits were estimated from the regression line using probit analysis.

Statistical Analysis

Probit analysis was employed to establish the dose–response relationship and to estimate the median lethal dose (LD_{50}) of lead acetate trihydrate. Percentage mortality at each dose level was converted to probit units using standard probit tables. The probit values were plotted against the logarithm (base 10) of the corresponding doses to generate a linear regression model. The regression equation was obtained using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA), from which the LD_{50} value (corresponding to probit 5.0) was derived. The coefficient of determination (R^2) was used to assess the goodness-of-fit of the model. The 95% confidence limits of the LD_{50} were estimated from the regression line using inverse prediction.

RESULTS

The dose–response relationship and probit transformation data for the determination of the median lethal dose (LD_{50}) of lead acetate trihydrate in Wistar rats are presented in Table 1. Administration of lead acetate produced a clear dose-dependent increase in mortality across the experimental groups. For clarity, administered doses of lead acetate trihydrate correspond to approximately 54.6% elemental lead (Pb).

No mortality was observed in the control group (0 mg/kg). However, exposure to 25 mg/kg resulted in 3 deaths out of 8 animals, corresponding to 37.5% mortality (probit = 4.67). Increasing the dose to 30 mg/kg led to 5 deaths (62.5% mortality; probit = 5.32), while 35 mg/kg produced 7 deaths (87.5% mortality; probit = 6.15). At the highest dose tested (40 mg/kg), 100% mortality was recorded; however, this value was excluded from probit regression analysis due to the upper-limit constraint of probit transformation. The progressive increase in percentage mortality with corresponding probit values indicates a consistent dose-dependent toxic response.

The regression plot illustrating the relationship between probit values and $\log_{10}$-transformed doses, as well as the graphical determination of the LD_{50}, is presented in Figure 1. Linear regression analysis of probit values against $\log_{10}$-transformed doses yielded the equation: $y = 10.081x - 9.4686$ ($R^2 = 0.986$). The high coefficient of

determination (R^2 = 0.986) indicates an excellent fit of the regression model to the experimental data, confirming the reliability of the probit transformation and dose–response relationship.

Using this regression model, the median lethal dose (LD_{50}), corresponding to probit 5.0, was estimated to be 27.2 mg/kg body weight of lead acetate trihydrate, equivalent to approximately 14.9 mg/kg of elemental lead (Pb), with a 95% confidence interval of 24.9 - 29.8 mg/kg (13.6 - 16.3 mg/kg Pb), indicating good precision of the estimate.

Table 1: Dose–response and probit transformation data for LD_{50} determination of lead acetate trihydrate in Wistar rats

Group	Dose (mg/kg)	$\log_{10}$ (Dose)	Total No. of Rats	Number of Deaths	Percentage Mortality	Probit Value
A	0.00	-	8	0	0	-
B	25.00	1.398	8	3	37.5	4.67
C	30.00	1.477	8	5	62.5	5.32
D	35.00	1.544	8	7	87.5	6.15
E	40.00	1.602	8	8	100	-

The LD_{50} value lies within the experimental dose range, further validating the robustness of the model.

Overall, these findings demonstrate that lead acetate trihydrate exhibits significant acute toxicity in Wistar rats in a dose-dependent manner.

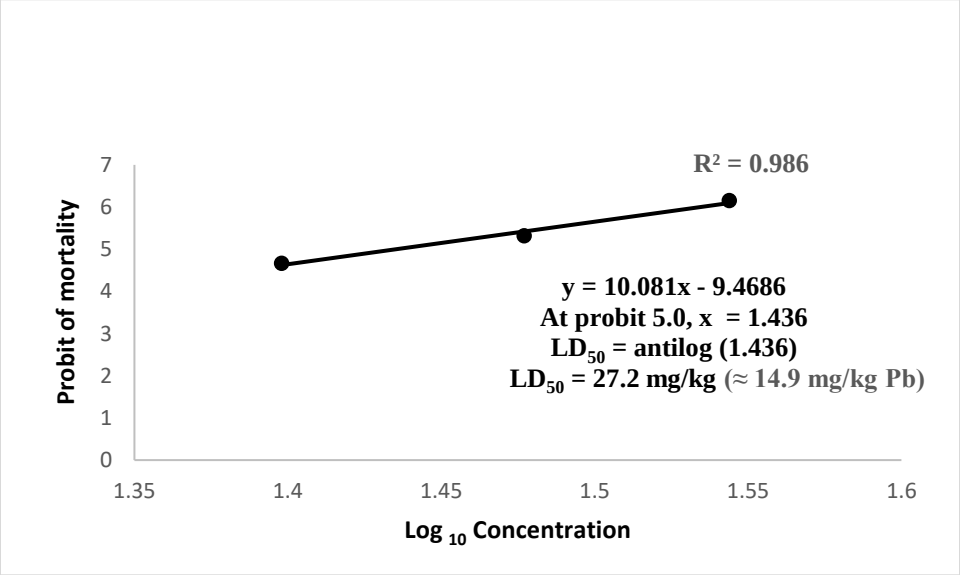

Figure 1: Probit regression plot of mortality versus $\log_{10}$ dose of lead acetate trihydrate in Wistar rats (n = 8). The regression line *($y = 10.081x - 9.4686$; $R^2 = 0.986$)* yielded an LD_{50} of 27.2 mg/kg (95% CI: 24.9–29.8 mg/kg), equivalent to 14.9 mg/kg elemental Pb.

DISCUSSION

The present study established the acute toxicity profile and median lethal dose (LD_{50}) of lead acetate trihydrate in Wistar rats using probit analysis. The findings demonstrate a clear dose-dependent increase in mortality, with an estimated LD_{50} value of 27.2 mg/kg (95% CI: 24.9 - 29.8 mg/kg), corresponding to 14.9 mg/kg elemental lead (Pb). The high coefficient of determination (R^2 = 0.986) obtained from the regression model indicates a strong linear relationship between probit-transformed mortality and $\log_{10}$ dose, confirming the robustness and reliability of the analytical approach [20], [22], [23].

The observed dose-dependent mortality pattern is consistent with the known toxicological behavior of lead compounds, which exert their effects through cumulative disruption of multiple physiological systems [19], [4]. Lead toxicity has been widely associated with interference in enzymatic processes, induction of oxidative stress, and disruption of cellular homeostasis. The progressive increase in mortality with increasing dose observed in this study supports these mechanisms, suggesting that higher concentrations of lead acetate (reflecting increased bioavailable elemental lead) overwhelm physiological detoxification pathways, ultimately resulting in systemic failure [19].

The LD_{50} value obtained in this study falls within the range reported for soluble lead salts in experimental animals, although variations across studies are common due to differences in species, age, route of administration, and experimental conditions [5], [13]. Expressing the LD_{50} in terms of elemental lead enhances comparability with other toxicological studies and regulatory benchmarks, which are often reported based on the active metal component rather than its salt form, thereby providing a more standardized basis for risk assessment [24]. The relatively narrow confidence interval reported here indicates good precision of the LD_{50} estimate, further supporting the reliability of the experimental design and statistical analysis.

Exclusion of the 100% mortality data point from the probit regression was methodologically appropriate, as extreme response values (0% or 100%) can distort probit transformation and compromise linearity. This approach aligns with established toxicological guidelines and ensures that the regression model accurately reflects the central portion of the dose–response curve, where estimation of LD_{50} is most reliable [21].

From a toxicological perspective, the relatively low LD_{50} value obtained in this study underscores the significant acute toxicity of lead acetate trihydrate. When considered as 14.9 mg/kg elemental Pb, this finding further emphasizes the potency of lead as a systemic toxicant. This is particularly important given the continued environmental and occupational exposure risks associated with lead compounds [4]. Acute exposure at sufficiently high doses can result in rapid onset of severe toxic effects, while lower, repeated exposures may lead to chronic toxicity through bioaccumulation [5], [1].

Despite the strengths of this study, certain limitations should be acknowledged. The sample size within each dose group was relatively small, which may influence the precision of mortality estimates at individual dose levels. Additionally, the study focused exclusively on acute toxicity and did not assess sublethal or chronic effects, which are highly relevant for environmental toxicants such as lead [2]. Future studies should incorporate larger sample sizes, additional dose levels, and longer observation periods to provide a more comprehensive toxicological profile. Investigations into biochemical, hematological, and histopathological endpoints would also enhance understanding of the underlying mechanisms of toxicity [13], [10].

In conclusion, this study provides a reliable estimate of the LD_{50} of lead acetate trihydrate in Wistar rats and confirms its pronounced acute toxicity in a dose-dependent manner. The inclusion of elemental lead equivalence strengthens the toxicological relevance of the findings and facilitates comparison with existing literature and regulatory standards. The findings contribute valuable data to the toxicological characterization of lead compounds and may inform risk assessment and regulatory decision-making regarding exposure limits and safety guidelines.

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Conflicts of Interest

The author declares no conflict of interest.

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