

Research Article

**Effect of Lead Acetate on Erythrocyte Osmotic Fragility in Male Wistar Rats**Oyedemi K.O.^{1*}, Alabi A. S.²¹Department of Physiology, College of Medicine and Health Sciences, AfeBabalola University, Ado-Ekiti, Nigeria.²Central Animal House, College of Medicine, University of Ibadan, Nigeria.*Corresponding author's E-mail: sinaoyedemi@yahoo.com

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ABSTRACT

This study aims at investigating the effect of lead acetate on erythrocyte osmotic fragility in male rats. Fifteen male rats (120-150 g) were divided into control (distilled water) and lead acetate-treated (100 ppm, 1000 ppm) groups (5 per group). The animals were orally treated on daily basis for five weeks. Erythrocyte osmotic fragility was determined by spectrophotometry method. Data were analyzed using descriptive statistics and ANOVA at $p = 0.05$. The results showed that treatment of rats for five weeks with high and low doses of lead acetate (100 ppm, 1000 ppm) produced no significant ($p > 0.05$) changes in erythrocyte osmotic fragility at all the NaCl concentrations (0.1% - 0.9%) relative to the control. These findings probably indicate that lead acetate has no effect on erythrocyte osmotic fragility of male rats.

Keywords: Lead acetate, Osmotic fragility, Erythrocytes, NaCl concentrations, Rats.**INTRODUCTION**

Lead acetate is a white crystalline chemical compound with a sweetish taste. It is made by treating lead (II) oxide with acetic acid¹.

Lead acetate has been reported to significantly stimulate glutamic-pyruvic transaminase and glutamic – oxalacetic transaminase in male rats². It has been reported to alter rats' development as well as affecting specific subsets of motor skills³. Lead acetate has also been reported to cause hypertension in male rats⁴.

Since lead acetate has been reported to cause significant alterations on the hematological functions of male rats⁵, this study therefore aims to investigate the effect of lead acetate on erythrocyte osmotic fragility in male Wistar rats.

MATERIALS AND METHODS**Experimental Animals**

Adult male rats weighing between 120 – 150 g bred in the Pre-Clinical Animal House of the College of Medicine, University of Ibadan were used.

They were housed under standard laboratory conditions and had free access to feed and water. They were acclimatized for two weeks to laboratory conditions before the commencement of the experiments.

All experiments were carried out in compliance with the recommendations of University of Ibadan Ethics committee (UI-ACUREC/App/2015/040) on guiding principles on care and use of animals.

Reagents

Lead acetate, sodium chloride (sigma), NaHPO_4 (Sigma), $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (sigma).

Experimental Design

Fifteen albino rats weighing between 120-150 g were divided into three groups, with each group consisting of five animals. The three groups of rats were subjected to the following oral treatments on daily basis for five weeks and the dosages of lead acetate used in this study were in accordance with those reported by⁶:

Group I served as the control and received 0.5 ml distilled water

Group II received 100ppm (low dose) lead acetate

Group III received 1000ppm (high dose) lead acetate.

Collection of Blood Samples

Twenty four hours after the last dosing of all groups, blood samples were collected from all the animals through the medial cantus with heparinized capillary tubes into EDTA bottles for erythrocyte osmotic fragility study.

Determination of Erythrocyte Osmotic Fragility

The erythrocyte osmotic fragility of rats was evaluated using the method of⁷.

One percent (%) sodium chloride (NaCl) solution was buffered with phosphate solution, Na_2HPO_4 (1.3 mg/mg) and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (0.24 mg/mg). Lower dilutions of NaCl solution (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8% and 0.9%) were prepared in test tubes and a tenth test tube contained only distilled water (0.0%). The pH of the distilled water (7.0) and those of the NaCl solutions (7.4) were measured using a pH meter (Digital pH meter, Labtech). Five millimeters of each concentration of NaCl was put in a test tube (9 in all) and 5 ml distilled water (0.0%) was put in the tenth tube. To each test tube was pipetted 0.02 ml of blood using a micropipette. The



contents were thoroughly mixed and allowed to stand for thirty minutes at room temperature (28 – 29°C). The test tubes were then centrifuge at 3,000 rpm for ten minutes. The Optical Density (O.D.) of each supernatant solution (a measure of the degree of hemolysis) was measured with a spectrophotometer (SM23A) at a wavelength of 540 nm using a tube of distilled water as blank.

The degree of hemolysis in the distilled water test tube was taken as 100% and the others were read in relation to it.

$$\text{Percentage hemolysis} = \frac{\text{O. D. of Test Solution}}{\text{O. D. of Standard Solution}} \times 100$$

A cumulative erythrocyte osmotic fragilitygram was obtained by plotting the mean percentage hemolysis for the three groups of rats against the concentrations of the NaCl solution.

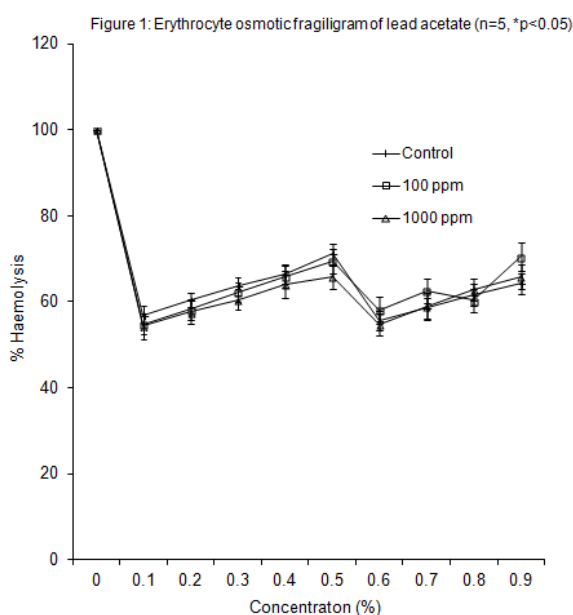
Statistical Analysis

The mean and standard error of mean (S.E.M) were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The effect of low and high doses of lead acetate on erythrocyte osmotic fragility after treatment of rats for 30 days is shown in Figure 1.

Treatment of rats for five weeks with both the low and high doses (100 ppm, 1000 ppm) of lead acetate produced no significant ($p > 0.05$) changes in erythrocyte osmotic fragility at all the NaCl concentrations (0.1 % - 0.9 %) relative to the control.



DISCUSSION

The osmotic fragility assay is a classical, rapid, useful and easy technique that has permitted to obtain relevant

information about the interactions of natural and synthetic drugs with cellular membrane⁸. It has also been reported that some drugs are capable of inducing alterations on the shape and physiology of the red cells⁹.

The results have shown that lead acetate caused no significant changes in erythrocytes osmotic fragility at various NaCl concentrations after five weeks of treatments. This probably indicates that lead acetate does not stabilize or strengthen the membrane integrity of red blood cells since it has been reported that erythrocytes osmotic fragility is used as a measure of the tensile strength of the red cell membrane¹⁰. The insignificant change in erythrocyte osmotic fragility produced by the compound probably indicates its ineffectiveness in the treatment of hemolysis since it has been reported that osmotic fragility refers to the degree or proportion of hemolysis that occurs when a sample of red blood cells are subjected to osmotic stress by being placed in a hypotonic solution¹¹.

The insignificant change in erythrocyte osmotic fragility induced by the compound probably indicates that it has no effect on red blood cells viability since it has been reported that the osmotic fragility of mammalian red blood cells is indicative of their viability¹². The insignificant change in erythrocyte osmotic fragility caused by the compound probably indicates it is ineffective in the treatment of hereditary spherocytosis and hypernatremia since erythrocyte osmotic fragility is often performed to aid with the diagnosis of diseases associated with RBC membrane abnormalities and some diseases linked to increased osmotic fragility induce hereditary spherocytosis and hypernatremia. Contrary result was reported by¹³ in whole blood incubated with *Lantana camara in vitro*.

In conclusion, this study has shown that lead acetate probably has no effect on erythrocyte osmotic fragility in male rats, *vis-à-vis*, it is probably ineffective in the treatment of hemolysis.

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