

## LEAD ACETATE INDUCED GENOTOXICITY IN DEVELOPING CHICK EMBRYO

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### ABSTRACT

The presence of toxic substances in natural environments can cause several alterations in organisms, which may include biochemical, cellular, morphological and teratological changes. Lead (Pb) compounds are known genotoxicants in affecting the integrity of chromosomes. The present study conducted was to determine the effects of lead acetate on genotoxicity and morphological analysis of chick embryo erythrocytes. Micronucleus formation in chick embryo cells provides a simple and rapid indirect measurement of the induction of structural or numerical chromosome aberrations that are resulted from exposure to lead acetate on chick embryo. Erythrocytes of peripheral blood of developing chick embryo (13<sup>th</sup> day) from 20µg to 40µg/egg treated lead acetate showed nuclear and no such phenomenon was seen in control group. The occurrence of micronucleus in various cells of organism is a good substitute for chromosomal assay. Our results observed under microscope on erythrocytes indicate that lead acetate induces chromosomal damage and nuclear abnormality, as well in developing chick embryo.

### Key words

Chick Embryo, Lead Acetate, Micro nuclei, Genotoxicity, Nuclear abnormality.

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### INTRODUCTION

Many toxic chemical substances of natural origin and others due to human activities are released into the environment daily. One of the several genotoxic substances known for mutagenic and carcinogenic is heavy metal that is lead [1]. Lead and its complex compounds produce severe organ damage in animals and humans [2]. Several studies have reported that lead toxicity is associated with impaired functioning of brain, liver, kidney, testes and the hematopoietic system [3] [4]. Chick embryos have complex metabolic competence; therefore they serve as good model system for genotoxic testing and are capable of covering metabolic activation and deactivation of xenobiotics.

The micronucleus test is used widely to evaluate DNA damage caused by clastogenic and aneugenic substances and plays an important role in genetic toxicology in evaluating the genotoxic potential of xenobiotics. This assay was developed for mice bone marrow erythrocytes analysis and was further used in various animal species [5] [6]. Wolf and Luepke [7] has described the formation of micronuclei in the erythrocytes of peripheral blood of incubated hen's eggs as a measure of genotoxicity and a positive response of direct acting mutagens and promutagens.

The MN assay, a simple and rapid indirect measure of induced structural and numerical chromosome aberrations, is scientifically accepted by the Organization for Economic Cooperation and Development, International Conference on Harmonization and European Union [8]. Micronuclei formations in cells are considered as a biomarker of damage of DNA of chromosome [9], genome instability and eventually of cancer risk.

Boller[10], Schmid[11]and Heddle [12]were introduced the micronucleus test, as one of the most frequently used genotoxicity tests in mammals. The occurrence of MN represents an integrated response to chromosome instability phenotypes and altered cellular viabilities induced by genetic defects and/or exogenous exposures to genotoxic agents [13]. The MN test evaluates the frequency of MN formation in a proliferating cell population in vitro [14] [15] as well as in vivo and in various tissues in ovary, bone marrow, peripheral blood, liver and in fetal cells of rodents and humans[16] [17].

The present study is aimed to detect the influence of lead acetate on genotoxicity of the chick embryo peripheral blood of erythrocytes.

## **MATERIALS AND METHODS**

### **Chemicals**

Pure lead acetate from Merck India Ltd, Giemsa and May-Grunewald stains from HiMedia laboratories, Mumbai were purchased. All other reagents used were of analytical grade and were procured from the local companies.

### **Source of Fertilized Eggs and Incubation Conditions**

Freshly laid *Bobcock* strain zero day old fertilized eggs were purchased from Sri Balaji hatcheries, Chittoor, Andhra Pradesh. The eggs were incubated horizontally and rotated (3h intervals) at  $37.5 \pm 0.5^\circ\text{C}$  with a relative humidity of 65% in an egg incubator.

### **Experimental Design**

Fertile eggs were divided into three groups: group A, Group B and Group C. Two groups A & B were administered as single dose of  $20\mu\text{g}$  and  $40\mu\text{g}/\text{egg}$  of lead acetate respectively, on day 7 of incubation. Group C received no lead acetate and served as healthy control. Eggs were set-up in an upright position with the blunt end at the top. On day 7, each egg was sterilized with 70% ethanol and egg shell was opened to obtain access to the air cell, where all the test samples were injected directly on to the inner shell membrane. The hole was covered by a wax to ensure the embryo's health until blood sampling takes place. The eggs were placed back into the humidified incubator. The eggs were further incubated in a horizontal position until the date of examination. Eggs were injected by the air sac method [18], on day 7 of incubation.

On day 13<sup>th</sup> of incubation, the egg shell was broken at the air chamber and erythrocyte of peripheral blood was collected for the measure of genotoxicity. The slides cleaned with 70% ethanol before smear preparation. Blood was spread out on slides immediately after blood sampling. Blood smear was prepared as soon as possible, within one hour of collection.

### **Staining of blood smears**

Micronucleus (MN) test was performed according to Chaubey [19]. The blood smears were air dried and stained on a staining rack using the following procedure:

Air dried blood smears were fixed in a jar of methanol for 5 min and stained using undiluted May-Grunwald for 3min. It is followed by immersion of slides in diluted May-Grunwald for 5

min, rinsed thrice in distilled water followed by staining with diluted Giemsa for 10 min and rinsed in distilled water thoroughly. Wipe the under surface of the slides to remove excess stain and air dried in a vertical position. Cleared for 5 min in xylene and mounted in DPX.

### Scoring of Micronuclei

Nearly 1000 cells were observed at random for each slide and approximately 3000 cells were studied under each group. The slides were observed using an Olympus BX60 microscope under bright field illumination at 100x (oil immersion). The examinations depicted in Figs.1A to 1C have been carried.

### Statistical analysis:

Data were analyzed using one way analysis of variance (ANOVA) to determine the significance of micronucleus test. Values were expressed as mean  $\pm$ SE. (n=6). Differences between means were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Lead is a ubiquitous environmental toxin, which can be detected in almost all phases of environment and biological systems because of the widespread occurrence of lead in the environment is primarily a result of anthropogenic activities. Much of the lead emitted into the atmosphere is in the form of inorganic salts.

Micronucleus analysis was performed on 3000 red blood cells from each blood embryo. RBCs could be detected in blood obtained from embryo grown at optimum temperature  $37.0 \pm 0.5^{\circ}\text{C}$ . A significant number of RBCs with micronucleus were seen in blood from lead acetate treated chick embryos. Micronucleus (MN) assay was developed as a short-term screening test. In this test, chromosomal aberrations are detected indirectly via chromatin less from the nucleus leading to MN in the cytoplasm of the cell. MN is defined as a small round-DNA containing cytoplasmic bodies formed during cell division by loss of both acentric chromatins. It was indicated that, for genotoxicity assay, the erythrocytes from chick embryo blood are relevant target cells and independent from their stage of maturity for environmental pollutant.

The results of the present study may indicate that lead acetate is an aneugenic agent, and one possible mechanism of MN formation in mononuclear RBC is that MN is formed in immature RBC before maturation process has taken place. This may be an interesting additional parameter in micronucleus assay in chick embryo system.

The criteria for the identification of MN were described as

1. MN must be clearly separated from main nuclei.
2. MN must be smaller than one third of the main nuclei.
3. MN must be on the same plane of focus and have the same color.

### Micronucleus test

Erythrocytes of control chick embryos were oval shaped and uniform in size with a single normal nucleus and showed no abnormalities in their morphology (Figure 1.A). The blood smears of  $20\mu\text{g}$  and  $40\mu\text{g}/\text{egg}$  lead acetate treated embryos presented micronucleated cells (Figure B) and statistically significant from control group.

Apart from the micronucleated erythrocytes, blood smears of  $20\mu\text{g}$  and  $40\mu\text{g}/\text{egg}$  lead acetate treated embryos showed abnormal cell such as sharp edged erythrocytes (Figure C).

Data presented in Table (1) showed that lead acetate treatment leads to formation of MN in chick embryo erythrocytes. Moreover, results presented in this study indicated that mononucleated chick embryo RBCs have developed micronucleus (Figure 1.B). The embryonic treatment with 20 $\mu$ g and 40 $\mu$ g/egg lead acetate was capable of inducing MN in erythrocytes which might be due to the cytotoxic nature of the lead acetate. Results demonstrated that the technique is simple, sensible and of easy application.

The analysis included the calculation of the frequency of micronuclei to measure genotoxicity by following formula

$$\text{MN\%} = \frac{\text{Number of cells containing MN} \times 100}{\text{Total number of cells scored}}$$

Mean frequency of total micronucleated erythrocytes was calculated for control and treated chick embryos. There was a 1.39 fold increases in frequency of MN-E in 40 $\mu$ g PbA (Lead acetate) treatment compared to 20 $\mu$ g PbA treatment. There was a significant increase ( $p < 0.05$ ) in the induction of MN-Es frequency in 40 $\mu$ g PbA treated embryos compared to 20  $\mu$ g PbA treatment.

Figure-1: Photograph of Peripheral Blood Erythrocytes Showing Micronuclei (100X) and Nuclear abnormality

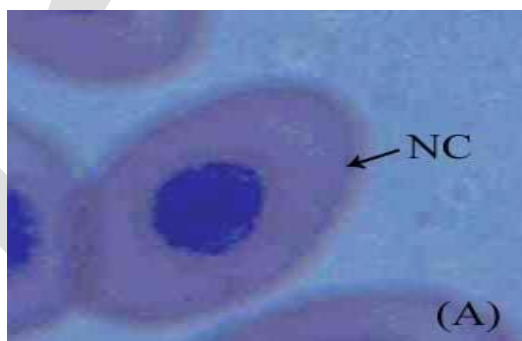


Figure: 1A. Control (NC: Normal cell)

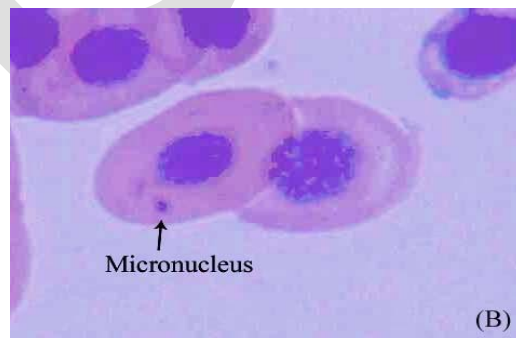


Figure: 1B: Micronucleated cell

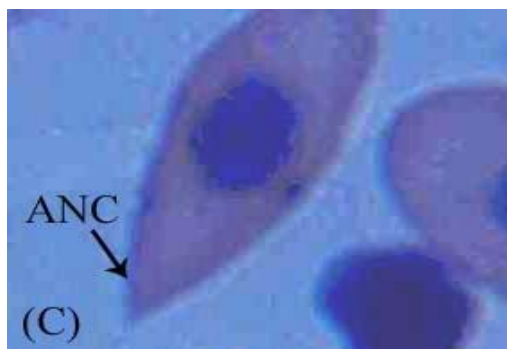


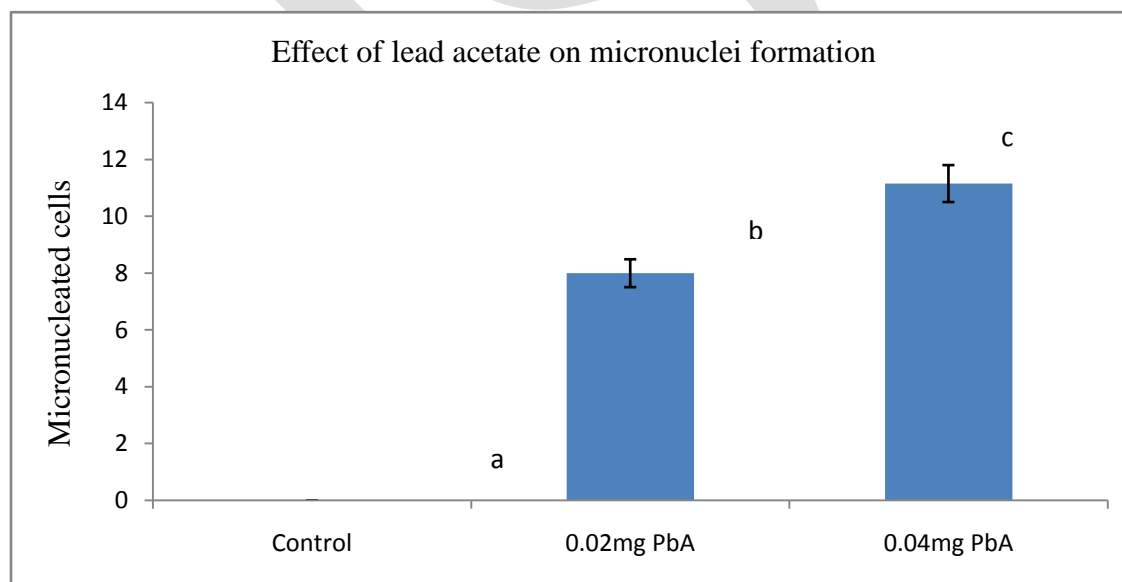
Figure: 1C. Sharp edged cell (ANC: Anisochromasia)

Table 1: Frequencies (%) of Micronuclei in 13<sup>th</sup> day old chick embryo peripheral blood erythrocyte exposed to lead acetate treatment.

S.No	Exposure doses	Total number of erythrocytes	Frequency of MN Mean(%) $\pm$ SD
1.	Control	3000	0
2.	20 $\mu$ g /egg Lead acetate	3000	7.995 $\pm$ 0.49
3.	40 $\mu$ g /egg Lead acetate	3000	11.15 $\pm$ 0.65

Each value represents the mean  $\pm$  SD (n=6).

Figure-2: Effect of lead acetate on micronuclei formation



Different letters are significantly different at the level of  $p < 0.05$   
Data expressed as mean $\pm$ SD.

## CONCLUSION

Lead induces a broad range of physiological, biochemical, and behavioral dysfunctions in laboratory animals and humans. According to the results obtained in the present study, MN tests employed for the genotoxic assessment has indicated that DNA damage induced by exposure to lead acetate in the developing chick embryos.

## REFERENCES

- [1] Matsumoto ST, Janaina R, Mario SM, Maria, AM (2005). Evaluation of the Genotoxic Potential Due to the Action of an Effluent Contaminated with Chromium, by the Comet Assay in CHO-K1 Cultures. *Caryologia* 58 (1): 40-46.
- [2] Pivey, A. *Environ. Health Perspect*; 115 (2007).
- [3] Bellinger, D.C. *Curr. Opin. Pediatr.* 20 (2008).
- [4] El -Nekeety, A.A., El -Kady, A.A., Soliman, M.S., Hassan, N.S. and Abdel -Wahhab, M.A. *Food Chem. Toxicol.*; 47(2009).
- [5] Ribeiro, L.R.L; Salvadori, F.M.D; Marques, K.E. *Mutagenese Ambiental*, ULBRA, Canoas, 355P(2003).
- [6] Dias M.V; Oliveira, M.R and Santelli, M.G. Using fluorescence for improvement of the quantitative analysis of micronucleus in cell culture. *Mutagenesis*, 565: 173-179(2005).
- [7] Wolf and Luepke. Formation of micronuclei in incubated hen's egg as a measure of genotoxicity. *Mutat. res.*, 394:163-175(1997).
- [8] Wolf, T; Rolf, N. C. & Luepke, P. N. Some new methodological aspects of the egg test for micronucleus induction (HET-MN). *Mutat. Res.*, 514:59-76 (2002).
- [9] Saleh, K., and M.A.A. Sarhan. Clastogenic Analysis of Chicken Farms Using Micronucleus Test in Peripheral Blood. *Journal of Applied Sciences Research* 3: 1646–1649 (2007).
- [10] K. Boller, W. Schmid, Chemische Mutagenese beim Sauger. Das Knochenmark des Chinesischen Hamsters als in vivo. Test system, *Humangenetik* 11, 35–54 (1970).
- [11] W. Schmid. The micronucleus test, *Mutation Res.* 31, 9–15(1975).
- [12] J.A. Heddle. A rapid in vivo test for chromosomal damage. *Mutation Res.* 18, 187–190(1973).
- [13] Iarmarcovai, G., S. Bonassi, A. Botta, R.A. Baan, and T. Orsiere. Genetic Polymorphisms and Micronucleus Formation: A Review of the Literature. *Mutation Research* 658: 215–233(2008).

- [14] Miller, B.M., E. Pujadas, and E. Gocke. Evaluation of the micronucleus test in vitro using Chinese hamster cells: Results of four chemicals weakly positive in the in vivo micronucleus test. *Environmental and Molecular Mutagenesis* 26: 240–247(1995).
- [15] Garriott, M.L., J.B. Phelps, and W.P. Hoffman. A Protocol for the in vitro micronucleus test-I. Contributions to the development of a protocol suitable for regulatory submissions from an examination of 16 Chemicals with different mechanisms of action and different levels of activity. *Mutation Research* 517: 123–134(2002).
- [16] Heddle, J.A. Micronuclei in vivo. *Progress in clinical and biological research* 340: 185–194 (1990).
- [17] Saleh, K., and H. Zeytinoglu. Micronucleus test in peripheral erythrocytes of rana ridipunda as an indicator of environmental pollution. *Anadolu University Journal of Science and Technology* 2: 77–82 (2001).
- [18] Blankenship, A.L., K.N.M. Hilscherova, K.K. Coady, S.A. Villalobos, K. Kannan, D.C. Powell, S.J. Bursian, and J.P. Giesy. Mechanisms of TCDD-induced Abnormalities and Embryo Lethality in White Leghorn Chickens. *Comparative Biochemistry and Physiology Part C-Toxicology Pharmacology* 136: 47–62 (2003).
- [19] Chaubey, R.C., H.N. Bhilwade, B.N. Joshi, and P.S. Chauhan. Studies on the Migration of Micronucleated Erythrocytes from Bone Marrow to the Peripheral Blood in Irradiated Swiss Mice. *International Journal of Radiation Biology* 63: 239–245(1993).