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Genotoxic effects of Mercuric Chloride in the Albino Rat, *Rattus norvegicus*

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Abstract

In the present study, toxicity of sublethal concentration of mercuric chloride on the karyotyping of Albino rat, Rattus norvegicus was observed, LD_{50} of mercury chloride was calculated and a sublethal concentration (1/20 LD_{50}) was gives orally to the rat 20 hours prior to the colchicines treatment, 2 hours after this treatment the bone marrow was separated from the femur of test animal and collected in the test tube. The slide was prepared and studied under the microscope. The investigation reveals the numerical and structural aberrations in the chromosomes of the bone marrow cells in the test animal.

Keywords; Mercuric chloride, Karyotyping, *Rattus norvegicus.*

Introduction

Morphology and number of chromosomes is species specific and a set of somatic chromosomes of an individual or species is called its karyotype[1]. The name karyotype is given to the whole group of characteristics that allows the identification of a particular chromosomal set of a cell or species as visualized during mitosis. In albino rat, *Rattus norvegicus* 21 pairs of chromosomes are present in the karyotype. Homologous pairs of identified chromosomes can be arranged in series of decreasing lengths, such arrangement is called as Idiogram[2]. Karyotype is characteristic of an individual species or genus and may be represented by an idiogram in which pair of homologues is ordered in a series of descending size. Any alteration from normal karyotype, on account of a toxicant may lead to several functional impairments. The selection of *Rattus norvegicus* for the experimental purpose is based on the fact that it is easy to rear in the laboratory, easy to handle and of short gestation period, further, albino rat being a mammal so can be used as a tool to similar references in other allied mammalian species.

Mercury has no biological role but is widespread in the biosphere and in food chains. Mercury is a dreadful poison and is absorbed readily through the respiratory tract, gastrointestinal tract and through skin. One of the mercury compounds, the mercuric chloride is used in this experiment. Mercuric chloride being a potent toxicant causes many physiological and metabolic disturbances in human and non-human animal models was selected as the experimental chemical in present study[3,4] and karyotyping is used as a technique to reveal the possible effects of mercury chloride intoxication in a mammalian model, *Rattus norvegicus.*

Material and Methods

Experimental animal: *Rattus norvegicus* has played a prominent role in the study of genetics. It has many features that enhance its value as model organisms for genetic analysis. Rats have very short generation time of just nine weeks. They are small enough so that thousands can live in a relatively small room. They have large litter of eight or more pups. Thus the albino rat provides a powerful model system for investigating the genetic basis of simple and complex human traits, especially those related to development and diseases. The healthy and active specimens of albino rats used as the test animal were acclimatized to the laboratory conditions for one month and kept in well aerated cages. The mean weight of rats was 150±10 grams.

Experimental Chemical: Mercury chloride is an odorless chemical compound with white crystal or powder appearance. Stable under ordinary conditions of use it slowly decomposes to metallic mercury in presence organic matter and sunlight and becomes volatile at 300^0C. Some properties of mercuric chloride are-

Table-1
Physical and chemical properties of mercuric chloride

Common formula	**$HgCl_2$**
Molecular weight	271.49
Solubility	7.4 g in 100 ml of water
Specific gravity	5.4
pH	3.2
Boiling poing	303 ^{0}C
Melting poing	276 ^{0}C
Vapour density	8.7

Preparation of sub-lethal dose: LD_{50} of mercury chloride in *Rattus norvegicus* was calculated as 9.26 mg/kg body weight. On the basis of LD_{50} value and the weight of albino rats, sub-lethal dose of 0.4 mg/body weight (1/20 LD_{50}) was calculated for karyotyping study in the test animal, which as prepared by dissolving 2mg $HgCl_2$ in 2.5 ml of deionized water of which only 0.5 ml (0.4 mg) was administered to the rat orally.

Methodology: In the experiment, three albino rats, *Rattus norvegicus* were taken as control and three others for experimental group. After acclimatization of animals for one month in quarantine, the rats of experimental groups were given acute treatment of mercury chloride (0.4 mg) taking consideration of LD_{50} of this chemical for albino rats[5]. 20 hours after mercury intoxication rats were injected with colchicine solution to arrest the chromosomes in their metaphase. 2 hours after colchicine treatment bone marrow was flushed out from the femur of rats in to the centrifuge tube containing isotonic Nacl solution. Homogenous suspension was made by gentle aspiration. After a series of centrifugations in isotonic solutions and fixatives suspension was refrigerated for 10 minutes and then allowed to fall on the clean slide which were dried and stained in 4% giemsa (figure 1). Slides of metaphasic chromosomes were prepared both from control and experimental group rats by using bone marrow harvest stained by giemsa. Isolation of bone marrow was carried out according to the methodology of Heddle[6] and chromosomal aberration assay was based on the methodology proposed by Preston[7]. Photomicrography of metaphase plates was done from both the control and treated group animals using digital camera and the micrographs thus obtained were studied to observe the changes.

Results and Discussion

Observations reveal the presence of 21 pairs of chromosomes in normal albino rat, *Rattus norvegicus*. Figure 2. The comparable to the rat chromosome ideogram adopted by Szpirer[8], which is based on the nomenclature rules for rat chromosome G-banding given by Levin[9]. The karyotype of mercury chloride treated rats showed a generalized reduction in number and size of chromosomes in bone marrow cells. Some of the cells appeared to be having loosened chromatin along with numerical and structural alterations includes deletion and duplication figure 3.

The observations of present investigation reveal functional impairment of cells in treated group. The numerical and structural alterations observed are in accordance to the decreased mitotic index[10]. The reduction in number and size of chromosomes clearly indicate the reduction in number of genes via the loss of codons that normally code for essential amino acids, the building blocks of several proteins, enzymes and proteinaceous hormones. The altered levels of these enzymes and hormones are anticipated to cause several physiological and metabolic disorders. Similar results of chromosomal aberration was observed in the bone marrow cells of mice given a diet supplemented with various heavy metals[11], parallel findings were also recorded in mice following zinc chloride toxicity[12] however cytogenetic damage in cultured cells following cobalt and zinc toxicity was estimated[13]. The findings are in accordance with the studies in which the toxicity and metabolism of mercury reviewed extensively[14] and same was also recorded in Albino rat after $HgCl_2$ intoxication[15].

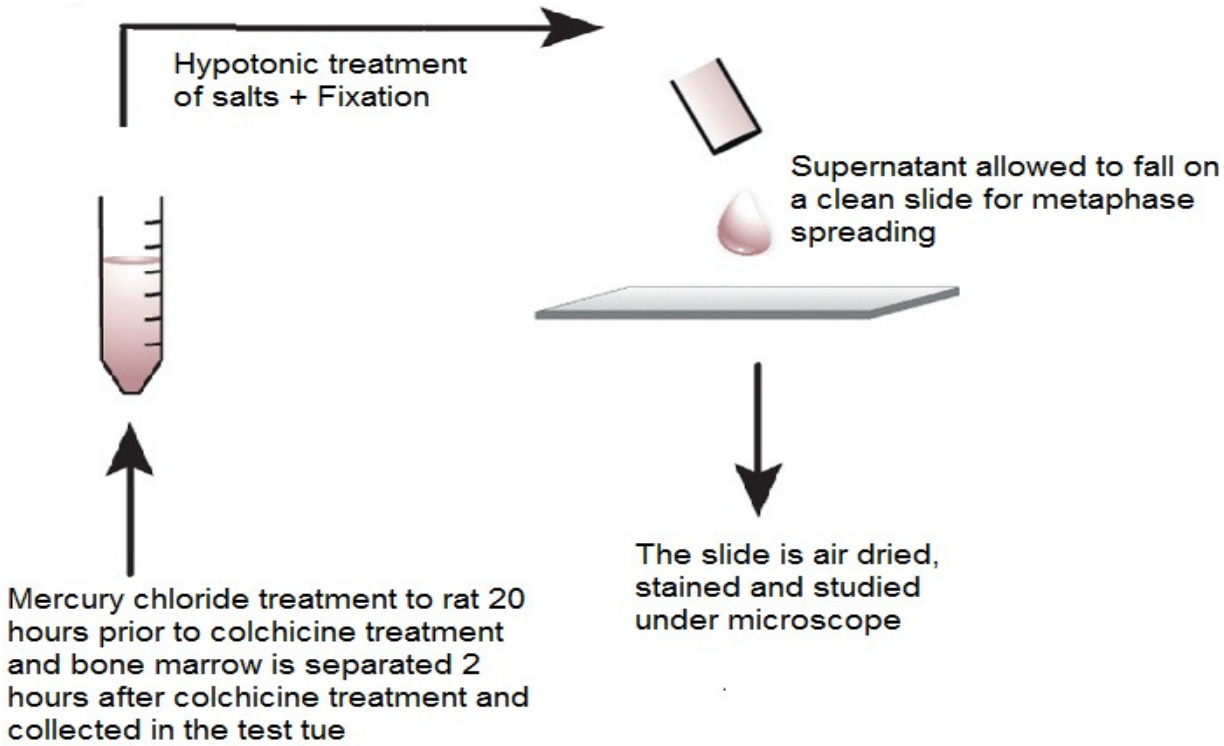

Figure-1
Methodology of karyotyping

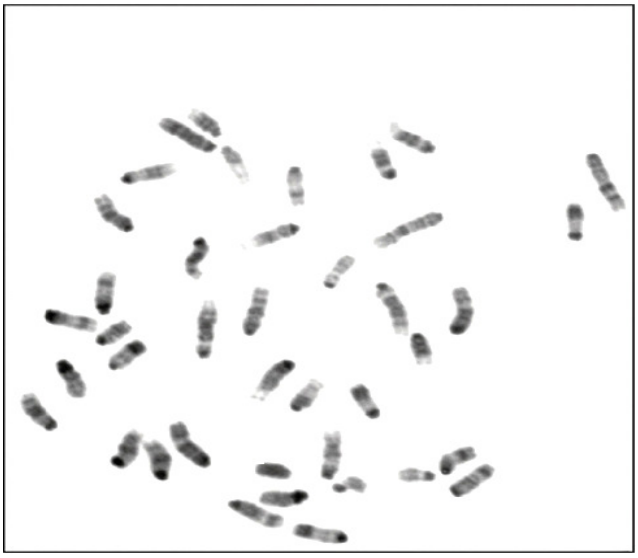

Figure-2
High magnification photomicrograph showing size of different chromosomes and distribution of chromatin material of control rat (*Rattus norvegicus*) in the bone marrow cells

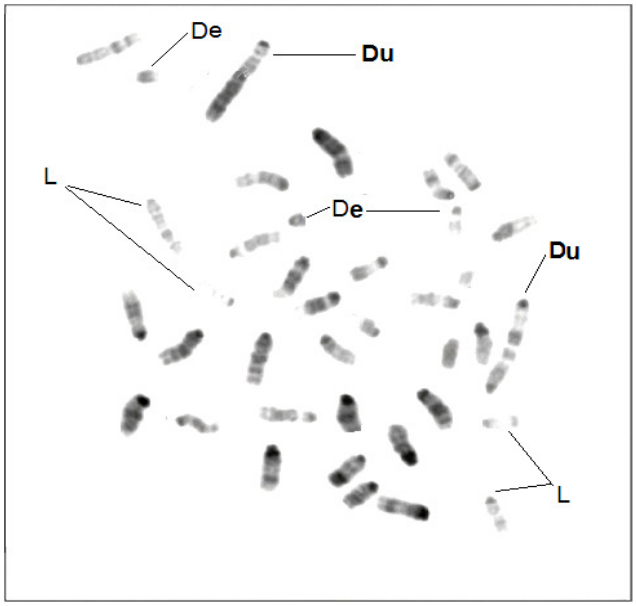

Figure-3
High magnification photomicrograph of chromosomes of $HgCl_2$ treated Albino rat (*Rattus norvegicus*) showing both reduced number of chromosomes, loosening of chromatin and structural changes in the bone marrow cells. (L=Loosening of chromatin, De=Deletion, Du= Duplication)

Conclusion

It is concluded that mercuric chloride is a dreadful poison which is toxic even at very low doses and causes a number of genotoxic effects in the experimental animals. The cytogenetic studies conducted during experimentation establish that in normal karyotype of rat there are 21 pairs of chromosomes. This number is decreased in the rat treated with mercuric chloride. The chromosomal aberration due mercuric chloride includes deletion, duplication and other numerical alteration and chromatin disturbances.

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