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Assessment of Chromosomal Aberration in the Bone Marrow Cells of *Swiss albino* Mice treated by Mancozeb

Kriti Tripathi, Wasim Raja and Sarfaraz Hanfi

Department of Cytogenetics, Genesis Institute of Biomedical Research,
Bhopal-460 024, India

Abstract: Mancozeb is widely used Dithiocarbamate fungicide and a polymeric complex of ethylenebis (Dithiocarbamate) manganese with zinc salt which is used as a fungicide to control the various diseases of seeds, fruits, vegetables, etc. In this study the effect of mancozeb at three dose levels 250, 500 and 750mg/kg body weight was found to be not mutagenic as compared to positive control (Cyclophosphamide) in bone marrow cells of *Swiss albino mice*. Single i. p. administered of cyclophosphamide (50 mg/kg) as a positive control induced different types of chromosomal aberrations in the bone marrow cells of *Swiss albino* mice. The mancozeb at the ¾, ½ and ¼ of Maximum Tolerated Dose (MTD) induced significantly prevented chromosomal aberrations in bone marrow cells of mice. The significant inhibition in chromosomal aberrations was noticed with Mancozeb at 250 mg/kg b. wt. Therefore, seems to have mancozeb not so mutagenic as compared to cyclophosphamide in Swiss bone marrow cells.

Key words: Cyclophosphamide · Mancozeb · Bone marrow · Mutagenicity · Chromosome

INTRODUCTION

Mancozeb is a polymeric complex of zinc and manganese salts of ethylenenebisdithiocarbamate (EBDC). It is commonly used for foliar application and seed treatment in agriculture. Mancozeb is grayish yellow powder that is stable under normal storage condition, but decomposes at high temperature due to moisture and acid [1]. Despite its low acute toxicity Mancozeb has been shown to produce significant toxicological effects on thyroid, gonads in male rats and chromosomes of bone marrow cells in mice [2, 3]. Exposure to Mancozeb causes normocytic type of anaemia, significant decrease in blood glucose and globulin levels and significant pathological changes in liver, kidney, spleen and heart [4, 5]. It has been reported that the administration of a fungicide sodium N-Methyl dithiocarbamate inhibits the secretion of lutenizing hormone thus affecting ovulation in rat [6]. No teratogenic effects were observed in a three generation rat study with mancozeb at a dietary level of 50mg/kg [7]. In a three –generation rat study with mancozeb at a dietary level of 50mg/kg/day there was reduced fertility but no indication of embryonic effects [7, 8]. Mancozeb was found to be mutagenic in one set of tests, while in another it did not cause mutations [8]. Mancozeb is practically nontoxic via the oral route with reported oral LD50 of greater than 5000mg/kg to greater than 11,200mg/kg in rats [7, 9]. No toxicological effects were apparent in rats fed dietary doses of 5mg/kg/day in a long-term study [7]. Therefore, we undertook the evaluation of preventive effect of Mancozeb using chromosomal aberration assay.

MATERIAL AND METHODS

Animals: The random breed, 6-7 weeks old male *Swiss albino* mice of weight 25 ± 2 gm body were used in the study. These mice were maintained under controlled conditions of temperature (25±2°C) and light (12 light: 12 dark). They were fed on standard mice feed procured from Brook Bond Lipton India Limited, Calcutta and water was given *ad libitum*.

Chemicals: Mancozeb was purchased from Bharat Pulverizing Mill Bombay India in formulation containing 75% technical grade of mancozeb and 25%, inert material. The cyclophosphamide was purchased from sigma chemical Co., U.S.A. and other chemical were procured locally.

Corresponding Author: Kriti Tripathi, Department of Cytogenetics, Genesis Institute of Biomedical Research, Bhopal-460 024, India. Mobile: +09425447174.

Chromosomal Aberration Assay: For chromosomal assay, three dose of Mancozeb i.e. 250, 500 and 750mg/kg b.wt were administered. Mancozeb were dissolved in double distilled water and administered as single dose in 0.2 ml per mouse i.p. to 6 animals.

Control mice, 6 in number were administered an equal volume of vehicle alone. The positive control group also received a single i. p. injection of 50 mg/kg CP in 0.9% saline. After 24 hr of given dose, the animals were sacrificed by cervical dislocations and bone marrow cells were harvested. Colchicine (4mg/kg b.wt.) was administered intraperitoneally 2 hr before the harvest of the cells. The slides prepared essentially as per modified method of Preston, *et. al.* (1987) [10]. Briefly, femur bones were excised and the bone marrow extracted in 0.56% KCL. The harvested cells were incubated at 37°C for 20 mins. and then centrifuged for 10 mins. at 1000 rpm. Cells were fixed in Carnoy's fixative (methanol:acetic acid = 3:1) and bursed opened on clean slides to release chromosome. The slides were stained with 5% Giemsa solution for 15 mins and then put in xylene and mounted with DPX. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1000 X (100 X 10) for each group. Different types of chromosomal aberration such as chromatid breaks, gaps, centromeric association, etc. were scored and expressed as % chromosomal aberrations. The statistical significance was determined using Student's 't' test.

RESULTS

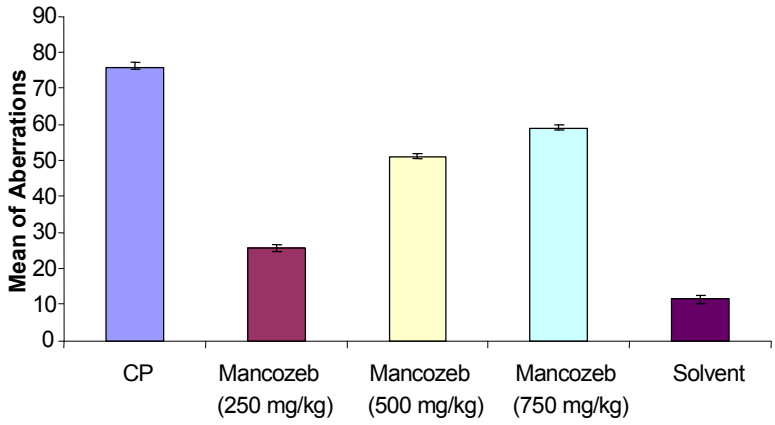
Data summarized in Table 1 the single intraperitoneal (i. p.) administration of 250, 500 and 750 mg/kg b. wt. dose of mancozeb failed to induce chromosomal aberrations after 24hr of exposure as compared to the control group. Whereas the single exposure of cyclophosphamide induced significantly different types of chromosomal aberrations. The degree of protection was 66.76, 32.72 and 22.33% respectively. A statistically significant ($p<0.05$) protection was observed with all the dose levels tested. In the positive control group cyclophosphamide induced different types of the chromosomal aberrations at the dose level tested. All kinds of observed aberrations like Breaks, Gaps, Fragmentations, Ring formation and Associations were found to be protected (Pic..1).

The mancozeb at ¾, ½ and ¼ of Maximum Tolerated dose (MTD) induced significantly different types of chromosomal aberrations in bone marrow cells of *Swiss* mice after 24hr of treatment.

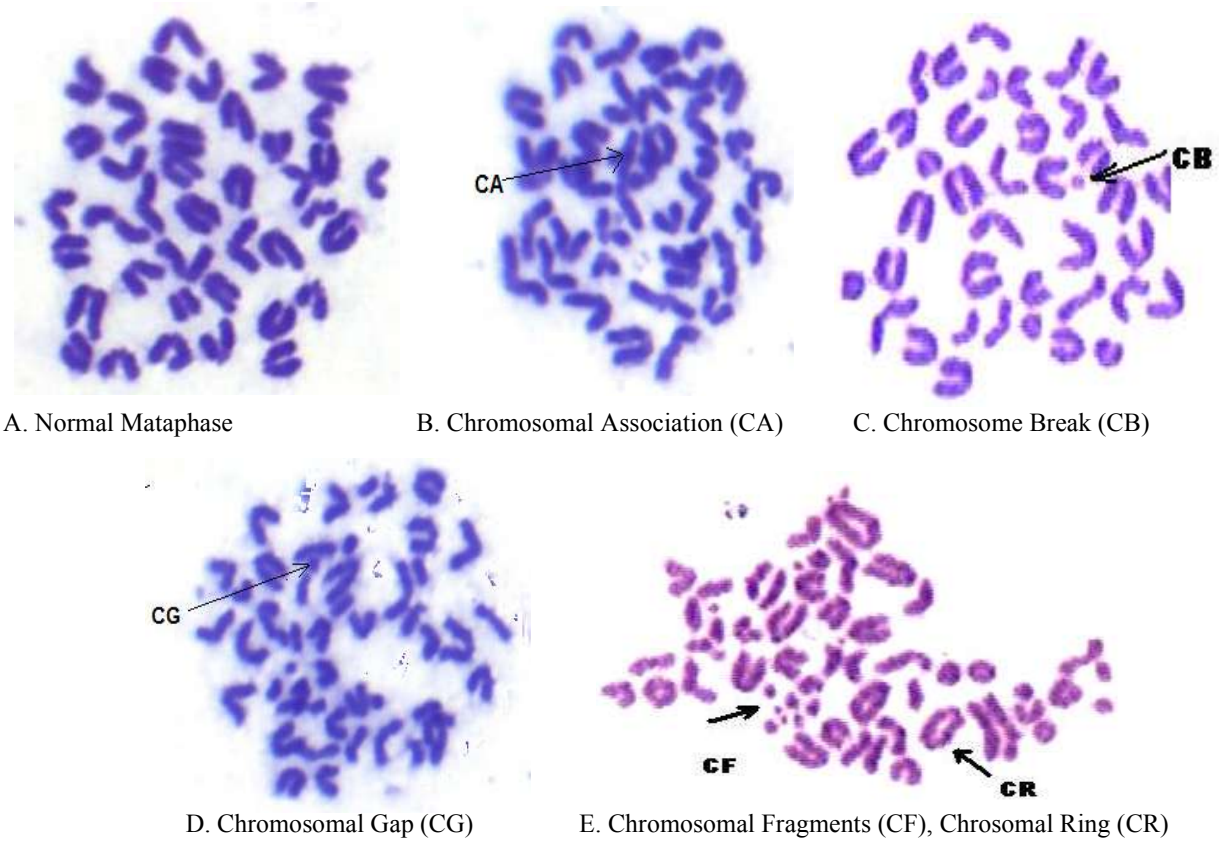
Table 1: Effect of Mancozeb in Chromosomal Aberration in Mice Bone Marrow Cells

			Different aberration in %					
S.N.	*Groups*	*Mean ± SE*	*Chro. Frag.*	*Chro. Break*	*Chro. Gap*	*Chro. Ring*	Chro.Asso.	*% Inhibi-tion*
1.	Cyclophosphamide 50 mg/kg	76.12 ± 0.25	44	2.12	11	6	13.0	-
2.	Mancozeb 250 mg/kg	25.30 ± 0.28*	11	4.90	1.3	2.34	5.46	66.76
3.	Mancozeb 500 mg/kg	51.21 ± 0.22*	35.0	2	5	3	5	32.72
4.	Mancozeb 750 mg/kg	59.12 ±0.31*	39	2	5	6	7	22.33
5.	Solvent DDW	11.33±1.20	4.33	5.00	2.00	Nil	Nil	-

*denotes statistically significant as compared to cyclophosphamide group at $p<0.05$. Each group having 6 animals.



Grp. 1: Showing the effect of Mancozeb in mean value ± SE of Chromosomal aberration in bone Marrow cells of *Swiss albino* mice



A. Normal Mataphase B. Chromosomal Association (CA) C. Chromosome Break (CB)

D. Chromosomal Gap (CG) E. Chromosomal Fragments (CF), Chrosomal Ring (CR)

Pic. 1 Photograph showing different types of chromosomal aberrations

DISCUSSION

Mancozeb is a fungicide widely utilized against wide variety of fungi. It has been known the use of pesticides has caused potential toxicity to biological system. Mancozeb metabolites, Carbon disulphide and Ethylene thiourea are involved in dysfunction of the nervous system [7]. In this study, results suggest that mancozeb does not cause mutagenicity in bone marrow cells of mice. The similar types of results were found by other workers [11-13]. Some studies were performed in other laboratories to assess the genotoxic potential of mancozeb but they failed to conclude it as a mutagenic compound due to conflicting results [12, 13]. Whereas few studies suggest the carcinogenicity of the compound [14-16]. It seems that non mutagenicity of the compound is not casually related with carcinogenicity of this compound. Researchers were found to be several carcinogens have non mutagenic effect.

Despite its low acute toxicity mancozeb has been shown to produce significant toxicological effects on thyroid, gonads in male rats and chromosomes of bone marrow cells in mice [2, 3]. It has been reported that the mancozeb is regarded as weak mutagen and has some carcinogenic activity in mouse [5]. Because of the importance of this compound and the number of people potentially exposed such as workers engaged in the production and use of the fungicide, people living in agricultural areas where compound is sprayed and people consuming polluted products. Therefore, the present study is too immensely because Mancozeb has been one of the most commonly used fungicides in commercial use.

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