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ORIGINAL ARTICLE

Mutagenic Effect of Ziram after Oral Exposure on Albino Rat

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ABSTRACT

Ziram is a dithiocarbamate fungicide used in agriculture field. Present study was undertaken to evaluate the genotoxicity of fungicide on non-targeted mammals, by measuring chromosomal aberration (CA) in bone marrow of Albino rats. LD_{50} was estimated by log-dose/ probit regression line method and found to be 1305.00 mg/kg.b.wt. Ziram dose equivalent to LD_{25} was administered orally for acute (1and 2 day) treatment and LD_5 for chronic (15, 30, and 60 days) treatment to rat through gabage tube. Control was also run simultaneously for each treatment with same quantity of vehicle (ground nut oil). Genotoxicity of Ziram has been found significant after chronic exposures, while non-significant changes were observed after acute and subacute exposures. This mutagenic property of Ziram might be due to its ability to cause degenerative changes in cellular and nuclear materials.

Key words: *Genotoxicity, Zirum, Chromosomal aberration, Albino rats, LD_{50}*

INTRODUCTION

In agriculture, fungicides are used to improve food production and to protect fruits, vegetables during storage and also applied directly to plants, trees, crops, cereals (Gupta, and Aggarwal, 2007) but causing systemic poisoning in humans (Mortazavi and Jafari- Javid, 2009). The important class of fungicides for controlling the fungi of agricultural crops was known to be ziram. It is commonly used as a protective fungicide either alone or in combination.

Despite its low acute toxicity, ziram a fungicide has been shown to produce several adverse effects on human and animal health, when repeated exposure can alter various functions (Ahmed, *et al.,* 2017). Reports are available on the toxicity of fungicides on the skin, liver, kidney, central nervous system, male and female reproductive system, and chromosomes of bone marrow cells in mice, rats and human (Edwards *et al.,* 1991; Georgian, *et al.,* 1983; Baligar and Kaliwal, 2001; Bindali and Kaliwal, 2002; Nordby, *et al.,* 2005; Domico, *et al.,* 2006; Tsang and Trombetta, 2007). In the present study the induction of structural chromosomal aberration *in vivo* in rat bone marrow by ziram has been investigated to confirm its genotoxicity.

MATERIALS AND METHODS

Wistar albino rats [*Rattus norvegicus* (Berkenhout)] have been selected from inbred colony. Healthy adult rats of almost equal size and weight (120±5) irrespective of sexes were selected randomly. The rats were maintained in polypropylene cages and acclimatized at temperature 25±5ºc, relative humidity 60±5% and a photoperiod of 12 hr/day. Each polypropylene cage was regularly cleaned and maintains proper hygiene. The rats were provided food and water *ad.libitum.* Ziram [zinc bis (dimethyldithiocarbamate)] was purchased from FIL Industries Ltd. Jammu in powder form and a solution was made in warm ground nut oil.

LD_{50} of ziram has been estimated by log-dose/probit regression line method given by Finney, (1971). Clastogenesity has been assessed after acute (1 and 2 days) and sub-chronic (15, 30 and 60 days) treatments. LD_{25} dose(694.77mg/kg b.wt.) was introduced orally through gavage tube for acute treatment, once and effect was observed after 24 hr and 48hr. LD_5 dose (284.31mg/kg b.wt.) was introduce for sub-chronic 60 days treatment and effect was observed after 15th, 30th and 60th days exposure. Ground nut oil was used as vehicle. Controls run for each treatment with same amount of vehicle *i.e.* ground nut oil. Recovery assessment for acute and sub-chronic treatment was carried out for 7 and 45 days simultaneously. Rats were sacrificed by chloroform anesthesia.

Bone marrow was isolated from femur by the method proposed by Heddle (1973). Chromosomal aberration assay was done as described by Preston *et. al.* (1987), incorporating cholchicine treatment, slide preparation through harvesting, hypotonic treatment, fixation and staining in giemsa. The metaphase scoring was done after acute (1 and 2 days) and sub-chronic (15, 30 and 60days) treatment exposures and 7 days acute and 45 days sub-chronic recovery. The chromosomal abnormalities were considered which includes chromosome and chromatid breaks and fragments of untraceable origin. Chromosome and chromatid gaps were also recorded but not included among aberration in the final evaluation. Percentage of aberration and frequency of aberrant cells have been calculated. The experimental data were analyzed for mean value and standard error (mean ± SE) for all groups, and comparison made by one way parametric ANOVA and followed by HSD Tukey test.

Table 1: *Chromosomal aberration* Estimation in Bone Marrow of Rat after Acute and Subchronic Ziram Intoxication and Recovery

S.No.	Treatment	Dose mg/kgb.w.	No. of individuals treated	Treatment time (days)	No. of Cells / animals	Chromosomal aberration					Total			% of aberration without gap		Frequency of aberrant cell	
						Gap		Break		Fragments	Without Gap	With Gap	No of aberrant cell	Mean± S.E.	Significance	Mean± S.E.	Significance
						ct	cs	ct	cs								
1.	Control	-	5	01	250/5	1	0	1	0	1	2	3	2	0.80±0.65		0.008±0.006	
2.	Acute	694.7	5	01	250/5	2	1	1	0	4	5	8	5	2.00±0.90	P<0.05↑	0.020±0.009	P<0.05↑
3.	Control	-	5	02	250/5	1	0	1	0	1	2	3	2	0.80±0.55		0.008±0.005	
4.	Acute	694.7	5	02	250/5	2	1	2	1	2	5	9	4	2.00±0.63	P<0.05↑	0.016±0.006	P<0.05↑
5.	Control	-	5	7	250/5	1	0	1	0	1	2	3	2	0.80±0.44		0.008±0.004	
6.	Recovery*	-	5	7	250/5	1	1	2	0	1	3	5	3	1.20±0.68	P>0.05↑	0.012±0.004	P>0.05↑
7.	Control	-	5	15	250/5	0	0	1	0	1	2	2	2	0.80±0.33		0.008±0.003	
8.	Sub-chronic	284.3	5	15	250/5	1	1	1	0	3	4	6	4	1.60±0.45	P<0.05↑	0.016±0.004	P<0.05↑
9.	Control	-	5	30	250/5	1	0	1	0	1	2	3	2	0.80±0.65		0.008±0.006	
10.	Sub-chronic	284.3	5	30	250/5	2	1	1	1	3	5	8	5	2.00±0.36	P<0.05↑	0.020±0.006	P<0.05↑
11.	Control	-	5	60	250/5	1	0	1	1	2	4	5	4	1.60±0.55		0.016±0.005	
12.	Sub-chronic	284.3	5	60	250/5	2	2	3	1	4	8	12	7	3.20±0.43	P<0.01↑	0.028±0.004	P<0.01↑
13.	Control	-	5	45	250/5	1	1	1	0	1	2	4	2	0.80±0.25		0.008±0.002	
14.	Recovery*	-	5	45	250/5	1	0	2	1	1	4	5	4	1.60±0.65	P<0.05↑	0.016±0.006	P<0.05↑

Dose was given once and effect was observed after 48 hours. *= Recovery assessment was carried out for 7 day and 45 days soon after 60 days treatment. ↑ = increase, ↓= Decrease, S.E. = Standard Error. ct=Chromatid break, cs= Chromosome break

RESULTS AND DISCUSSION

The rats of experimental sets were treated with different conc. of ziram and mortality numbers with percentage of rats for each dose were noted after 96 hours. The mortality percentage showed a corresponding increase with the increased dose of ziram. The calculated value of LD_{50} for ziram is 1305.00 mg/kg body weight. The toxicity of ziram was found to be dose dependent.

Percentage of aberrations (without gap) have been calculated and compared with their respective control value for significance level (table I). Increase in the percentage of aberration in the order of ($p<0.05$) after acute (1 and 2 days) and sub chronic (15 and 30 days), while ($p<0.01$) after chronic (60 days) treatments. A non-significant ($p>0.05$) increase have been observed in the percentage of aberration after 7 and 45 days recovery. Result of the present study reveals that ziram caused significant increase of chromosomal aberration with compare to their control values in bone marrow of rats during chronic treatment. Finding indicates *in vivo* clastogenic and spindle poisoning action of ziram. The present finding is gain supported by findings of Mosswso *et al*. (1994) who reported clastogenic effect after ziram (fungicide) exposure on Chinese hamster cells. Ziram causes an increase in the number of chromosomal aberration in bone marrow cell of mice treated with 100 mg/kg oral dose (National library of medicine, 1993).

This mutagenic property of Ziram might be due to its ability to cause degenerative changes in cellular and nuclear materials. Hydrolytic enzymes may release from damaged lysosomes. These hydrolytic enzymes get entered in nucleus and may cause DNA damage by their digestive action inside nucleus which in turn leads to chromosomal aberration.

Genotoxicity of ziram has also been assessed after chronic dosing to reveal cumulative effect. Further recovery assessment is also observed for 7 days after acute treatment and 45 days after sub chronic treatment. Increase in percentage of chromosomal aberration has been found to be non-significant ($p>0.05$). The decrease in aberration frequency with recovery assessment is probably due to non-availability of the critical concentration of genetically reactive metabolites of ziram at the target DNA molecule. A critical concentration of reactive metabolites of chemical compound in the target tissue or cell is extremely important for the production of any mutation (B.E. Matter (1976).

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