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Research Article

EVALUATION OF CYTOTOXICITY OF DICHLORVOS PESTICIDE IN LABORATORY MOUSE

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ABSTRACT
An assessment of cytological and cytogenetic effects was undertaken using laboratory mice as an experimental model. Dichlorvos was tested employing micro-nucleus test, mitotic index and chromosomal abnormalities. The frequency of poly-chromatic (P) and normo-chromatic (N) erythrocytes and presence of micro nuclei in tested animals showed that this pesticide has distinct cytotoxic effects. The results of this study suggested that dichlorvos is a genotoxic agent. Analysis of the mitotic index both in the male and the female mice corroborated the result of micro nucleus test. The in-vivo evaluation of bone marrow chromosomes for the presence of chromosomal aberrations indicated that the pesticide induced structural abnormalities and cells with such abnormalities were distinctly higher in treated animals that in controls. On the basis of these observations it has been summarized that dichlorvos should be graded as cyto and geno toxic agent.

Key Words: *Mouse, Dichlorvos, Mitotic Index, Chromosomal Abnormalities, Cyto and Geno Toxic*

INTRODUCTION
Today pesticidal drugs are playing an important role in enhancing the food production. The use of pesticides substantially reduces losses of food during various stages of food production storage and distribution. These measures have contributed in increasing production ion farm animals also but pesticides have been held responsible in increasing the toxic pesticide burden in animals owing to residues. Further residues of the pesticides through the food chain are translocated into other non target organisms including man.

Many constituents present in the pesticide exert deleterious effects resulting in toxicity, pathological alterations, morbidity and mortality.

These may affect the various stages of cell cycle and even cause profound genetic changes. There are numerous reports on the presence of pesticidal residues in agricultural produce, animal feed, milk, egg, fish, human tissue, etc. (Agnihotri, 1983; Rangaswami, 1983 and Goel, 1986). It has been established that certain pesticides or their metabolites cross the placental barrier and reach the neonate. Therefore, a study on the effects of pesticides on animals particularly from the standpoint of genotoxic effects assumes special importance. The objective of this paper is to focus on these changes, particularly cytotoxicity and chromosomal profile.

MATERIALS AND METHODS
The cytological and cytogenic effects of commonly used pesticide dichlorvos were studied in laboratory mouse (*Mus musculous*) which was used as an experimental model. Three dose levels 5, 10, 15 ppm were injected in the animals of both sexes which formed group 1, 2, 3. The animals of the group 4 were administered EMS-150 and served as positive control. The fifth group was not given and treatment and served as control.

Somatic chromosomes were prepared from the flushed bone marrow cells of the animals which were injected colchicin to arrest the cell division at metaphase stage. Chromosomal slides were prepared following the hypnotic treatment, air drying and staining with Giemsa.

Bone marrow smears of the various groups were scored for the frequency micronuclei in the polychromatic (P) and normochromatic (N) cells. The ratio of these was established.

The good metaphase plates were carefully examined for the incidence of chromosomal aberrations. The chromosomal anomalies were duly characterized.
The data have been presented on mitotic index, incidence of micro nuclei and chromosomal aberrations in the tables.

RESULTS AND DISCUSSION

A preliminary assessment of cytological and cytogenic effects of the dichlorvos revealed that the ratio of polychromatic and normochromatic cells are almost identical in all the three treated groups which received progressively higher doses of pesticide but markedly different in the positive control group which was given EMS (150 ppm).Ems is a known mutagen and a cytotoxic agent .
The incidence of micronuclei was observed in the polychromatic and normochromatic cell which suggests a disturbance in mitotic cycle.

Table 1: Frequency Of Micronucleus In Bone Marrow Erythrocytes Of Dichlorvos Treated Mice

Group	Dose (ppm)	Total cells counted	No of p. cells	No of N. cells	P/N ratio	MN in P cells		MN in n cells		Overall frequency of cells with micronucleus (%)
						No	%	no	%	
I	5	1172	571	601	0.95^{NS}	3	0.53	3	0.49	0.51
II	10	1185	568	617	$0.92^{N€S}$	4	0.71	2	0.32	0.51
III	15	1143	548	595	0.92^{NS}	5	0.91	2	0.34	0.61
IV	EMS 150	1230	493	737	0.67^{*}	16	3.24	11	1.49	3.01^{**}
V	_	1120	577	543	1.06	4	0.69	1	0.18	0.45

*MN: Micronucleus; ** Significant At 1 % Level; * Significant At 5 % Level; NS: Non Significant*

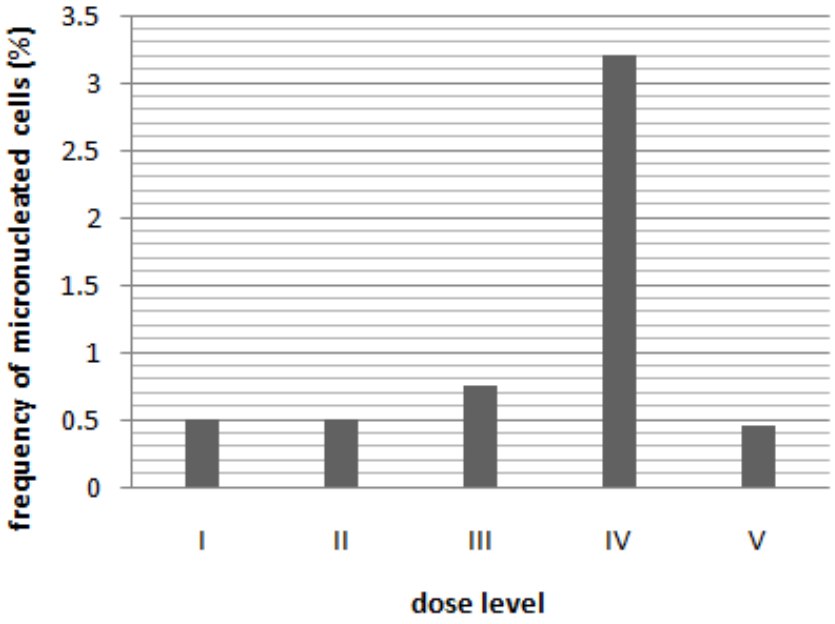

Figure 1: Micronucleus test with Dichlorvos

Mitotic activity was affected as was evident from the reduction in mitotic index in dichlorovos treated groups, the effect was dose dependent.

Table 2: Mitotic Index of Bone Marrow Cells of Mice Treated With Dichlorvos

Drug	Sex	Group	dose	No of cells counted	Mitotic index
Dichlorvos	Female	I	5	2000	19^{NS}
		II	10	2000	18.15^{NS}
		III	15	2000	17.75^{**}
		IV	EMS 150	2000	11.00^{**}
		V	Untreated	2000	19.55
	Male	I	5	2000	18.85^{NS}
		II	10	2000	17.15^{*}
		III	15	2000	17.00^{**}
		IV	EMS 150	2000	9.75^{**}
		V	untreated	2000	19.65

*** Significant At 1% Level; * Significant At 5% Level; NS: Non Significant*

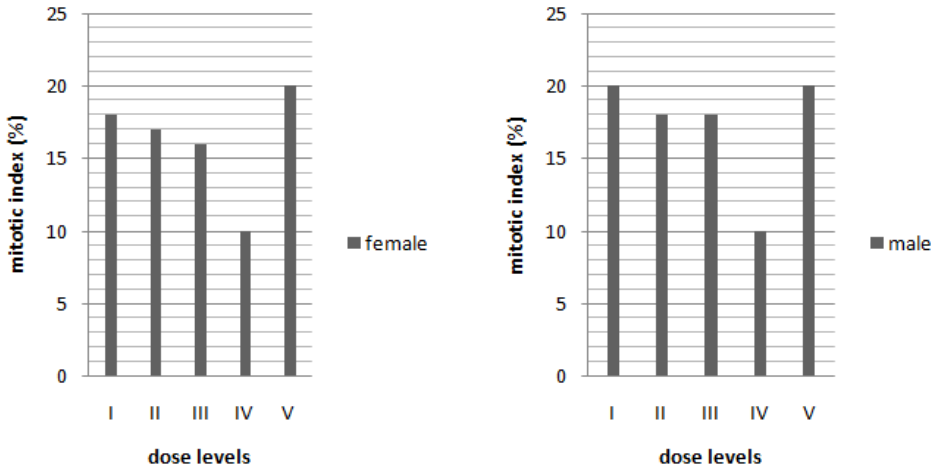

Figure 2: Mitotic index for dichlorvos treated mice

The types of chromosomal abrasions which were scored included chromatid gaps, chromatid breaks, iso-chromatic breaks, acentric fragments ,centromeric breaks and multiple gaps.

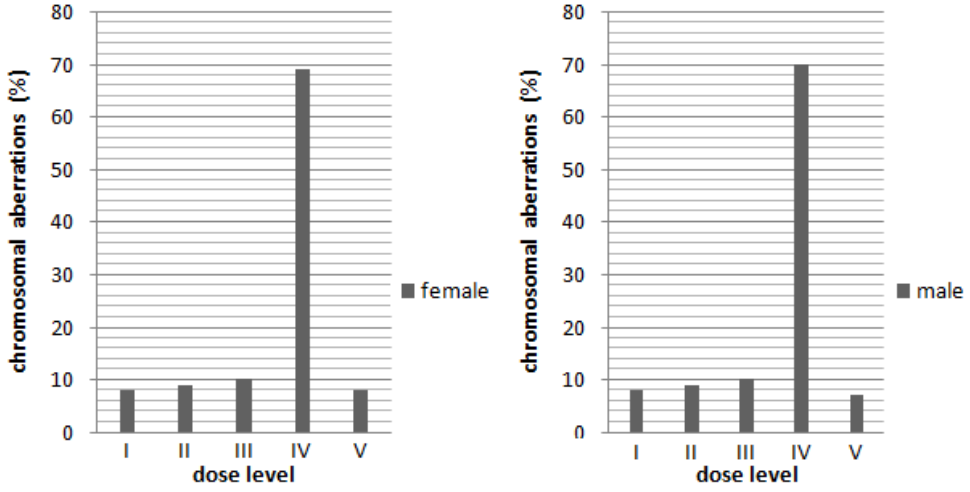

Figure 3: chromosomal aberration test with dichlorvos

Table 3: frequency of chromosomal aberrations in dichlorvos treated mice

Sex	group	Dose (ppm)	Cell counted	Total aberration	Type of chromosomal aberrations							CENT B	MULT GAP
					CG	CB	ICG	ICB	ACF	PULV	POLY		
Female	I	5	200	6.5^{NS} (13)	2.5 (5)	0.5 (1)	1.0 (2)	0.5 (1)	1.0 (2)	-	-	1.0 (2)	-
	II	10	200	7.5^{NS} (15)	2.0 (4)	1.0 (2)	1.5 (3)	-	1.0 (2)	-	-	2.0 (4)	-
	III	15	200	8.0^{NS} (16)	1.5 (3)	1.5 (3)	1.0 (2)	0.5 (1)	1.0 (2)	-	-	2.5 (5)	-
	IV	EMS 150	200	67.00** (134)	4.0 (8)	1.5 (3)	1.5 (3)	1.0 (2)	1.5 (3)	35.5 (71)	-	11.5 (23)	10.5 (21)
	V	Untreated	200	3.5 (7)	1.0 (2)	0.5 (1)	1.0 (2)	-	-	-	-	1.0 (2)	-
Male	I	5	200	7.0^{NS} (14)	1.5 (3)	1.0 (2)	2.0 (4)	-	0.5 (1)	-	-	2.0 (4)	-
	II	10	200	7.5^{NS} (15)	2.0 (4)	1.5 (3)	2.0 (4)	-	1.0 (2)	-	-	1.0 (2)	-
	III	15	200	8.5^{NS} (17)	2.5 (5)	1.5 (3)	1.0 (2)	0.5 (1)	1.5 (3)	-	-	2.0 (4)	-
	IV	EMS 150	200	70.5** (141)	5.5 (11)	1.5 (3)	2.5 (5)	1.0 (2)	2.0 (4)	32.5 (65)	-	9.0 (18)	16.5 (33)
	V	Untreated	200	5.0 (10)	1.0 (2)	1.0 (2)	1.5 (3)		0.5 (1)	-	-	1.0 (2)	-

** Significant in 1% level*

The pesticide affects cells and cellular processes. However, the changes may remain imperceptible for a long time. It has been reported that certain pesticides on entering the body suppressed the mitotic activity of the cells. The decrease in the proliferation is sometimes dose dependent (Fahrig, 1974; Bruin, 1976; Manna and Vardhan, 1977 and Manna, 1986).

The dichlorvos also exerted a mito-inhibitory effect on the bone marrow cells of the mice.The micro nucleus test is a vital indicator in the screening of cytogenetic effects (Doller and Schmid, 1970; Degraebe *et al.*, 1979). Both polychromatic and normochromatic erythrocytes of the bone marrow exhibit the presence of the micro-nuclei. It was also established that micro-nuclei are legging fragments of the chromosomes affected by mutagen during erythropiosis.

The publications on the damage to the morphology of the chromosomes as a result of the pesticide exposure are numerous. The frequency and type of abrasion presumably depends on the dose level and duration of exposure to the pesticide. (Hardel, 1979; Hardell and sandstorm, 1979; Czeizel *et al.*, 1975)

The presence of chromosomal abrasions affirms the clastogenic effect of dichlorvos.

This study makes an endeavor to use multiple tests to assess the genotoxicity of dichlorvos. This approach is likely to surmount the possibility of false positive or false negative result encounteres when only one test system is used.

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