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CLASTOGENICITY OF LABORATORY MIXTURE OF LINDANE AND CARBARYL IN MICE

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ABSTRACT

A laboratory mixture of two insecticides, viz., carbaryl and lindane (1:1) was tested for its clastogenicity in the bone marrow erythrocytes of swiss albino mice. The insecticidal mixture was injected intraperitoneally at three dose levels as a suspension in 1% gum acacia, and chromosome aberrations assay and micronucleus test were performed in both sexes separately. Ethylmethanesulphonate (EMS) was taken as positive reference mutagen. An increase in the frequency of micronucleated erythrocytes was observed following treatment with the test chemical mixture. In chromosome aberrations assay, a significant induction was observed at 185 mg/kg bw. at 24h post-treatment time. The female mice appear more tolerant to the test mixture than the male mice.

Key words : Clastogenicity, lindane, carbaryl, mice, micronuclei, chromosome aberrations, erythrocytes

Use of pesticides in agriculture, though a necessity, poses a great danger to environment and human health. Many of them, in addition to being toxic, have mutagenic, carcinogenic and teratogenic effects. It is thus essential to evaluate such chemicals for their genotoxicity. In the present study, clastogenicity of a mixture of two widely used insecticides, lindane or gamma-HCH (58-89-9) and carbaryl (63-25-2) (commercially available as Sevidol) was tested.

The two constituents of Sevidol have been individually tested for their mutagenicity, but contradicting reports are available. Carbaryl was found to be mutagenic in *Vicia faba* [1], but showed negative results in dominant lethal assay in swiss mice [2]. However, meiotic chromosomes were found to be damaged by carbaryl

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in mice [3]. Lindane caused chromosome aberrations in human lymphocytes and rat fibroblasts in vitro [4] but was non- mutagenic in the bone marrow cells of rats [5].

Sevidol dissolved in dimethylsulphoxide (DMSO) has been reported to induce micronuclei in the bone marrow erythrocytes of mice [6]. It is thus essential to confirm the mutagenic effects of Sevidol in an inert solvent such as aqueous gum acacia. Hence this study was conducted to determine the genotoxicity of mixture of lindane and carbaryl dissolved in aqueous solution of gum acacia.

MATERIALS AND METHODS

Healthy, disease free Swiss albino mice (body weight 20-25 g, age 6-8 weeks) procured from the animal house of Haryana Agricultural University, Hisar, were grouped in different cages (five animals/cage) isolating males and females. The test chemical was prepared by mixing carbaryl and lindane in equal proportion by weight. Working solution was prepared afresh each time by suspending in 1% gum acacia in distilled water, and volumes not exceeding 0.1 ml were injected intraperitoneally (i.p.) into mice. DMSO and chloroform were found not to be suitable due to their toxicity to mice at concentrations necessary for dissolving the test chemical. Ehylmethanesuphonate (EMS, 180 mg/kg b.w.) was taken as a positive control and 1% gum acacia in distilled water as the solvent control. Test doses were selected on the basis of LD50/24 (survival of 50% animals 24 h after treatment) Twenty animals per dose in three replicates were used for this purpose. The mean LD50/24 was found to be 160.65 mg/kg b.w. for male and 186.74 mg/kg b.w. for female mice. The three test doses taken for the clastogenicity tests were 185 (LD50/24), 90(1/2LD50/24) and 45(1/4LD50/24)mg/kg b.w.

Cytological studies

I. *Chromosome aberrations* : Animals were pre-treated with colchicine (6mg/kg b.w.) 2h prior to sacrificing them. The chromosome spreads were prepared by standard procedure [7] and cells with chromosome breaks, chromatid breaks, multiple breaks and rings scored. The polyploid cells were scored separately.

II. *Micronucleus test* : A treatment routine of two injections, 24h apart, was followed. The animals were sacrificed 6h after the second injection, and cells were stained with Giemsa and May-Grunwald. The slides were scored independently by two investigators. One thousand polychromatic as well as normochromatic erythrocytes were scored for the presence of micronuclei from each animal, and results were subjected to students' t-test. The ratio

of polychromatic to normochromatic erythrocytes (P/N ratio) was calculated in order to determine the cytotoxicity of test chemical.

RESULTS AND DISCUSSION

The mean LD50/24 values of the test mixture in the two sexes were interestingly different, the female mice showing more tolerance than the male mice. We took the LD50 dose of female mice as the highest reference dose for the clastogenicity tests.

A significant difference was observed in the frequency of micronucleated erythrocytes between control and treated animals at all the doses tested, showing a dose-dependent increase up to the highest dose tested (185 mg/kg b.w.). The micronucleus frequency observed at this highest dose was comparable to that induced by EMS at 180 g/kg b.w. The ratio of polychromatic to normochromatic erythrocytes (P/N ratio) was unaffected at all the doses, although at 45 mg/kg dose, a slight but statistically insignificant decrease was observed. This implies lack of expression of any cytotoxic effect of the test mixture at the time point tested. Absence of suppression of erythropoiesis by the test mixture might have facilitated the early expression of micronuclei in the normochromatic erythrocytes observed at 30h

Table 1. Incidence of micronuclei (MN) and cytotoxicity in bone marrow erythrocytes of treated mice

Dose (mg/kg b.w.)	No. of animals		Polychromatic cells with MN (%)	Normochromatic cells with MN (%)	Polynormochromatic cells with MN (%)	P/N ratio
Sevidol						
45	5	M	1.35 ± 0.21*	0.82 ± 0.28*	2.17 ± 0.07*	0.73 ± 0.13
		F	1.05 ± 0.08*	0.75 ± 0.08*	1.80 ± 0.10*	0.73 ± 0.13
90	5	M	1.80 ± 0.30*	1.02 ± 0.15*	2.82 ± 0.13*	0.97 ± 0.11
		F	1.15 ± 0.11*	0.92 ± 0.15*	2.07 ± 0.13*	0.91 ± 0.14
185	5	M	3.-7 ± 0.24*	1.37 ± 0.16*	4.44 ± 0.30*	1.07 ± 0.08
		F	2.32 ± 0.25*	1.15 ± 0.28*	3.47 ± 0.17*	1.01 ± 0.06
EMS (180)	5	M	2.70 ± 0.20*	1.15 ± 0.15*	3.85 ± 0.34*	1.54 ± 0.12*
		F	2.25 ± 0.25*	1.10 ± 0.15*	3.35 ± 0.16*	1.54 ± 0.12*
Gum Acacia (1%)	5	M	0.12 ± 0.05	0.10 ± 0.01	0.22 ± 0.02	1.03 ± 0.18
		F	0.15 ± 0.08	0.11 ± 0.02	0.26 ± 0.02	1.02 ± 0.16

1000 cells scored per animal; M = Male; F = Female; *Significant at 5 per cent level.

post-treatment. A difference in the induction of micronuclei was observed between the two sexes, although it was statistically not significant. Yet it could be as a result of the moderately higher tolerance shown by female mice as observed in the toxicity tests conducted (data not presented).

The chromosome aberrations assay carried out in mice (Table 2) at the same dose-levels as in the micronucleus test suggests significant induction of chromosome abnormalities in both the sexes. In male mice, this frequency was 3.5%, while in female mice, it was 2.4% at 185 mg/kg b.w. dose. The lower freqency of chromosome aberrations in female mice at relatively higher dose is again suggestive of the difference in tolerance level of the two sexes observed in toxicity test as well as micronucleus assay.

Table 2. Incidence of chromosomal aberrations in the bone marrow cells of treated mice.

Dose (mg/kg b.w.)		Cells with chromosomal aberrations per 1000 cells				Total cells with chromosomal aberrations (%)	Polyploid cells (%)
		Chromosome breaks	Chromatid breaks	Ring chromosomes	Multiple breaks		
Sevidol							
45	M	6	8	6	0	2.0	1.1
	F	7	9	5	2	2.3	0.4
90	M	7	6	7	0	2.0	0.9
	F	6	6	4	0	1.6	0.5
185	M	11	13	8	3	3.5	1.0
	F	9	8	7	0	2.4	0.8
EMS (180)	M	14	17	9	4	4.4	1.5
	F	13	15	10	2	4.0	1.1
Gum acacia (1%)	M	0	1	2	1	0.5	0.2
	F	0	1	1	0	0.2	0.2

Number of cells scored = 4000; M = Male, F = Female

A generally milder genotoxicity response observed in female mice in the micronucleus test and chromosome aberrations assay supported by the higher LD

50/24 value calculated from the mortality data indicates an enhanced tolerance of female mice toward the test chemical as compared to the male mice. Moreover, as the human system is generally more sensitive to mutagens as compared to the mouse test system [8], the present study suggests that Sevidol be used with utmost care by workers to prevent excessive exposure since it has a mutagenic potential.

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