Genotoxic effects of dithane M-45 on the bone marrow cells of mice in vivo

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Summary. Genotoxic effects of dithane M-45 were studied on the bone marrow cells of male albino mice (Lacca strain) in vivo. Different doses (30 mg, 40 mg and 300 mg/kg b.wt) of dithane M-45 were injected intraperitoneally and their effects were investigated after time intervals of 1, 2, 5 and 10 days. The chromosomal aberrations observed in the bone marrow cells of male mice after treatment with dithane M-45 were fragments, rings, dicentric chromosomes, terminal chromatid deletions, chromatid gaps and breaks. In addition to these chromosomal aberrations, physiological effects such as uneven stretching of chromatin material, end-to-end chromosomal associations, exchange configurations, clumping, stickiness and centromeric associations were also observed.
Key words. Chromosomal aberrations; fungicide; dithane M-45.

Fungicides are being used extensively for the control of fungal diseases of agricultural and horticultural crops. Many workers have reported the cytotoxic/genotoxic effects of different fungicides on plant and animal systems[1-4]. Dithane M-45 is a broad-spectrum dithiocarbamate fungicide and a residual pollutant. As this fungicide is frequently used for the eradication of scab disease of apple, it is desirable to assess its genotoxic effects on the mammalian system.

The present study was conducted on the bone marrow cells of male albino mice, *Mus musculus* (Lacca strain) aged 8–10 weeks. Each individual weighed 25–30 g. The mice were divided into four groups of four animals each. The animals of three groups were treated with the fungicide, and those of the fourth group served as controls. The animals of each treated group were injected with 0.3 ml of one concentration of dithane M-45 solution, as given in table 1. Controls were injected with 0.3 ml of distilled water.

Chromosomal preparations of bone marrow cells were prepared according to the method of Scribner et al.[5]. Well-spread plates were observed for chromosomal aberrations.

The diploid chromosome number in *Mus musculus* is 40. Results obtained in the present investigations (table 2) indicate that all tested doses of the fungicide dithane M-45 (30 mg, 40 mg and 300 mg/kg, b. wt) induce chromosomal aberrations in the bone marrow cells of mice. The different types of chromosomal aberrations observed are fragments, centromeric associations, exchange configurations (fig. 1); dicentric chromosomes, terminal chromatid deletions, isochromatid gaps (fig. 2); chromatin stretching (fig. 3); rings (figs 2,4); chromatid gaps and breaks, and hypo- and hyperdiploidy (table 2). Similar types of chromosomal aberrations after treatment with fungicides benomyl and vitavax have recently been reported in the bone marrow cells of rat[6].

As dithane M-45 is an ethylene-bis-dithiocarbamate compound, its reactivity is associated with the N-H bond

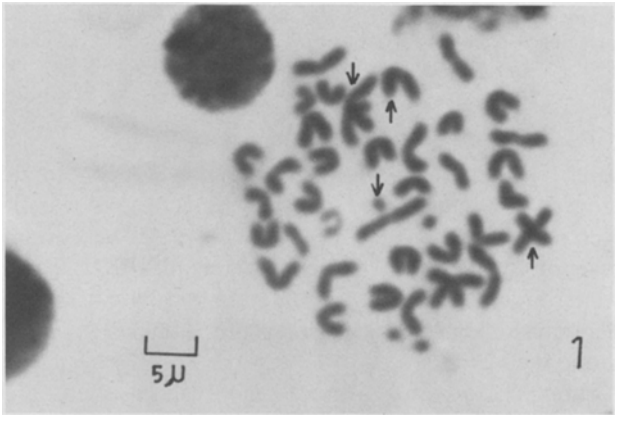

Figure 1. Metaphase plate showing centromeric association, terminal chromatid deletion and fragments (30 mg/kg b.wt, after two days of treatment).

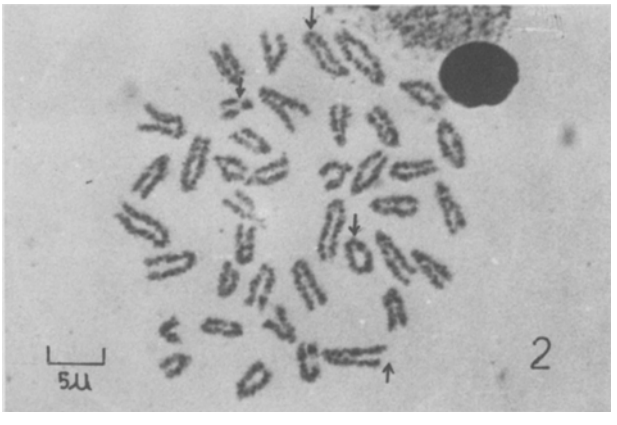

Figure 2. Metaphase plate showing dicentric chromosomes, rings, isochromatid gap and terminal chromatid deletion (40 mg/kg b. wt, after 1 day of treatment).

Table 1. Doses of dithane M-45 and post-treatment study intervals

Dose in mg/kg b.wt	Post-treatment study interval (in days)
30	1,2,5 and 10
40	1,2,5 and 10
300	1,2,5 and 10
Control	1,2,5 and 10

Table 2. Effect of dithane M-45 on the chromosomes of bone marrow cells of mice. In the control, no chromosomal aberrations were recorded. ChFr-Chromosomal fragments (MB)-Multiple breaks); RC-Ring chromosomes; DC-Dicentric chromosomes; TChtd De-Terminal chromatid deletions; Chtd Ga-Chromatid gaps; Chtd Br-Chromatid breaks; T-Total.

Dose (mg/kg b.wt intraperitoneally)	Post treatment interval (in days)	Metaphase plates observed	Number of metaphase plates showing chromosomal aberrations						
			ChFr (MB)	RC	DC	TChtdDe	ChtdGa	ChtdBr	T
30	1	50	2	2	1	1	4	3	13
	2	50	2	2	1	2	6	2	15
	5	50	2	1	–	1	5	4	13
	10	50	–	1	–	–	2	1	4
40	1	50	3	2	2	3	4	–	14
	2	50	2	3	2	2	5	–	14
	5	50	3	3	–	3	4	3	16
	10	50	1	1	–	2	1	2	7
300	1	50	4	6	–	4	11	3	28
	2	50	5	3	–	4	3	4	19
	5	50	3	2	–	1	4	1	11
	10	50	3	2	–	–	5	–	10

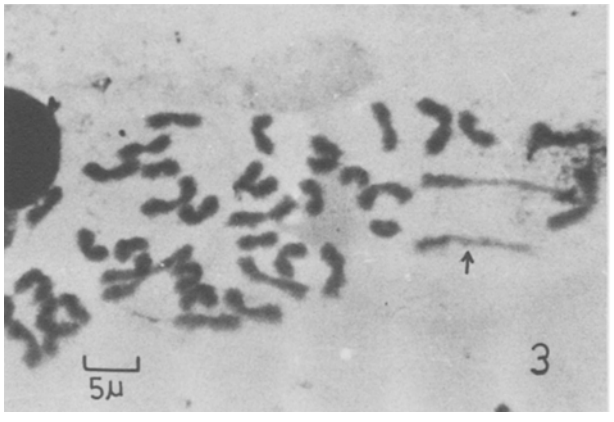

Figure 3. Metaphase plate showing chromatin stretching (40 mg/kg b.wt, after 10 days of treatment).

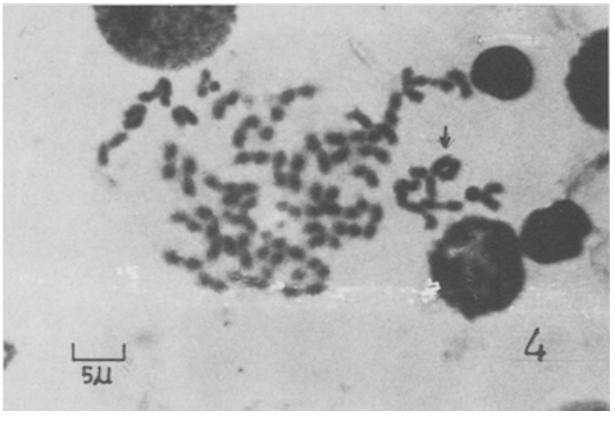

Figure 4. Metaphase plate showing rings (300 mg/kg b. wt, after 5 days of treatment).

present in it. Its main site of action is on enzyme systems, where it reacts readily with sulphydryl groups on the enzymes. Epstein and Legator[7] were of the view that some carbamates, after their metabolic activation to N-hydroxy-carbamates, cause inactivating DNA alterations, which can result in chromosomal breaks and other alterations.

The frequencies of aberrations observed in the present study after 1 and 2 days of treatment were almost the same (table 2). A decrease in the frequency of aberrations after 5 and 10 days of treatment may be due to the fact that cells with severe chromosomal damage might have been deleted in the cell cycles immediately following the treatment. Ring chromosomes (figs 2,4) were clearly seen at all doses and after all the time intervals. Ring formation is probably due to the breakage of chromatids and fusion of the broken ends. Most of the rings observed had the centromere in the middle region.

It was observed that the most vulnerable or sensitive region in the chromosomes appeared to be either the heterochromatic region of the centromere or the telomere. Centromeric fusion and centromeric interactions leading to the formation of star-shaped or X-shaped configurations were frequently observed after all the time intervals studies, with all the different doses of dithane M-45 (fig. 1). The exchange configurations and centromeric associations indicate the high sensitivity of the centromeric regions to the fungicide treatment. In some cases stretching of centromeric regions was also observed. The erratic behaviour of chromosomes with all the doses of dithane M-45 resulted in an imbalance of chromosome number, i.e. hypo- or hyperdiploidy. The occurrence of more hypodploid cells as compared to hyperdiploid cells may be because the highly-affected chromosomes might have been deleted, which would have resulted in the production of hypodiploid cells in the next cell division. Gaps of the chromatid type were also observed for all the doses. The genetic significance of gaps is not clear, but they are clear indicators of the genotoxic potential of the chemicals[8].

Chromatin stretching, clumping and stickiness were also induced by dithane M-45. According to Bhunya and Behera[3], such effects indicate that the fungicide may have acted upon the protein moiety of the chromosomes.

Badr [1] reported chromosomal stickiness to be a major abnormality produced by dithane M-45 and denmart in root tip cells of *Allium cepa*. Fragments of both the centric and the acentric type were observed in the present investigation. The acentric fragments originated either as terminal or interstitial deletions of chromosomes, as is clearly indicated by the unequal length of the chromosomes. It is assumed that fragments frequently undergo reunion with the broken ends of the chromatid arms to form various configurations. Acentric fragments were rare, which shows that most of the fragments either undergo fusion, or get lost in the following cell cycles. Nandi [2] reported fragmentation of chromosomes in root tips of *Allium cepa* treated with ceresan and agrosan GN. Similar breaks were also reported in the root tip cells of *Allium cepa* after treatment with denmart and dithane M-45 [1].

From the preceding discussion, it is clear that the fungicide dithane M-45 induced a number of different types of chromosomal aberrations in the bone marrow cells of male mice at the doses tested in the present investigations.

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Chromosome-breaking activity of extracts of the mushroom *Paxillus involutus* Fries ex Batsch

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Summary. Dry and presoaked seeds of *Nigella damascena* were treated with aqueous extracts of the mushroom *Paxillus involutus*. At the first mitosis after the onset of germination, metaphase chromosomes showed damage independent of the origin of the mushrooms. The damaging substance(s) is (are) thermostable. Except a few achromatic gaps, all the lesions observed are of the chromosome type, i.e. are induced at the pre-synthetic G1 stage.
Key words. Chromosome damage; mushroom extracts; *Paxillus*; *Nigella*.

Although some poisonous properties of mushrooms have been known since far into antiquity, the carcinogenic and mutagenic potentialities of some extracts, even those from some edible kinds such as *Agaricus bisporus* or *Gyromitra*, have only recently been suspected [1, 2].

These findings were promptly confirmed for the genus *Lactarius* [3] especially in the commercial pickled species *Lactarius necator* [4]. In this last species, a substance named necatorin showed a high mutagenicity [5]. The occurrence of such mutagens/carcinogens is probably more widespread than was originally expected; for example they exist in several families of Basidiomycetes, and various alteragenic molecules have been already isolated [6].

In these studies, the mutagenicity of extracts was always demonstrated in an Ames' test, using *Salmonella typhimurium* strains with or without S9 mix. This fraction seems to be able to deactivate the mutagens. The fact that clastogenic potentialities have not been investigated prompted new experiments in this field.

Although the species *Paxillus involutus* Fries ex Batsch was considered edible for a long time, more recent, not infrequent cases of poisoning have allowed the identification of a *Paxillus* syndrome, found to be mainly due to a phenol derivative named involutin, the chemical and pharmacological properties of which are now well known [7]. In fact, preliminary experiments with *Paxillus* extracts on plant cells [8] clearly suggested an effect at the chromosome level in higher organisms, and suggested that it would be worthwhile to extend the experiments.

Material and methods

Nigella damascena (var. blue Miss Jekyll) seeds were used to test the clastogenicity of extracts. The special advantages of this plant material for clastogenic studies have been previously described [9]. Mushrooms were harvested in September-October in various habitats avoiding external contaminations such as leaves, fungi, etc., and kept at −20 °C till the beginning of experiments. To prepare extracts, thawed mushrooms were first sliced, then pounded in a mortar in a 0.14 M NaCl sterile solution (at 22 °C), filtered and kept at 4 °C.

To test the thermostability of potential mutagens, extracts were heated in an oven (80 °C/5 h) or evaporated (50 °C for 4 days) before being cooled down to room temperature. After treatment, (100 seeds in 5 ml extract, 5 h at 22 °C), seeds are allowed to germinate under stan-