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GENOTOXIC EFFECT OF HOSTATHION IN MICE

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SUMMARY

The genotoxic potential of technical hostathion was studied in the in vivo mouse system. The test parameters used were chromosomal aberrations assay and mitotic index in bone marrow cells; and meiotic chromosomal aberrations assay and meiotic index in germinal cells. Three dose levels (2.8, 4.2 and 5.6 mg/kg. b.wt.) were used for 3 and 5 days subacute treatments after oral administrations. Chromosome aberrations were observed in bone marrow cells and in diakineses-metaphase I cells from the testes. The dose and time yield effects statistically analysed. The results demonstrated that hostathion induced a significant structural chromosomal damages in somatic and germinal cells as well as peridiploidy of numerical aberrations in somatic cells which were dose- and time dependent at all dose levels. A dose dependent significant decrease was observed in mitotic and meiotic indices of mice cells. It was concluded that hostathion has genotoxic effects. Therefore, the use of this pesticide in our life must be restricted.

INTRODUCTION

Hostathion is an important organophosphorous pesticide widely used in Egypt to control sucking and biting pests which attack many agricultural crops such as cotton, vegetables and fruits. Like other organophosphorous pesticides, hostathion is an alkylating compound, besides the presence of the biological active trizolyl group (hetercyclic nucleus) of configuration similar to biomolecules (Gomaa et al., 1979) and therefore could be mutagenic/ carcinogenic (de Hondt et al., 1989; Abdel Aziz et al., 1993; Sierra- Torrea et al., 1998;

Gomes et al., 1999). To our knowledge, the genotoxicity of technical grade of hostathion was not previously known. However, active and formulated trizophous has been investigated in a few number of test system, although the results in mammalian system have led to contradicting conclusions. According to the available literature two reports indicated to clastogenic effects induced by formulated and active trizophous in bone marrow cells of rats and mice respectively (Sherif, 1983 and Sharaf et al., 1990). On the other hand, negative results were found by Pilinskaya et al., (1980), who reported that active trizophos did not induce chromosomal aberrations in mammalian cells. In Drosophila melanogaster, Velazquez et al., (1990) observed that the treatment with trizophous led to a weak increase in the non-disjunction frequencies compared with the control, but it gave negative results in the total and partial sex chromosome losses. As we mentioned before, there is an amazing lack of genotoxicity studies on technical hostathion grade and contradictory cytogenetic reports on formulated and active trizophous of hostathion pesticide. So, the present study is carried out to screen the genotoxicity of hostathion (technical grade 40 H).

Hostathion has been tested for it's ability to induce chromosomal aberrations in the bone marrow and spermatocyte cells as well as testing mitotic and meiotic activities of male mice following in vivo oral administration.

MATERIAL AND METHODS

Hostathion was used in its commercial form (40%). The different concentrations used were prepared by emulsification in water. Male swiss mice (Mus musculus) weighing about 25 grams obtained from Egyptian Organization for Biological Products and Vaccines were used. Animals were kept in light and temperature controlled room with food and water supplied ad libitum.

Hostathion was administrated orally at three dose levels, 2.8, 4.2 and 5.6 mg/kg b.wt. representing, low, median and high doses. The used doses are 1/20, (1/20 + 1/10)/2 and 1/10 LD50 , where the LD50 dose was determined in the present study to be as 56 mg/kg (using the methods of Abdel Aziz et al. ,1993). The doses of hostathion were given daily for two different times 3 and 5 consecutive days. A group of five animals was used for each treatment, in addition to an untreated group of five animals which served as control. Animals were sacrificed 24 hours after the last injection. Chromosomes from bone marrow and spermatocyte cells were prepared following the methods of Yosida and Amano (1965) and Brewen and Preston (1978) respectively. 50 metaphases were studied/ animal for scoring different types of aberrations. The mitotic and meiotic indices of bone marrow and spermatocyte cells respectively were investigated by recording the number of dividing cells/1000 cells /animal.

Statistical Analysis :

The experiment followed complete randomized design (C.R.D.). The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran, (1980) using Mstat- C programme. Least signficant differences (LSD) were used to compare between means of treatments according to Walter and Duncan (1969) at probability 5%.

RESULTS

The results of the cytogenetical examination in bone marrow and spermatocyte cells of mice injected orally with the three tested doses of hostathion (2.8, 4.2 and 5.6 mg/kg. b.wt. are listed in tables (1-6). Chromosome aberrations and depression of mitotic and meiotic indices were observed .

In somatic cells, chromosome aberrations consisted of structural and numerical ones. Structural aberrations included chromatid gaps (Fig. 1) , breaks, and deletions, as well as chromosomal gaps, centromeric attinuations and endomitosis . Numerical aberrations were peridiploidy and polyploidy . In germinal cells, only structural aberrations were observed and represented by X-Y univalents (Fig. 2), autosomal univalents and breaks. Results of mutagenicity testing revealed a significant increased of chromosomal aberrations in bone marrow (Table 1) and germinal (Table 4) cells after treatment with different dose levels of hostathion. For bone marrow, the significant structural aberrations were in the forms of chromatid gaps, chromatid breaks (only at high dose), deletions, centromeric attinuations and endomitosis as well as total structural aberrations. The significant numerical aberrations were peridiploidy. For germinal cells, the significant structural aberrations were in the forms of X-Y univalents and autosomal univalents as well as total structural aberrations.

The effect of time showed that chromatid gaps, endomitosis and total structural aberrations as well as peridiploidy in bone marrow (Table 2) and autosomal univalents and total structural aberrations in spermatocyte cells (Table 5) increased significantly with increasing the time.

The effect of interaction between doses and times showed that the total structural aberrations and peridiploidy of somatic cells (Table 3) as well as total structural aberrations and autosomal univalent of germinal cells (Table 6) were significantly increased as the dose and time increased (except of the low dose treatment for 3 days). The maximum number of aberrated cells was reached after using the high dose for 5 days.

Cells with more than one aberrations in somatic (Table 1) and germinal (Table 4) cells showed a slight dose dependent increase. This increase was statistically non - significant (at low dose) and

Table (1): Mean percentages and statistical analysis of chromosomal aberrations in bone marrow cells of mice treated with different doses of hostathion.

Dose (mg)	No. of examined animals	No. of examined cells	Structural aberrations						Total structural aberrations	Numerical aberrations		Total numerical aberrations	C.W.A.	Mitotic index
			Chromatid type			Chromosome type				Peridi-ploidy	Poly-ploidy			
			Gap	Break	Deletion	Gap	Centromeric attenuation	Endomitosis						
0	5	250	0.20^{d}	0.00^{b}	1.00^{c}	0.20	0.6^{b}	0.00^{b}	2.00^{d}	2.00^{c}	0.00	2.00^{c}	0.00^{c}	65.80^{a}
Low	10	500	1.10^{c}	0.50^{b}	2.10^{b}	0.40	1.30^{ab}	0.40^{ab}	5.80^{c}	3.70^{b}	0.30	4.20^{b}	0.20^{cb}	49.90^{b}
Medium	10	500	2.80^{b}	0.60^{b}	2.50^{b}	0.40	1.50^{a}	0.70^{a}	8.40^{b}	4.50^{a}	0.40	4.90^{ab}	0.60^{ab}	43.70^{c}
High	10	500	3.80^{a}	2.70^{a}	1.70^{a}	0.30	2.00^{a}	0.90^{a}	13.00^{a}	4.90^{a}	0.20	5.10^{a}	0.80^{a}	36.80^{d}

- Statistical analyses of results were done according to Duncan's multiple range tests.
- Means with different letters within each column are significant at 5% level.
- C.W.A.: Cells with more than one aberration.

Table (2): Mean percentages and statistical analysis of chromosomal aberrations in bone marrow cells of mice treated with hostathion at different times.

Time (Days)	No. of examined animals	No. of examined cells	Structural aberrations						Total structural aberrations	Numerical aberrations		Total numerical aberrations	C.W.A.	Mitotic index
			Chromatid type			Chromosome type				Peridi-ploidy	Poly-ploidy			
			Gap	Break	Deletion	Gap	Centromeric attenuation	Endomitosis						
3	15	750	1.70^{b}	0.75	2.20	0.20	1.20	0.30^{b}	6.35^{b}	3.40^{b}	0.30	3.80	0.50	50.90^{a}
5	15	750	2.25^{a}	0.65	2.45	0.45	1.50	0.70^{a}	8.25^{a}	4.15^{a}	0.15	4.30	0.30	47.20^{b}

- Statistical analyses of results were done according to Duncan's multiple range tests.
- Means with different letters within each column are significant at 5% level.
- C.W.A.: Cells with more than one aberration.

Table (3): Mean percentages and statistical analysis of chromosomal aberrations in bone marrow cells of mice treatedwith hostathion (interaction between doses and times).

Time (Days)	Dose (mg)	No. of examined animals	No. of examined cells	Structural aberrations						Total structural aberrations	Numerical aberrations		Total numerical aberrations	C.W.A.	Mitotic index
				Chromosome type			Chromosome type				Peridi-ploidy	Poly-ploidy			
				Gap	Break	Deletion	Gap	Centromeric attenuation	Endomitosis						
3	0	5	250	0.20	0.00	1.00	0.20	0.60	0.00	2.00^{d}	2.00^{c}	0.00	2.00	0.00	65.80
	Low	5	500	0.40	0.60	2.20	0.20	0.80	0.00	4.20^{cd}	3.20^{bc}	0.40	4.00	0.00	53.60
	Medium	5	500	2.60	0.80	2.00	0.20	1.40	0.60	7.60^{c}	4.00^{ab}	0.60	4.60	0.80	45.20
	High	5	500	3.60	1.60	3.60	0.20	2.00	0.60	11.60^{b}	4.40^{ab}	0.20	4.60	1.20	39.00
5	0	5	250	0.20	0.00	1.00	0.20	0.60	0.00	2.00^{d}	2.00^{c}	0.00	2.00	0.00	65.80
	Low	5	500	1.80	0.40	2.00	0.60	1.80	0.80	7.40^{c}	4.20^{ab}	0.20	4.40	0.40	46.00
	Medium	5	500	3.00	0.40	3.00	0.60	1.60	0.80	9.20^{bc}	5.00^{ab}	0.20	5.20	0.40	42.00
	High	5	500	4.00	1.80	3.80	0.40	2.00	1.20	14.40^{a}	5.40^{a}	0.20	5.60	0.40	34.60

- Statistical analyses of results were done according to Duncan's multiple range tests.
- Means with different letters within each column are significant at 5% level.
- C.W.A.: Cells with more than one aberration.

Table (4): Mean percentages and statistical analysis of chromosomal aberrations in spermatocytes of mice treated with different doses of hostathion.

Dose (mg)	No. of examined animals	No. of examined cells	Structural aberrations			Total structural aberrations	C.W.A.	Meiotic index
			X-Y Univalents	Autosomal Univalents	Breaks			
0	5	250	0.60^{d}	1.00^{c}	0.20	1.80^{d}	0.00^{c}	33.8^{a}
Low	10	500	1.70^{c}	2.30^{b}	0.50	4.50^{c}	0.40^{bc}	28.9^{b}
Medium	10	500	3.90^{a}	2.30^{b}	0.40	6.60^{b}	0.80^{ab}	19.3^{c}
High	10	500	2.80^{b}	6.20^{a}	0.40	9.30^{a}	1.00^{a}	13.90^{d}

- Statistical analyses of results were done according to Duncan's multiple range tests.
- Means with different letters within each column are significant at 5% level.
- C.W.A.: Cells with more than one aberration.

Table (5): Mean percentages and statistical analysis of chromosomal aberrations in spermatocytes of mice treated with hostathion at different times.

Time (Days)	No. of examined animals	No. of examined cells	Structural aberrations			Total structural aberrations	C.W.A.	Meiotic index
			X-Y Univalents	Autosomal Univalents	Breaks			
3	15	750	0.20	2.55^{b}	0.40	4.60^{b}	0.65	24.55
5	15	750	2.50	3.65^{a}	0.35	6.50^{a}	0.45	23.40

- Statistical analyses of results were done according to Duncan's multiple range tests.
- Means with different letters within each column are significant at 5% level.
- C.W.A.: Cells with more than one aberration.

Table (6): Mean percentages and statistical analysis of chromosomal aberrations in spermatocytes of mice treated with hostathion (interaction between doses and times).

Time (Days)	Dose (mg)	No. of examined animals	No. of examined cells	Structural aberrations			Total structural aberrations	C.W.A.	Meiotic index
				X-Y Univalents	Autosomal Univalents	Breaks			
3	0	5	250	0.60	1.00^{d}	0.20	1.80^{c}	0.00	33.8
	Low	5	250	0.80	1.600^{cd}	0.60	3.00^{c}	0.80	30.0
	Medium	5	250	3.80	2.20^{cd}	0.40	6.40^{c}	1.00	19.8
	High	5	250	2.80	4.20^{b}	0.40	7.20^{b}	0.80	14.6
5	0	5	250	0.60	1.00^{d}	0.20	1.80^{c}	0.00	33.8
	Low	5	250	2.60	3.00^{bc}	0.40	6.00^{b}	0.00	27.8
	Medium	5	250	4.00	2.40^{cd}	0.40	6.80^{b}	0.60	18.8
	High	5	250	2.80	8.20^{a}	0.40	11.40^{a}	1.20	13.2

- Statistical analyses of results were done according to Duncan's multiple range tests.
- Means with different letters within each column are significant at 5% level.
- C.W.A.: Cells with more than one aberration.

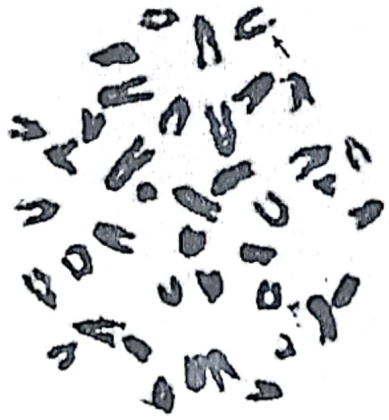

Fig. (1): Bone marrow metaphase spread of treated male mice showing a chromatid gap.

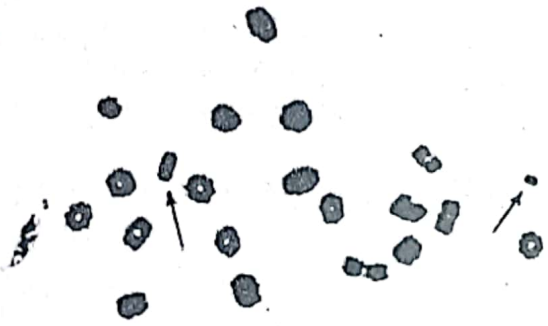

Fig. (2): Spermatocyte-1 showing x-y univalent in treated male mice.

significant (at median and high doses). However the aberrated cells decreased as the time increased (Tables 2 and 5), but this decreased was insignificant.

The effect of interaction between doses and times showed that cells with more than one aberrations were increased as the dose and time increased for somatic (Table 3) and germinal (Table 6) cells. These increases were statistically non-significant.

Generally chromosome aberrations due to the effect of hostathion were higher in somatic cells than in germ cells .

The mitotic index was significantly depressed due to the effect of all tested doses (Table 1) for all the times (Table 2) of the treatments.
Also, the meiotic index was significantly depressed at all tested doses (Table 4). The number of cell division was decreased as the time increased, but this decreased was statistically non - significant (Table 5).

The effect of interaction between doses and times showed that mitotic (Table 3) and meiotic (Table 6) activities were decreased as the dose and time increased . These decreases were statistically insignificant.

DISCUSSION

The present study showed that hostathion has induced chromosomal aberrations and depressed of mitotic and meiotic indices in mice cells. The induction of chromosome aberrations are in parallel with the results obtained from the experiment performed in vivo on rats and on mice using the pure chemicals of hostathion in formulated forms (Sherif, 1983) and active trizophos (Sharaf et al., 1990) respectively. However, those results can not be compared with those of the present investigation, because the used form of pesticide was different. On the other hand, the present results in somatic and germinal cells are in agreement with the experiments performed with other technical organophosphorous (OP) pesticides .

A significant increase of chromosomal aberrations was induced in somatic cells, of mice due to the effect of gardona (Amer and Ali, 1992), Pirimiphos- methyl (Abdel Aziz and El-Fiky, 1997) and Phenothoate (El -Nahas et al., 1997) compared with untreated groups.

Chromosome aberrations were also demonstrated in spermatocyte cells of mice after treatment with OP pesticides such as 3-methyl-4- nitrophenol (Nehez et al., 1985 b), methanidophos (Sun and Huang, 1988); Quinalphos (Rupa et al., 1991) and tamaron (Abdel Aziz et al., 1993).

The induction of chromosomal aberrations due to the effect of hostathion may be attributed to the biologically active trizolyl group (Gomaa, 1973 and Gomaa et al., 1979) and to the chemical

alkylating agents (Wild, 1975). Gomaa (1973) assumed that the decomposition product of trizophos (3-hydroxy-S-trizol) react with a biochemical radical to form a purine analogue to form DNA adduct leading to anomalies in the chromosomes as a result of disturbance of DNA replication. Also, Wild (1975) reported that the chemical alkylating agents of all organophosphates can interact with cellular DNA leading to its cytotoxic or genotoxic effects.

As indicated in the present results the mean values of chromosomal aberrations due to the effect of hostathion were higher in somatic cells than in germinal cells. Similar results were observed in mice cells after treatment with other organophosphorous insecticides such as curacron (Ramadan, 1986) and tamaron (Abdel Aziz et al., 1993). The decrease of chromosomal aberrations in germinal cells might be related to gonadal barriers which reduce the risk of exposure of germ cells against chemical and toxins compared to somatic cells (Russell, 1978).

In the present study the percentages of cells with more than one kind of aberrations (in somatic and germinal cells) showed a significant increase as the dose increase and in the same time decreased as the time increased, this could be explained on basis that such damaged cells were eliminated from the population (Schmid et al., 1971and Gomez - Arroyo et al ., 1987).

The depression in mitotic index which was observed in the present study due to the effect of hostathion occurred also after treatment with malathion insecticide (Balaji and Sasikala, 1993). Also, El-Nahas et al., (1997) found a significant depression in mitotic activity of maternal and embryonic cells of mice exposed to phenthoate.

The present study also, demonstrated that the treatment with hostathion has depressed of meiotic index. Similar results were also observed in rats and mice treated with Quinalphos (Ray et al., 1992) and dimethoate (Hoda and Sinha, 1993) respectively.

The observed dose and time - dependent depression of mitotic and meiotic activities in the present study may be attributed to the cumulative and cytotoxic effects of the insecticide (Al- Omar et al ., 1986 and Skaare et al ., 1988).

In conclusion, the present study, indicated that the hostathion has genotoxic effect and consequently it may have a potential risky effect on the health of human and animals. Therefore, the use of the pesticide against insects in agricultural field must be controlled.

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