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# *In Vitro* Effect of Fenthion on Human Lymphocytes

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Fenthion is an organophosphorus insecticide which is extensively used in control of leaf hoppers, cutworms, mites on vegetable crops. It has been reported that organophosphorus pesticides cause a significant increase in sister chromatid exchanges in mammalian cell lines (Nicholas et al. 1978, Chen et al. 1981). A significant increase of chromosomal aberrations has been reported in rural population exposed to pesticides (Paldy et al. 1987). Organophosphorus pesticides malathion, diazinon, dimethoate, phosdrin and dursban induced sister chromatid exchanges in human lymphoid cells (Sobti et al. 1982). Exchange type of aberrations has been reported in fluoriculturist who were exposed to organophosphorus, organochlorine pesticides (Dulout et al. 1985). In the present investigation an attempt has been made to evaluate the cytogenetic effect of fenthion in human lymphocyte cultures **in vitro**.

## MATERIALS AND METHODS

Intravenous blood was collected from healthy donors aseptically using heparin as anticoagulant. Lymphocyte cultures were initiated in RPMI 1640 medium supplemented with 20% human AB serum 0.5% phytohemagglutinin 0.1% dicrysticin. Fenthion (98%) technical grade was obtained from A.P.Agricultural University, Hyderabad. Cells were treated with 0.5, 1.5, 2.5 and 5.0 µg/culture medium for 24 and 48 hrs. of duration. The insecticide was dissolved in ethylalcohol. Control cultures were maintained simultaneously with and without ethylalcohol-control I & II respectively. All the cultures were incubated at 37°C. Colchicine was added two hours before harvesting the cultures. The method followed was that of Moorhead et al. (1960). A separate set of cultures were maintained for the study of sister chromatid exchanges (SCE's), Bromodeoxy uridine (Brdu) at concentration of 3 µg/ml was added to the cultures at the time of initiation. The cells were treated with the test compound for 24 and 48 hrs. duration were allowed to complete at least two cell cycles in the presence of Brdu. The cultures were harvested and slides were prepared according

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Table 1. Incidence of chromosomal aberrations in human lymphocyets treated with fenthion 24 and 48 hrs.

| Treatment µg/culture | | Total Metaphases scored | Chromatid Gaps | Chromatid Breaks | Iso chromatid Gaps | Iso chromatid Breaks | Fragments | Total No.of aberrations Excluding gaps | Aberration/ cell Excluding gaps |
|---|---|---|---|---|---|---|---|---|---|
| 24 hours | | | | | | | | | |
| Control | I | 200 | 2(1.0) | 2(1.0) | 0 | 0 | 0 | 2(1.0) | 0.01 |
| Control | II | 200 | 3(1.5) | 2(1.0) | 0 | 0 | 0 | 2(1.0) | 0.01 |
| 0.5 | | 200 | 5(2.5) | 4(2.0) | 0 | 2(1.0) | 0 | 6(3.0) | 0.03 |
| 1.5 | | 200 | 8(4.0) | 7(3.5) | 4(2.0) | 3(1.5) | 0 | 10(5.0) | 0.05* |
| 2.5 | | 192 | 4(2.1) | 7(3.6) | 6(3.1) | 5(2.6) | 2(1.0) | 14(7.3) | 0.07* |
| 5.0 | | 196 | 7(3.6) | 9(4.5) | 4(2.0) | 4(2.0) | 2(1.0) | 15(7.7) | 0.08 |
| 48 hours | | | | | | | | | |
| Control | I | 200 | 5(2.5) | 3(1.5) | 2(1.0) | 0 | 0 | 3(1.5) | 0.02 |
| Control | II | 200 | 4(2.0) | 4(2.0) | 0 | 0 | 0 | 4(2.0) | 0.02 |
| 0.5 | | 200 | 4(2.0) | 6(3.0) | 2(1.0) | 3(1.5) | 0 | 9(4.5) | 0.05* |
| 1.5 | | 200 | 6(3.0) | 8(4.0) | 6(3.0) | 4(2.0) | 0 | 12(6.0) | 0.06* |
| 2.5 | | 198 | 4(2.0) | 9(4.6) | 0 | 6(3.0) | 2(1.0) | 17(8.6) | 0.09* |
| 5.0 | | 190 | 8(4.2) | 10(5.7) | 2(1.1) | 4(2.1) | 4(2.1) | 18(9.5) | 0.09 |

* $p < 0.05$. Values in parentheses are percentages.

Table 2. Frequency of SCE in human lymphocytes treated with fenthion for 24 and 48 hrs.

| Treatment μ g/ml | No. of Metaphases scored | 24 hrs. treatment SCE/cell ± S.E.M. | 48 hrs. treatment SCE/cell ± S.E.M. |
|---|---|---|---|
| Control I | 50 | 5.02 ± 0.4 | 4.42 ± 0.2 |
| Control II | 50 | 4.68 ± 0.3 | 4.91 ± 0.5 |
| 0.5 | 50 | 6.92 ± 0.4 | 7.14 ± 0.3 |
| 1.5 | 50 | 8.0* ± 0.2 | 9.04* ± 0.6 |
| 2.5 | 50 | 8.46* ± 0.4 | 9.92* ± 0.5 |
| 5.0 | 50 | 10.02* ± 0.6 | 10.38* ± 0.3 |

* $p < 0.05$

to the method of Perry and Wolf (1974) and Moorhead et al (1960) for differential staining and chromosomal aberration respectively. All the experiments were repeated twice. One hundred metaphases were scored for chromosomal aberrations and twenty five metaphases for sister chromatid exchanges for each concentration and in each experiment. The significance of chromosomal aberrations was analysed using the chisquare test and for SCE's student's test was used.

## RESULTS AND DISCUSSION

The results on the incidence of chromosomal aberrations is given in table - I, and frequency of sister chromatid exchanges is given in table - II. The predominantly observed aberrations in this study were single chromatid gaps and breaks, suggesting that the chemical acts mostly in the late S or $G_2$ phase of the cell cycle. Chromatid gaps were found more frequently than iso chromatid gaps in all the treated groups. The significance of gaps is not very clear, but Stoain and Raicu (1975) have shown that gaps are produced due to loss of despiralization of both DNA & chromosomal protein. Quite possible such a phenomenon may in turn interefere with the normal functioning of the chromosomes. A higher aberration frequency was observed in chromosome groups A and B. This increase seems to be related to their large size. It has been reported that the large chromosomes suffer more damage than shorter chromosomes when exposed to a mutagenic agent (Promchainant 1975). Presence of fragments at higher concentrations reveals the increased mutagenic potential of the test compound. The higher incidence of sister chromatid exchanges at all concentrations reveals that the chromosomes are more sensitive for damage. It is assumed that the increase of SCE is a consequence of some kind of damage in DNA. The possible mutagenic nature of some organophosphates has been suggested by the work of Epstein and Legator (1971) who reported the mutagenicity of trimethyl phosphate a simple organophosphate. The reports on the mutagenicity of fenthion are few. Water et al (1980) reported negative effects of fenthion in salmonella, E.Coli and also sacchromyces. In contrast to this there are reports on positive effects of fenthion. Chen et al (1982) reported the induction of SCE in chinese hamster V 79 cells. The present study reveals that fenthion causes chromosome damage in human lymphocyte cultures.

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