CYTOGENETIC EFFECTS OF ORGANOPHOSPHATE PESTICIDES ON GOAT LYMPHOCYTES IN CULTURE

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Summary

Cytogenetic effects of malathion and parathion, the organophosphorus pesticides were studied on goat lymphocytes in culture. The mitotic indices (% of blast cells + cells at metaphase) of goat lymphocytes showed corresponding decrease with the increase in dose of pesticides. Malathion had significant effect only at 150 μg/ml or higher dosages while, parathion caused antimitotic effects even at the lowest dose (5 μg/ml) tested. The clastogenic effects of malathion were significant ($p \leq 0.05$) at 100 μg/ml. In higher doses, the effects were highly significant ($p \leq 0.01$). The frequency of metaphase plates with chromosomal abnormalities were highest (22.0%) at 200 μg/ml. The incidences of chromosomal abnormalities were significant ($p \leq 0.05$) in parathion treated series even at 5 μg/ml dose level. At 10 μg/ml or higher dose levels the difference between treatment groups and controls were more pronounced ($p \leq 0.01$). Various types of chromosomal abnormalities were encountered in goat lymphocytes treated by malathion and parathion. However, the incidence of gaps, breaks, acentric fragments, dicentric chromosomes were higher than other types of structural and numerical abnormalities.

(**Key Words** : Goat, Organophosphorous Pesticides, Mitotic Index, Chromosomal Aberrations)

Introduction

Goats like other domestic animals are prone to ticks, mites and other body pests in the tropics. Organophosphates have replaced in part the organochlorine pesticides in use in last three decades owing to their relatively quick biodegradation (Van Boa et al., 1974). Malathion, parathion and other organophosphates have often been accused of causing acute toxicity in man and animals, that may be fatal whereas the prolonged exposure at smaller doses cause genetic effects (Gupta and Sahai, 1982; Czeizel et al., 1987).

Mutagenic effects of parathion have been reported in rats in a multi-generation study by Gupta and Sahai (1982). The reduction in mitotic index and DNA labeling index of peripheral lymphocytes exposed to organophosphates have been reported by Petropolis and Kamra (1978). Various types of structural and numerical chromosomal aberrations have been reported in peripheral blood cultures of man, hamester and farm animals exposed to organophosphorus pesticides (Alam et al., 1974; Gupta et al., 1988, 1994).

In this paper, the cytogenetic effects of malathion and parathion on goat lymphocytes in culture have been presented.

Materials and Methods

Pesticides

Commercial grade parathion (Metacid-50) or O,O-dintrophenyl phosphotioate, MW 291), was obtained from Bayer India Ltd. Malathion (O,O-dimethyl phosphodithioate of diethyl mercaptic succinate, MW 330) was procured from The Pesticide India, Ltd. The pesticides were dissolved in hanks basal salt solution to get the following concentrations which have been worked out on the basis of their toxic levels determined in earlier studies.

Malathion : 500, 750, 1,000 and 1,250 μg/ml

Parathion : 50, 75, 100 and 125 μg/ml

Experimental design and sampling procedure

Peripheral blood samples were collected from goats maintained at National Dairy Research Institute, Karnal,

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India. The blood was collected in sterile heparinized vacutainers (Becton and Dickinson, USA), by jugular vein puncture using 21 g needle. In each experiment with malathion and parathion, the 40 samples were divided in to 5 groups of eight vials in each. Group I to IV were treatment groups and the group V served as control.

Experimental procedure

The culture medium TC 199 (Bios, India) was reconstituted using L-glutamine (Sigma, USA), Phytohemagglutinin (PHA, Sigma, USA), Sodium benzyl penicillin, streptomycin and sterile cattle serum. The media was filtered through the millipore filters (0.2 micron) into sterile culture vials (4.5 ml in each). To every culture 0.5 ml of whole blood was inoculated. The cultures were incubated for 72 h at 37°C. In the first experiment, 0.1 ml solution of malathion in above mentioned concentrations were added to the cultures of treatment group I, II, III and IV respectively to achieve the final dose level of 50, 100, 150 and 200 μg/ml of the medium. In the second experiment, 0.1 ml of parathion was added per vial to obtain the final dosages of 5, 10, 15 and 20 μg/ml in treatment groups I to IV respectively. In both experiments, the pesticide was added 24 h before harvesting the cultures. Group V cultures (control) in both the experiments remained untreated.

The cultures were harvested by adding colchicine (250 μg/ml) and incubated for 1 hr. The chromosomal slides were prepared by using the hypotonic solution (0.075 M KCl), acetic acid: methanol (1 : 3) fixative and air drying protocols described earlier (Gupta et al., 1988). The slides were stained with 2% Giemsa (Phosphate buffer, pH 6.8) for 30 min. and mounted in DPX.

Analytical and statistical methods

The chromosomal slides were examined under phase contrast microscope in oil immersion to identify and classify the types of chromosomal aberrations. The frequency of blast cells and cells at metaphase were recorded to study the mitotic index of peripheral lymphocytes in all the treatment groups as well as in controls. The data were analysed to determine the differences between control and treatment groups using χ^2 test at 5 and 1% levels of significance (Snedecor and Cochran, 1967)

Results

Effects of pesticides on mitotic index of lymphocytes

The mitotic index of peripheral blood lymphocytes was expressed as percentage of blast cells and cells at metaphase or anaphase. The figure 1 shows a corresponding decrease in the mitotic index of goat lymphocytes in culture with increase in the dose of malathion and the differences between the treatment groups and control were significant ($p \leq 0.05$) at 100 μg/ml or higher dosages. In contrast, the parathion caused significant ($p \leq 0.5$) effects even at 5 μg/ml (table 1). At 10 μg/ml and higher doses, the differences between treatments and control were highly significant ($p \leq 0.01$).

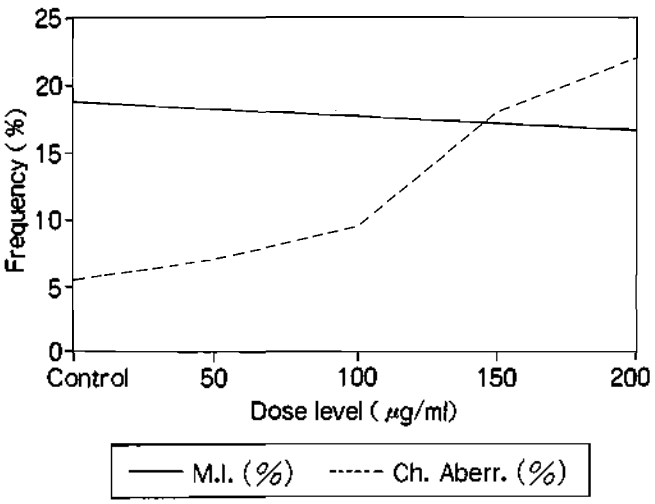

Figure 1. Frequency (%) of mitotic index (M.I.) and chromosomal aberrations in relation to dose level of malathion on goat lymphocytes *in vitro*.

TABLE 1. MITOTIC ACTIVITY OF GOAT LYMPHOCYTES TREATED WITH PESTICIDES

Pesticide	Dose (μg/ml)	Cells scored (No)	Blast cells (%)	Metaphase cells (%)	Mitotic index (%)
Malathion	Control	2402	17.44	0.96	18.48
	50	2256	17.83	0.98	18.31
	100	2194	17.14	0.87	18.02
	150	2187	16.10	0.82	16.92*
	200	2064	15.70	0.82	16.52*
Parathion	Control	2370	18.19	0.97	19.16
	5	2240	16.92	1.03	17.90*
	10	2366	16.02	0.85	16.48**
	15	2223	13.41	0.54	13.94**
	20	2061	12.66	0.44	13.10**

* Significant ($p \leq 0.05$) ** Significant ($p \leq 0.01$)

Effects of pesticides on chromosomal profile

The frequency of chromosomal aberrations in goat lymphocytes in culture varied from 5 to 5.5 percent in controls of both the experiments. In the malathion treated series, the frequency of chromosomal abnormalities increased to 9.5 percent at 100 μg/ml (table 2) and the difference were significant ($p \leq 0.05$). At higher doses the frequency of induced chromosomal abnormalities were highly significant ($p \leq 0.01$). The incidence of chromosomal breaks, dicentric chromosomes and translocations were higher than other types of abnormalities encountered (figure 3).

TABLE 2. FREQUENCY (%) OF CHROMOSOMSOMAL ABNORMALITIES IN GOAT LYMPHOCYTES TREATED *IN VITRO* WITH PESTICIDES

Pesticide	Dose (μg / ml)	Metaphase cells with anomalies (%)	Types of chromosomal aberrations						
			Gap	Break	ACF	DIC	CF	PV	Num
Malathion	Control	5.50	1.0	1.0	0.5	1.0	1.0	0.5	0.5
	50	7.0	0.5	1.5	0.5	2.5	1.5	0.5	0.5
	100	9.50*	1.0	2.0	1.5	2.0	2.0	1.0	0.5
	150	18.00**	2.0	2.5	2.0	6.0	3.0	2.0	0.0
	200	22.00**	2.5	4.0	2.0	7.0	4.0	2.0	0.5
Parathion	Control	5.00	1.0	0.5	1.5	1.0	0.5	0.0	0.5
	5	8.50*	1.0	1.5	2.0	1.5	1.5	1.0	1.0
	10	17.00**	3.0	4.5	3.5	3.0	2.0	1.5	1.0
	15	29.50**	4.5	7.0	6.5	4.5	3.0	2.5	1.5
	20	36.50**	7.0	7.5	3.0	5.5	3.5	5.5	2.5

* Significant ($p \leq 0.05$) ** Significant ($p \leq 0.01$).
ACF : Acentric fragment; DIC: Dicentric chromosome; CF: Centric fusion; PV: Pulverization; Num: Numerical aberration.

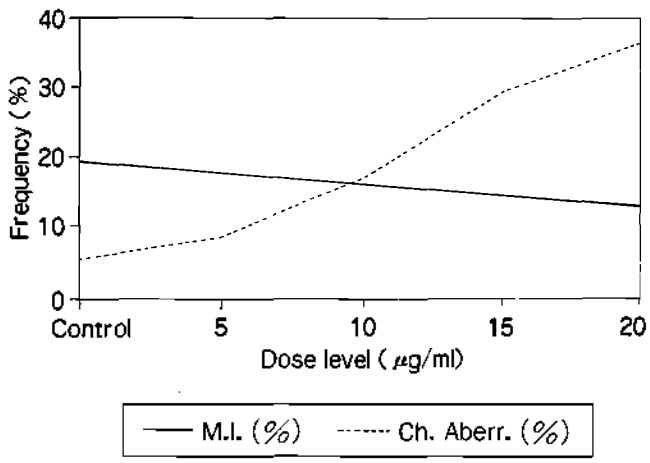

Figure 2. Frequency (%) of mitotic index (M.I.) and chromosomal aberrations in relation to dose of parathion on goat lymphocytes *in vitro*.

In the experiment with the parathion treatment, a dose related increase in the frequencies of metaphase cells with aberrations was recorded (figure 2) and the differences between treatment and control groups were significant ($p \leq 0.05$) even at 5 μg/ml dose (table 2). In other treatment groups at 10, 15 and 20 μg/ml dose levels, the incidence of chromosomal anomalies were 17, 29.5 and 36.5 percent respectively. The differences between these treated series and control were highly significant ($p \leq 0.01$). The cells with chromosomal gaps, breaks, acentric fragments and dicentric chromosomes were preponderant on other types (figure 4).

Discussion

Effects of pesticides on mitotic index of lymphocytes

Pesticides cause a reduction in mitotic activity of cells due to delay in the synthetic activity of cell cycle. This has been reported to be due to the base substitution by one or more alkyl radical of the compound (Freese, 1971). In this study, the pesticides were added to the cultures when these were 48 h old and the cells have already completed nearly two mitotic cycles. This protocol was adopted primarly because these pesticides were found to

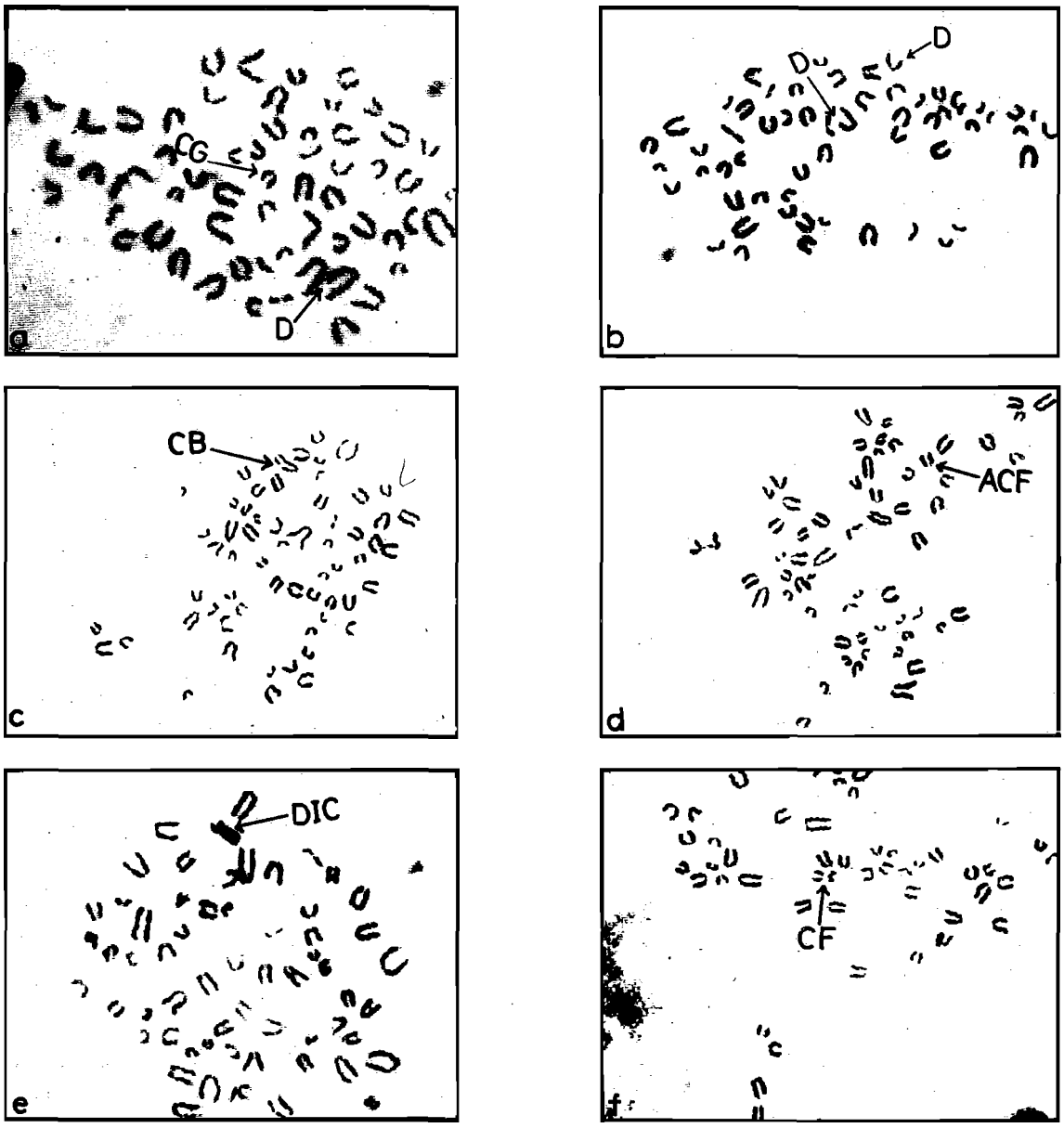

Figure 3. a-f Metaphase plates showing chromosomal aberrations in malathion treated goat lymphocytes in culture. a-chromatid gap (CG) and terminal deletion (D): b-terminal deletion (D); c-chromatid break (CB); d-acentric fragments (ACF); e-dicentric chromosome (DIC), f-centric fusion (CF).

interfere with the activity of PHA in inducing the cell division in non dividing peripheral blood lymphocytes and cultures normally failed when these were added in the beginning during earlier trials. In the already dividing cells, a dose related decrease in the mitotic indices was observed under the influence of malathion and parathion treatment. The antimitotic effects of malathion, parathion and other related organophosphates have been reported by Petropolis and Kamra (1978), Simon et al. (1978) and Gupta et al. (1988, 1994). In this study the effects of parathion were much higher than malathion even at low doses. This could have been due to the supply of higher number of alkyl reactive groups in parathion treatment than that of malathion which might have interefered with synthetic activity of goat lymphocytes in culture and ultimately delayed the cell cycle. Fahrig (1974); Blevins et al. (1977) and Simon et al. (1978) reported that pesticides induced unscheduled DNA synthesis in human and

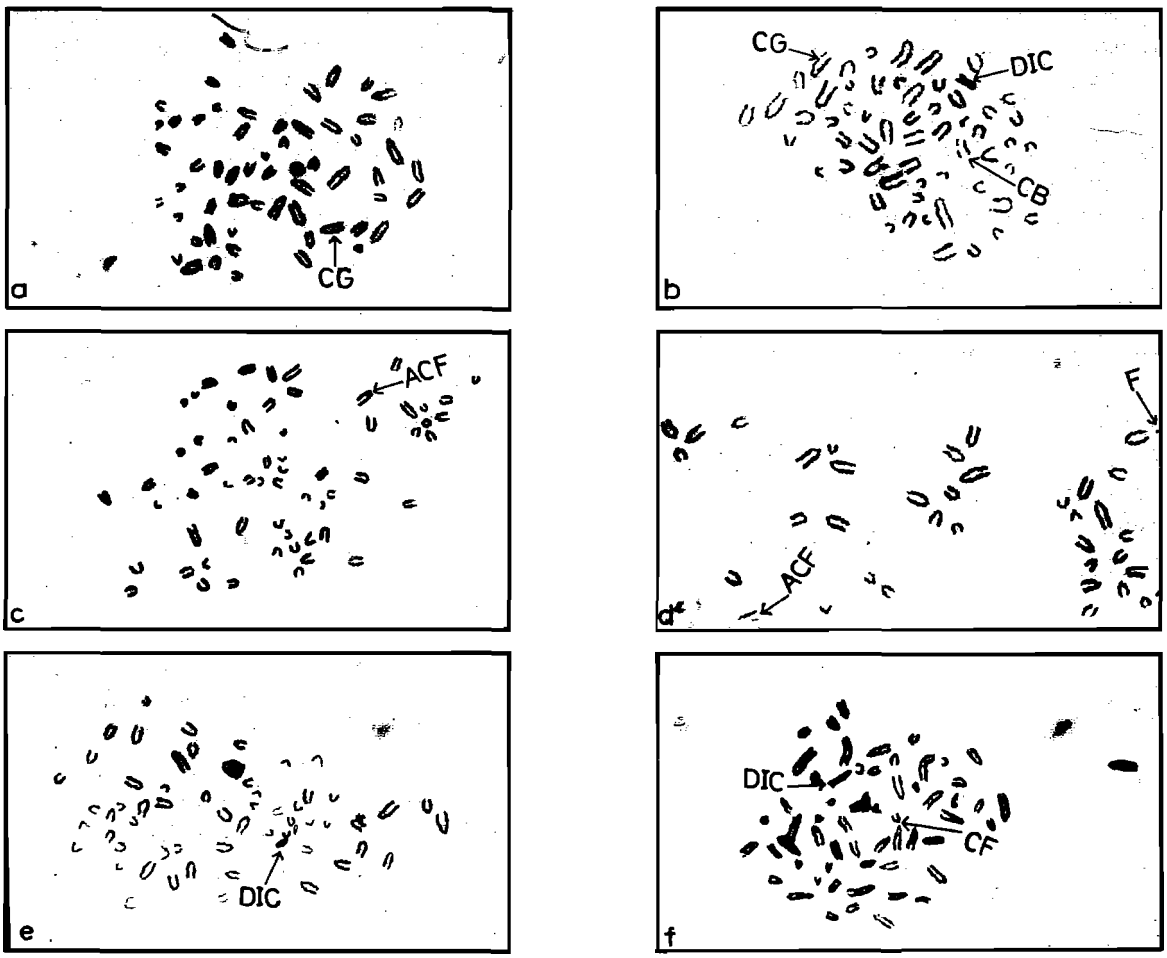

Figure 4. a-f Metaphase plates showing chromosomal aberrations in parathion treated goat lymphocytes in culture. a-chromatid gap (CG): b-chromatid gap (CG), chromatid break (CB) and dicentric chromosome (DIC): c-acentric fragment: d-acentric fragments (ACF) and chromatid fragment (F): e-dicentric chromosome (DIC) : f-dicentric chromosomes (DIC) and centric fusion (CF).

hamester lymphocytes in culture. Leurs and Rohrborn (1963) held that the mutagenic potential of a compound depends upon the number of free alkyl radicals released per molecule in the reaction and they act like base analogues.

Effects of pesticides on chromosomal profile

The chromosome damaging effects of organophosphorus pesticides are considered to be due to replacement of one or more DNA base pairs by the free alkyl radicals carried by them. This essentially causes a breakdown in DNA backbone resulting in one or other type of structural or numerical chromosomal abnormalities in the cells (Datta et al., 1970). In the present study, both malathion and parathion caused significantly higher incidences of chromosomal aberrations in goat lymphocytes treated *in vitro* and a dose related response was noticed. In malathion treated series, the frequency of breaks, dicentrics and translocations were higher than other types of aberrations in 100 μg/ml or higher dose levels. Probably, malathion caused breakage in the nucleoproteins of goat chromosomes both at chromatids as well as at centromeric position. This could have resulted into the reunion of broken fragments with sticky ends in different configurations giving rise to higher incidences of dicentrics and translocations. Similar incidence of chromosomal aberrations have been reported in malathion treated buffalo lymphocytes in culture by Gupta et al. (1988).

The frequencies of induced chromosomal aberrations in goat lymphocytes treated with parathion were significant ($p \leq 0.05$) even at the lowest dose of 5 μg/ml

and a regular increase was observed with the corresponding increase in dose level inoculated in the cultures (figure 2). Chromosome damaging potential of parathion in small doses have been reported both *in vivo* and *in vitro* studies by Lutz-Ostertag et al. (1969), Gupta and Sahai (1982) and Gupta et al. (1994). In this study the frequencies of chromosomal aberrations observed in two experiments indicated that parathion has much stronger chromosome damaging effects than malathion. Incidences of chromosomal gaps, breaks, acentric fragments, dicentric chromosomes and pulverization were more frequent than other types of structural and numerical abnormalities. Parathion might have caused severe damage to the DNA of goat lymphocytes probably due to the nucleophillic base substitution by the alkyl radicles giving rise to higher frequency of chromosomal gaps and breaks at chromatids or centromeric positions.

From this study it appears that parathion has higher genotoxic effects on goat lymphocytes *in vitro* than malathion. In an earlier study also, Gupta and Sahai (1982) have reported the high mutagenic potential of parathion at low doses in somatic and germ cells of rats *in vivo*. Thus the malathion formulations could be relatively safer drugs at rather low doses than those of parathion, for the control of body pests in goats and other farm animals.

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