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HEPATO-RENAL AND CYTOGENITIC STUDIES ON THE EFFECT OF THE INSECTICIDE CHLOROPYRIFOS METHYL (RELDAN), THE FOLIAR FERTILIZER, FF (MULTICRI I) AND/OR THEIR MIXTURE ON RATS

By

O.C. BAYOUMI*; A. ABDEL-AZEEZ**; Kh. KHILO**; A.A. EL-FIKEY and SH.A.HEMEDA***

* Dept. of Pesticides, Fac. of Agric, Kafr El-Sheikh Tanta Univ.
** Dept. of Physiology and Biochemistry, Fac. of Vet. Med. Kafr El-Sheikh Tanta Univ.
*** Dept. of Animal Husbandry, Fac. Vet. Med. Alex. Univ.

SUMMARY

The stability of chloropyrifos methyl (Reldan) insecticide, Multicri I (FF) and/or their mixture mixed with rats' diet were studied at invervals of time using thin layer chromatographic method. Results showed that no degradation or interference were found up to seven days after treatment, however, different degradations were found during the next experimental period (21 days). The biochemical studies showed that the compounds tested and/or their mixture had a great effect on different serum enzymes activities. Also the cytogenitic studies focused the chromosomal damages induced by Reldan, multicri I and their mixture which evaluated by derermining the frequencies of different type of chromosomal aberrations in bone marrow cells. These aberrations were presentented by chromatid breaks, chromosomal breaks (ring chromosome) and chromosomal fragments.

INTRODUCTION

The large use of the organophosphorus pesticides have played an important role for controling the nuisible pests and also represent one of the main causes of environmental pollution. As well as the use of chemical fertilizers present different problems for human health. The organophosphorus compound had the potential for causing an acute toxicity indicated by signs of cholinergic poisoning and a delayed neuropathy that may develops days to weeks after exposure of some animals species, or human to these compounds (Davis and Kichardsum, 1980, Imamura et al.,1983; Abdel-Baki et al; 1993).

Also the genotoxic effects of many pesticides were reported by many authors (Dulout et al. 1983 and Youssef et al., 1985). Dulout et al. 1983 reported that the organophosphorus insecticide malathion had a significant increment of abnormal metaphase gaps breaks and chromatid exchange. The present work aimed to focus the effect of organophosphorus insecticide, Reldan; the foliar fertilizer Multicri 1 and/or their mixture on some enzyme activities on rats and on the metaphase chromosome of bone marrow cell and on the stability of these compounds at intervals with time, when mixed with rats diets.

MATERIAL AND METHODS

1- Chemicals used:

a- Chloropyrifos methyl (Reldan) 50% O,O dime-

thyl O. (3,5,6 Trichloro -2 pyridyl) phosphorothioate.

b- Multicri I (Liquid foliar fertilizer, F.F.) each liter contains 6.52g Zinc, 4.44g Manganese, 2.08g Iron, 0.69 Copper and 7.98g Sulfur.

Stock solution of Reldan, Multicri I and/or their mixture were prepared in water or in acetone using different volumetric flasks. Solutions were stored in a refrigerator. Appropriate dilution of stock solution were used preparing samples for different assays.

2- Animals and treatments of diet:

Mature albino rats' weight of 100-120 gm were used in the current study. Animals were fed daily on the mixture of equal parts of crushed wheat flour bread and crushed maize.

Milk powder was used mixed with water one time weekly.

For control treatment rats' diets were treated with 200 ml of distilled water for each kilogram and then were kept to dry at room temperature. The same procedures were made with Reldan insecticide (4.25 gm a./200 ml) and also for Multicri I (2.5 ml f.f/200 ml)

For the joint action effect, rats; diets were treated with 200 ml of distilled water contain 4.25 gm of Reldan insecticide and 1.25 ml of multicri I for each kiolgram. Rats reared under controlled conditions for the experimental period.

3- Acute toxicity tests

A total of forty rats were used in the study. Rats were allocated into four groups each group contained five males and five females. Animals within group I were used as control [received no treatment] while those of group two received diet treated with the pesticid group three received diet treated with the foli fertilizer and group four received diet terate with a mixture of pesticide and doliar fertiliz

Animals were fed daily on 10 gm of treated o untreated diets. The treatment were continue for 21 days, then the animals prepared for di ferent biochemical and cytogenitical studies.

4- Biochemical experiments:

Rats reared on polluted diets and/or non treate diets were sacrificed at the end of experiment Blood samples were collected in heparinzie tubes and entrifuged at 4000 rpm. for 15 min utes. The activities of Aspartate amino trans aminase (AST) Alanine amino transaminas (ALT), alkaline phosphates (ALKPase) an urea were determined on the plasma using th colorimetric method of kits obtained from Egyptian American Co. for labratory services.

5- Cytogenetical studies:

Chromosomal preparations were made fro the bone marrow cells following the procedu described by Macgregor and Varley (1983 Each rat was injected intra-peritonialy wi colchicine (1 mg/kg) to stop cell division at th metaphase stage (Herbert, 1983), then ra were sacrificed 2hr. later. The bone marro was dissected out and finely minced by a sma scissor then immersed for 5 minutes in prev ously autoclaved phosphate buffer saline of th following composition (gm/litter) :NaCl 8 Ma2HPO4 (7H20) 4.23 and KH2PO4 0.47.

The treated bone marrow cells were placed potassium chloride (0.075 M) for 25 minutes 37°C and then centerfuged at 1000 rpm for 1 minutes. The supernatent was discarded an the cell sediment was fixed with methano glacial acetic acid (3:1) after two addition

centrifugations in fresh fixative, drops of cell suspension were placed on clean slides and left for air drying. The slides were stained with 4% Giemsa for 20 minute for conventional examination.

Cytogenetic observations were performed by analysing 50 well spread metaphase cell per animal and determining the frequency of abnormal metaphase.

Statistical analysis was performed according to Snedecor and Cochran (1980) using Chi-square test.

6- Thin-layer chromatographic analysis of chemicals used:

Thin -layer chromatoplates were prepared by the method described by Bayoumi et al. (1983) Reldan and Multicri I or their mixture (Coresponding to large 2,4, 20,40 mg) using a stock solution 1 mg/ml acetone or water were applied as 25 mm spots to the bottom edge of silica gel TLC-plates.

Three solvent systems were used for the separation of reldan, Multicri I and/or their mixture extracted from treated rat diet the composition of these solvent systems were as follows:

(a) benzene : methylene chloride: acetone (4:1:1).

(b) chloroform: ethylacetate: acetone (3:1:1)
(c) chloroform: acetone: acetonitrile (4:1:1).

The development was stoped at 7.5cm above the front edge of the plate. The air dried chrolatoplates were then treated with iodine vapours and the mean Rf values of detectable spots were recorded for each sample. Each assay was replicated at least three times.

7- Extraction of insecticide, foliar fertilizer and their mixture from rats diets:

Rats diets used for the toxicity tests were analysed at intervals with time during the experiental period (3,7,14,21 days) using thin-layer chromatographic methods.

50 gm of rat diets samples were extracted three times with 150 ml chloroform. The chloroform extracts were drained on an anhydrous sodium sulfate which was subsequently washed with 50 ml of chloroform. The combined filtrates were evaporated to dryness and the residue was quantitavely dissolved in 25 ml acetone.

Aliquots (100 ul) of different extracts were used for TLC analysis to determine the possible degradation of the compounds tested using iodine vapours as detectants.

RESULTS AND DISCUSSION

Currently Reldan insecticide, the foliar fertilizer Multicri I are registered for use in Egypt so the determination of the side effects of these compounds are important to protect our environment from their hazards after treatments.
From the Rf values presented in Table (1) it was evident that the best overall separation of Reldan was obtained by using the solvent system (chloroform: Ethyl-acetate:acetone 3:1:1) with Rf value of 0.82 and without any interferance with rats, diets extracts. The detection limits based on the revealation of spots by iodine vapours was sensitive for detecting 2 ug of Multicri I and 4ug for Reldan (Table 2).

This detection method requires at least 5 minutes to visualizes the spots of a high concentratios more than 40 ug while 15 minutes was found necessary to visualize the spots of low concentrations below 10 ug.

Table (1): Thin-layer chromatography Rf values of Reldan, Multicri I and Their mixture in different solvent systems.

Compounds	Solvent system *		
	1	2	3
Reldan (a)	0.82	0.86	0.87
Multieri I (b)	0.00	0.00	0.00
a + b	0.82	0.86	0.87

1= Chloroform: Ethyl acetate: acetone (3:1:1)
2= Benzene: Methylene chloride: acetone (4:1:1)
3= Chloroform: acetone: acetonitrile (4:1:1).

Table (2): Sensitivity of detection of Reldan and Multicri I by iodine vapors.

Compounds	Concentration (µg)			
	2	4	20	40
Reldan	ND	W	G	VG
Multicri I	N	W	G	VG

W= week detection ND= no detection
VG= very good detection G= good detection

Table (3): Thin-layer chromatographyic analysis of Reldan, Multicri I and their mixture at intervals in rats' diets.

Compounds	Weeks after treatment						
	0	1	2		3		
Reldan (1)	0.82	0.82	0.82	0.66	0.82	0.66,	0.14
Multieri I (2)	0.00	0.00	0.00		0.00		
(1) + (2)	0.82	0.82	0.82	0.66	0.82	0.66,	0.14

Table (4): Effect of reldan, Multicri I and their mixture on urea & some enzyme activities in serum of rats determined as inhibtion %.

Enzymes / Chemical used	Urea	ALT	AST	Alkpase
control	0.00	0.00	0.00	0.00
Reldan (1)	13.30	71.42	22.60	67.85
Multicri (2)	13.30	78.57	56.84	44.40
(1) + (2)	21.70	75.00	26.84	33.30

The residues of previous compounds or their mixture in rats, diet were determined at time intervals as previously described . Results presented in Table (3) showed the marked degradation of insecticide two weeks after diets treatments.

The biochemicals studies made on the treated rats with the previous compounds and/or their mixture were presented in Table (4) as inhibition percent of some enzymes (AST, ALT.Alk Pase, and urea) activities. In the period of the experiments, rats showed normal behaviour, while the analysis of their enzymes activities showed a decrease in ALT, AST, ALKPase.

Table (5): Cytogenetic analysis of metaphase chromosomes of bone marrow cells of rats treated with Reldan, Multicri I and their mixture.

Type of treatment.	No. of examined cells	No. of Nbnormal cells		Structural in chromosomal aberriations in abnormal cells					
				Chromatid break		Ringchromosome		Fragment	
				No.	%	No.	%	No.	%
Reldan (1)	50	22	44	12	24	7	14	9	18
Multicri I (2)	50	25	50	13	26	9	18	7	14
Mix (1 + 2)	50	32	64	15	30	12	24	10	20
Control	50	2	4	2	4	--	0	1	2

Table (6): Chi-square analysis of data obtained from cytogical examination.

Treatment	No. of examined cells	Abnormal cells	Chi-squar values
Reldan (1)	50	22	8.762**
Multicri (2)	50	25	24.556**
Mi x (1x2)	50	32	37.478**
Control	50	2	

** Highly significant difference between treated and control group.

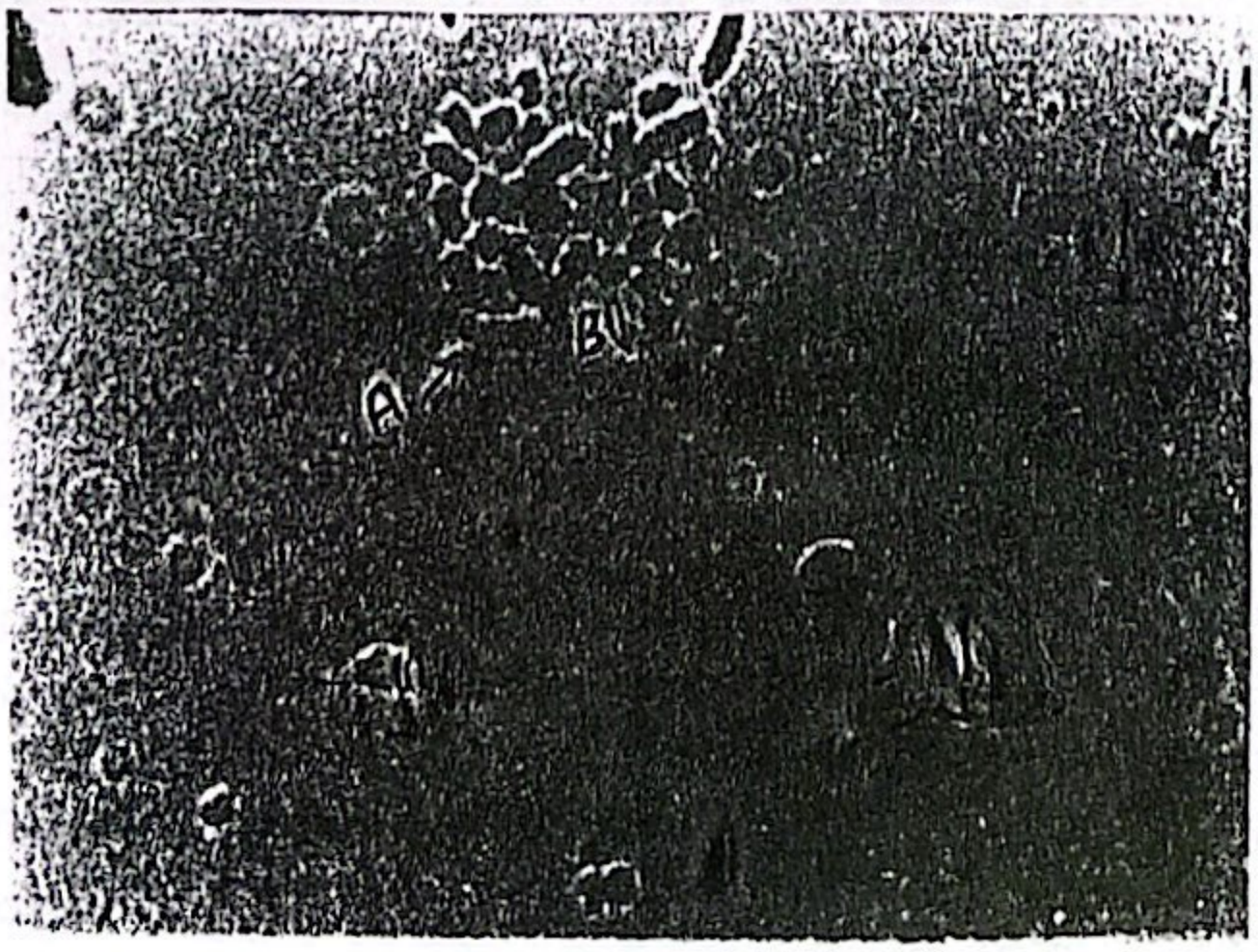

Fig. (1): Metaphose chromosome of marrow bone marrow cells.
lesion: A chromatid breaks. B. gap

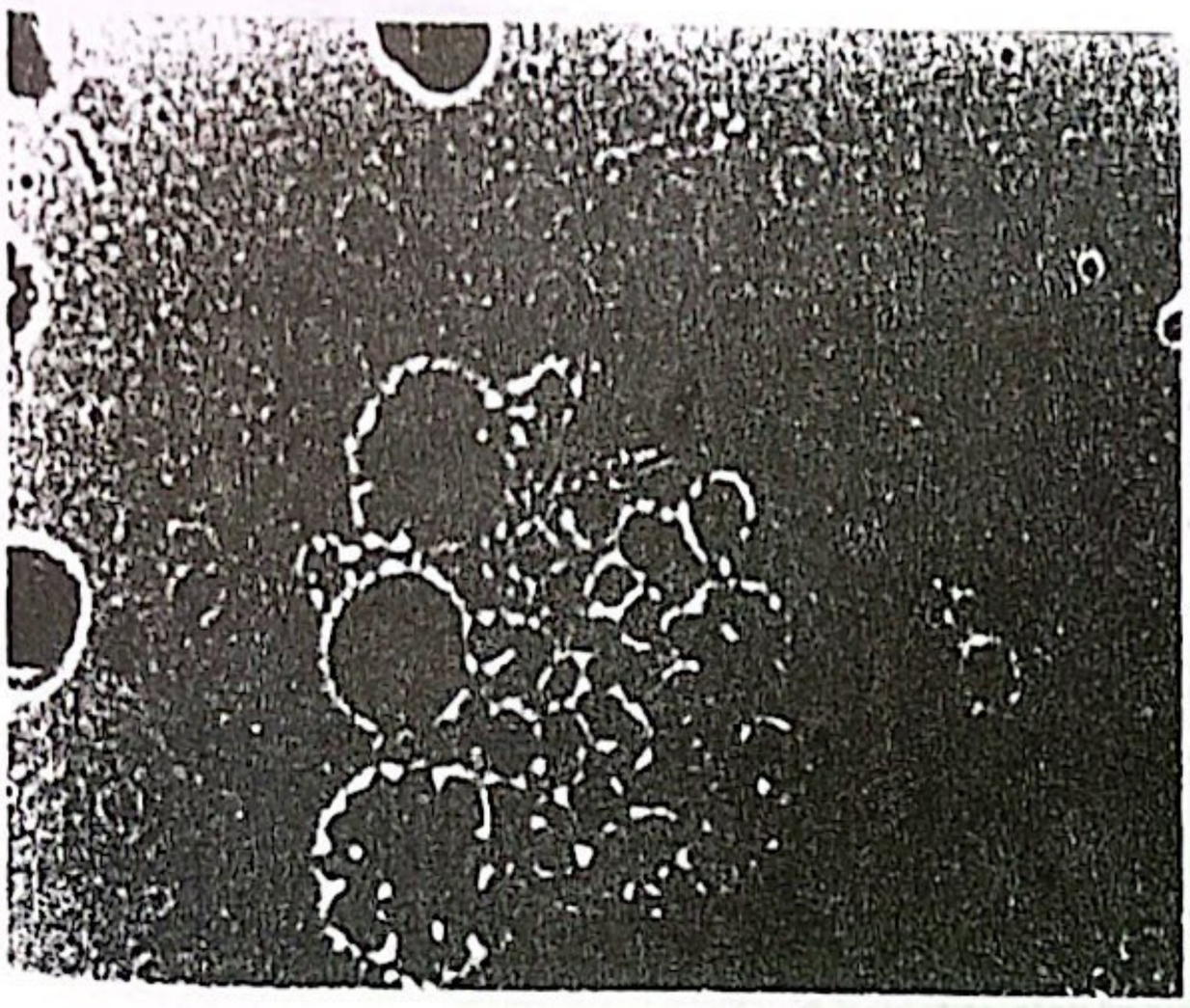

Fig. (2): Metaphase chromosome of bone marrow cells.
lesion: Ring chromosome.

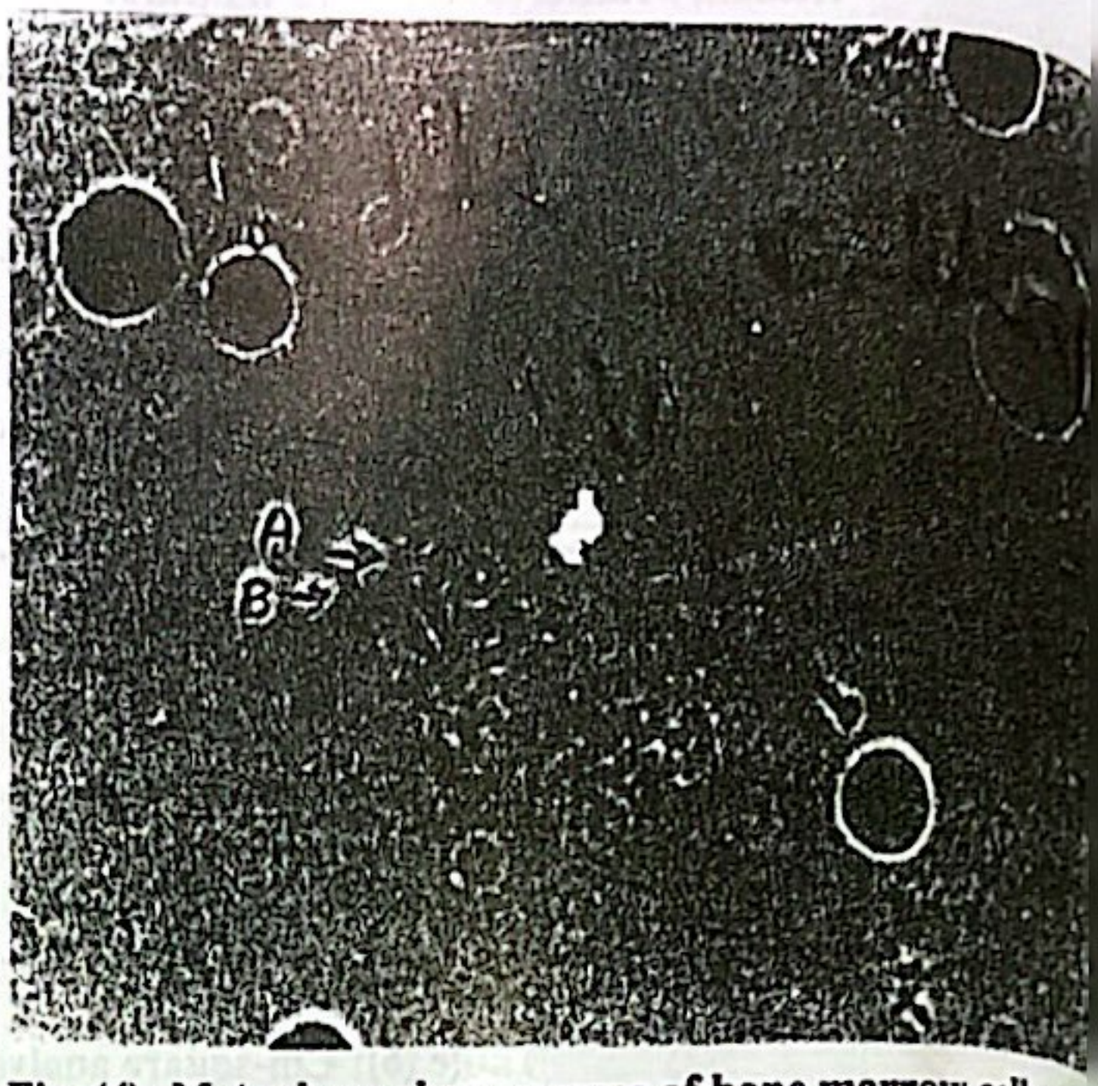

Fig. (4): Metaphase chromosome of bone marrow cells
lesion : A chromatid breaks.
B. Ring chromosomes.

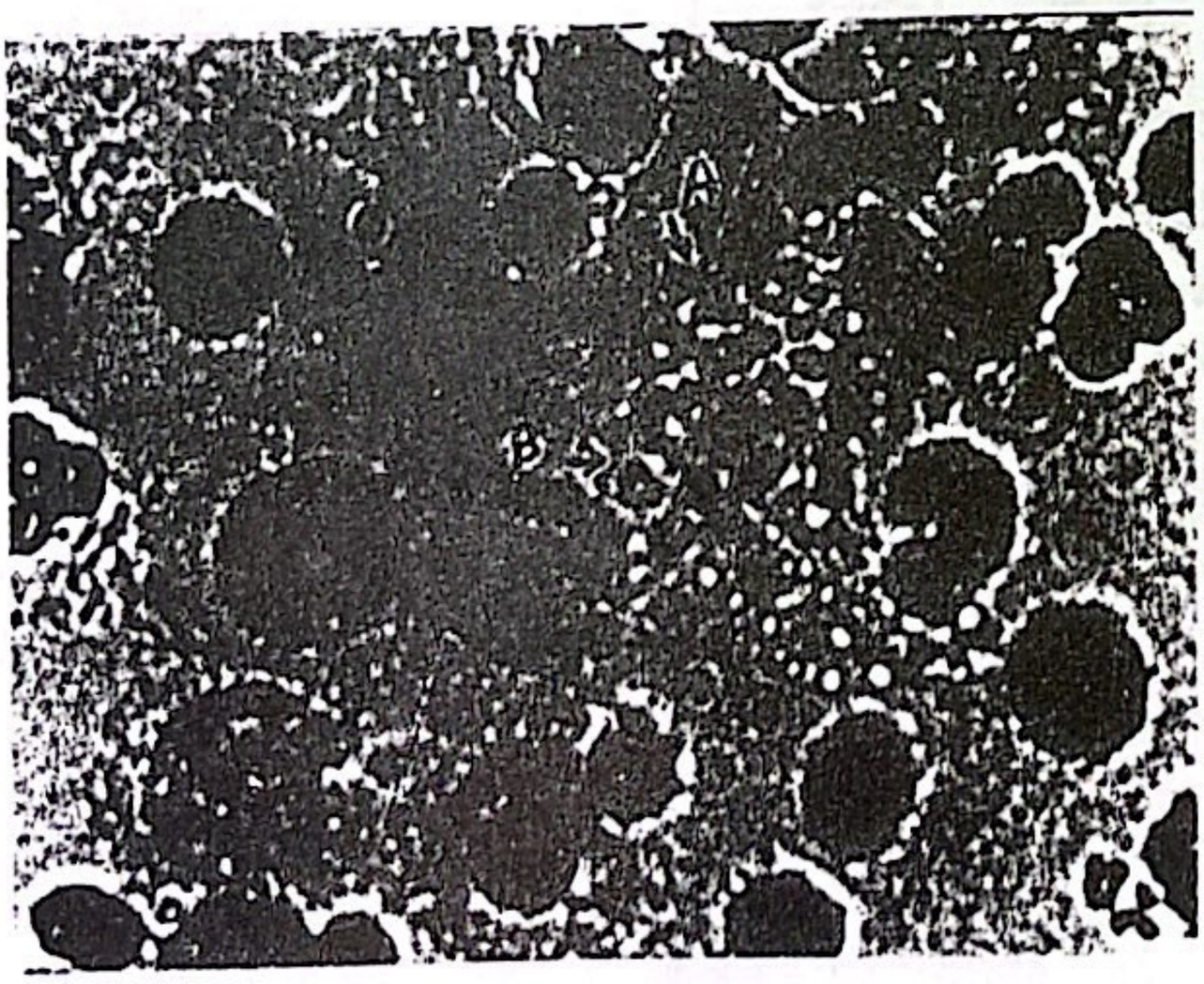

Fig. (3): Metaphase chromosome of bone marrow cells.
lesio : A chromatid breaks.
B. chromosome fragment

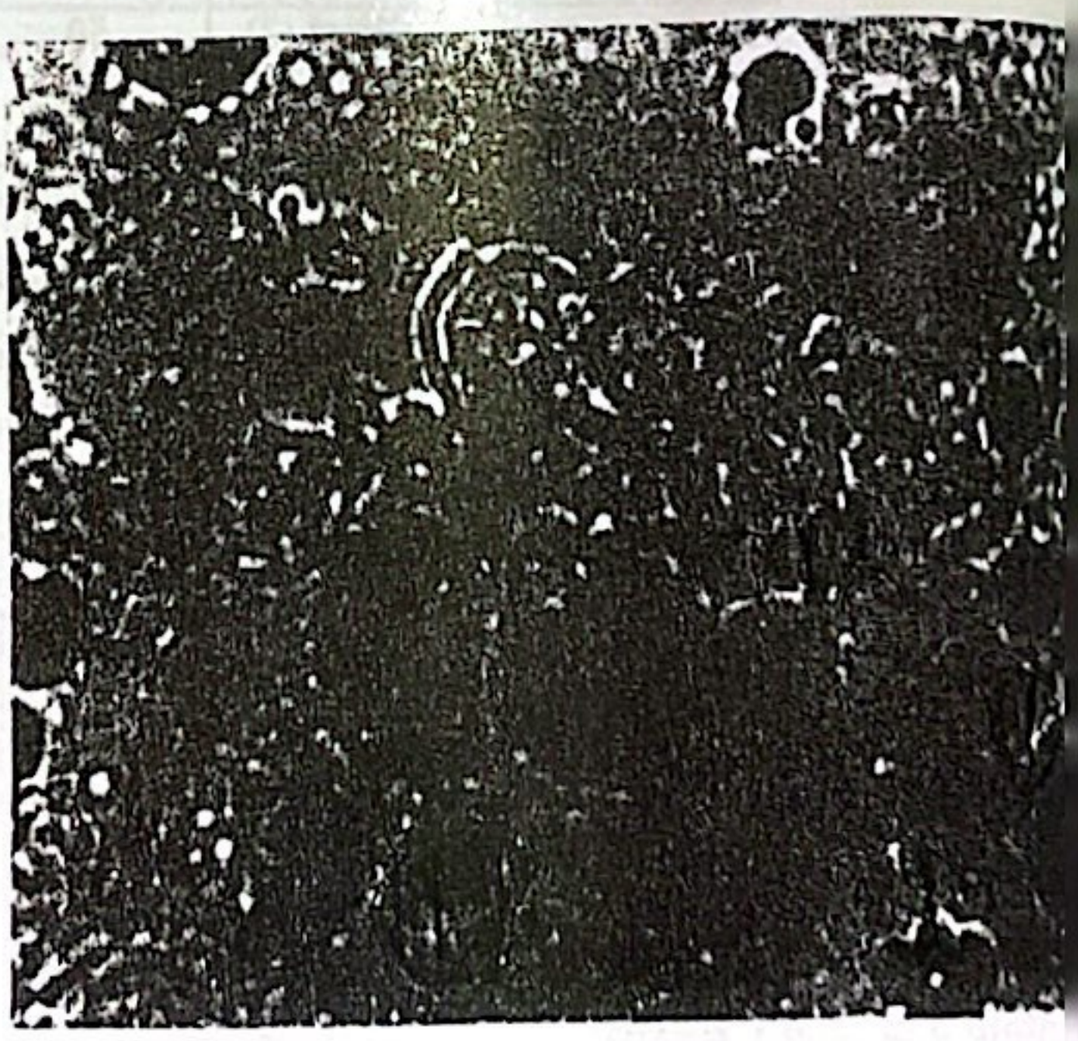

Fig. (5): Metaphase chromosome of bone marrow cells
control group.

These phosphatase and urea activities. These changes in serum concentration of AST, ALT, ALKPase and urea activity occur essentially as a result of some processes involving the body tissues (Ford and Lowerence 1975 and Hofmann and El-Amrous; 1974). The obtained results tend to support the findings of Sasinovich et al (1973) who observed a high decrease in the activity of serum and liver ALT and alkaline phosphatase in rats treated with the organophosphorus insecticides chlorophos. Also our findings are in agreement with the results obtained by Enan et al. (1982) who noted an inhibtion of AST and ALT in rats due to the same organophosphorus compound. In the same time Ambrose et al. (1970) reported that there was a significant inhibition of alkaline phosphatase of mallard hen treated by some organophosphorus compounds. In contrary to our findings different authors reported that the use of some organophosphorus compounds increased the concentration and the activity of some enzymes like AST. ALT and alkaline phosphatase and urea in tested rats (Abbassy et al 1987, Tag Eldin 1988 and Abdel-Baki 1993). Figure (5) shows the metaphase chromoseme of nontreated control group. Table (5) summarizes the results obtained from cytogenetic analysis examination of metaphase chromosomes of bone marrow cells of rats treated with the organophosphorus insecticide (Reldan), the foliar fertilizer Multieri I and their mixture revealed the presence of 44%, 50% and 64% of abnormal cells as 22.25 and 32 out of 50 cells. Many types of structure chromosomal aberrations were observed. These aberrations represented by chromatid breaks (Fig.1,4) ring chromosome (chromosomalbreaks) (Fig.2,4) and chromosomal fragments (Fig.3). Chi-squar test of the data revealed highly significant effect of the reldan, Multicri I and their mixture in relation to control one (table 6). The percentage of chromosomal aberrations were higher in the case of mixture of these compounds (64%). Moreover the rate of occurrence of chromatid breaks were more than other types of aberration in all treatment, the effects of many pesticides on chromosome structure have been studied by many authours.

Dulout et al. (1983) reported that malathion induced subchromatide and chromatid-type aberrations in relation with the dose used. Fehheimer (1968), reported that many chemical agents have been found to influence the occurrence of chromosomal aberrations.

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