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CYTOTOXIC EFFECTS OF THE PYRETHROID INSECTICIDE (MATOX)® WITH REFERENCE TO ITS INFLUENCE ON THE REPRODUCTIVE HORMONE

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SUMMARY

Male rats exposed to the aerosols of the pyrethroid insecticide (Matox)® at a level of one tablet/36 m^3 for 10 hours daily up to 2,4 and 8 weeks revealed a significant decrease in weight of testis, epididymis, seminal vesicle and prostate glands after 4 and 8 weeks. The sperm motility percentage was significantly decreased after 2,4 and 8 weeks of exposure to Matox aerosols while the sperm cell concentration was significantly decreased after 4 and 8 weeks of exposure to Matox aerosols.

Male rats exposed to Matox aerosols showed a significant decrease in the level of LH and testosterone hormone after 4 and 8 weeks. Meanwhile, the FSH level was significantly reduced only after 8 weeks of exposure.

Cytogenetic studies revealed that Matox have neither visible effect on chromosomes structure and arrangement nor numerical chromosomal aberrations. However, the miotic division of testicular cells in Matox treated group were highly significantly decreased when compared with that of control one.

Haematological studies showed that Matox aerosols induced a significant decrease in R.B.Cs., W.B.Cs. counts, Hb content and PCV after 2,4 and 8 weeks.

INTRODUCTION

The pyrethroids are a class of natural and synthetic organic compounds which have been used commercially for many years because of their well known insecticide properties. These agents are still highly effective and often included in many currently available household sprays (Gosselin et al., 1984).

The synthetic pyrethroids are rapidly absorbed and distributed through body tissue, including the brain (Gosselin et al., 1984). The previous studies about the neurotoxicity of the pyrethroids have indicated interaction of these compounds with the gamma amino butyric acid (GABA) receptors and other C.N.S. stereo-specific receptors; the calmodulin-phosphodiesterase system and sodium channels in the mammalian brain (Lawrence and Casida, 1983; Rashatawer and Matsumura, 1985 and Soderlund, 1985).
The natural pyrethoids had a teratogenic effect on the genital tract of the birds (Lutz-Ostertage and Lutz, 1974). and may had antiandrogenic properties (Brody et al., 1983). Haematological and enzymatic change had been reported in rabbits (Hassan et al., 1988) rats (Fakhry et al., 1990) and mice (Ibrahim et al., 1991) exposed to aerosols of the pyrethroid insecticide (Ezalo)®.

The purpose of this study was to evaluate the change in spermatogenesis, hormonal profile, haematological parameters as well as the changes on chromosomes structure and their arrangement in albino male rats after exposure to Matox aerosols for 2,4 and 8 weeks.

MATERIAL AND METHODS

Matox® contain pynamin forte , it was included

by the Sumintono Chemical Co., Japan. It is a pyrethroid, allethrin (2-methyl-40 x 0-3 (2-propenyle)-2-cyclopentenyle-2,2- dimethyle-3-(2-methyle-1-propenyle) cyclopropane carboxylate.

Matox® tablets produced by Kafr El-Ziat Insecticide and Chemical Co. Egypt were used. Each tablet contained pynamin fort 40 (100 mg) and Kerosene (20 mg). Electric heater, turned on and then it started to release its aerosols.

Sixty male albino rats weighing from 200 to 250 gm. were obtained from Animal House, Fac. Vet. Med., Alex. Univ. Egypt, and acclimatized to our laboratory before use. rats were fed a balanced diet and water ad lib.

Rats were divided into 2 equal gorups, one group was kept as a control and the other group was exposed to Matox® aerosol at a level of one tablet /36 m^3 size room for 10 hours daily. Ten rats from each group were sacrificed after 2,4 and 8 weeks of treatment and blood samples were collected for haematological examination. Erythrocyte and leucocyte counts were performed using the Improved Neubauer method (Schalm, 1975), Hb content was estimated as cyanomethaeglobin after Crosby et al. (1954), hematocrit value was determined by the microhaematocrit technique (Schalm, 1975). Serum was obtained and stored frozen at -20°C until assayed for LH according to Karonen et al. (1978) and FSH according to Travis (1980) using Farmos Diagnostic Kits and testosterone according to Isamil (1980) using Kits of Diagnostic Product Coporation, Los Angeles, USA.

At the same time, the testis, epididymis, seminal vesicle, and prostate glands were dissected out, grossly examined and weighed. The index weight (I.W.) of the organs was calculated according to the equation of Bearden and Fluquarg (1980)

$$I.W. = \frac{\text{organ weight}}{\text{body wight}} \times 100$$

The motility percentage and the sperm cell concentration was estimated after Bearden and Fluquary (1980).

Chromosomal preparations were made from the bone marrow and testicular cells following the methods described by Macgregor and Varely (1983) as follow:-

The rats were injected I/P with colchicine (1mg/kg), then sacrified after $1\frac{1}{2}$ -2 hours. The bone marrow and testicular tissues were dissected out and minced with a small scissor, then immersed for 15 minutes in phosphate buffer saline. The treated bone marrow and testicular cells were then suspended in 0.56% Kcl solution for about 25 minutes at 37°C and centrifugation was performed at 800 r.p.m for 8 minutes. Fixation of the sediment was made several times using a mixture of ethanol and glacial acetic acid (3:1), the precipitated cells were dropped on a clean previously cooled slides, then air dried and stained with Gimsa stain (4%) in phosphate buffer.

Meiotic index was calculated according to Brusich (1980) as the following:

$$\text{Meiotic index} = \frac{\text{Number of divided cells}}{\text{Number of divided and non divided cells}} \times 100$$

The data were statistically analyzed using Student t-tst and Chi-square (Snedecor and Cochran, 1980).

RESULTS

The continuous exposure of male albino rats to Matox® aerosols 10 hours daily resulted in a significant decrease ($P < 0.05$) in index Weight (I.W.) of the testis, epididymis, seminal vesicles and prostate glands and sperm cell concentrations after 4 and 8 weeks of treatement (Table 1). Moreover, spermatozoal motility % was significantly decreased after 2,4 and 8 weeks of exposure to Matox® aerosols (Table 1).

FSH concentration in male rats exposed to Matox® aerosols was significantly decrease ($P<0.05$) after 8 weeks (Table 2). Meanwhile, LH and testosterone levels were significantly decreased after 4 and 8 weeks of treatment (Table 2).

Cytogenetic examination of metaphase chromosomes of bone marrow and testicular cells revealed that there was no visible effects of Matox® aerosols on chromosome structure of numbers in compare with control group. (Fig 1,2). Matox® aerosols caused highly significant decrease of meiotic index (Table 3).

Haematological examination revealed that treated rats showed a significant decrease in RBCs, WBCs, PCV and HB content after 2,4 and 8 weeks of treatment (Table 4).

Table (1): Effect of Matox® aerosol on I.W. of reproductive organs, spermatozoal motility % and sperm cell concentration.

Parameter	Period	Control	Treated
Testes (I.W.)	2nd week	1.41±0.04	1.33±0.02
	4th week	1.39±0.06	0.82±0.02*
	8th week	1.49±0.02	0.79±0.00*
Epididymis (I.W.)	2nd week	0.50±0.01	0.48±0.01
	4th week	0.48±0.01	0.26±0.02*
	8th week	0.52±0.02	0.22±0.01*
seminal vesicle (I.W.)	2nd week	0.45±0.01	0.42±0.02
	4th week	0.43±0.01	0.23±0.02*
	8th week	0.39±0.03	0.12±0.01*
Prostate gland (I.W.)	2nd week	0.21±0.02	0.18±0.02
	4th week	0.26±0.01	0.17±0.02*
	8th week	0.25±0.01	0.12±0.03*
Sperm motility %	2nd week	80.00±3.50	63.75±2.10*
	4th week	82.00±5.00	43.33±4.50*
	8th week	75.50±6.20	52.50±5.00*
Sperm cell concentration x 10^6	2nd week	1.73±0.04	1.66±0.07
	4th week	1.80±0.08	0.55±0.02*
	8th week	1.45±0.07	0.43±0.01*

Mean ± S.E.
* Significantly different (P < 0.05).

Table (2): FSH, LH and testosterone concentration following exposure to Matox® aerosols.

Parameter	Period	Control	Treated
FSH (IU/L)	2nd week	3.10±0.04	3.15±0.05
	4th week	3.02±0.02	2.98±0.03
	8th week	3.40±0.02	2.20±0.02*
LH (IU/L)	2nd week	3.81±0.03	3.59±0.06
	4th week	3.60±0.03	0.25±0.02*
	8th week	3.52±0.01	0.10±0.03*
Testosterone (ng/ml)	2nd week	2.50±0.02	2.32±0.03
	4th week	2.22±0.01	1.45±0.01*
	8th week	2.71±0.02	1.31±0.03*

Mean ± S.E.
* Significantly different (P < 0.05).

Table (3): Chi-Square test showing the effect of Matox® aerosols on division of testicular cells (Meiotic index) in rats.

Group	Dividing Number	cell %	Non dividing Number	cell %	Total
Control	86	58.10	62	41.90	148
Treated	23	19.30	96	80.70	119

X^2 = 39.47 highly significant.

Table (4): Haematological parameters of male albino rats after exposure to Matox® aerosols.

Parameter	Period	Control	Treated
RBCs (x $10^6/mm^3$)	2nd week	5.63±0.19	4.49±0.17*
	4th week	5.71±0.07	4.15±0.17*
	8th week	5.62±0.15	0.10±0.23*
WBCs (x $10^3/mm^3$)	2nd week	8.83±0.17	7.20±0.07*
	4th week	8.85±0.18	6.65±0.29*
	8th week	8.78±0.18	7.27±0.28*
Hb (%)	2nd week	65.0±2.06	41.33±1.45*
	4th week	65.67±1.87	4.00±0.58*
	8th week	66.38±2.57	41.50±1.41*
PCV %	2nd week	35.17±1.01	23.69±1.69*
	4th week	34.17±1.17	23.83±1.42*
	8th week	34.67±1.02	24.17±1.14*

Mean ± S.E.
* Significantly different (P < 0.05).

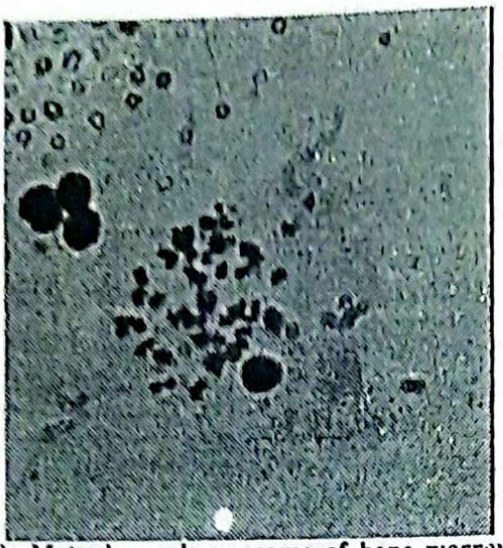

Fig.(1): Metaphase chromosome of bone marrow cell from treated group showing no chromosomal abnormalities.

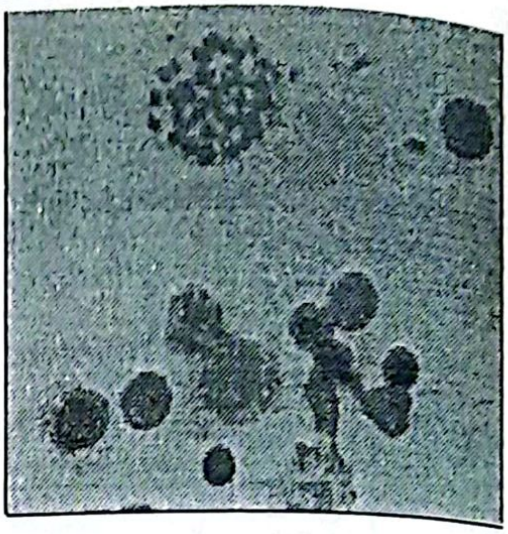

Fig.(2): Metaphase chromosome of bone marrow cell from control group.

DISCUSSION

The mutagenic and toxic potentials of environmental chemicals on male germ cells has become an important area of environmental concern.

Pyrethroids can interact competitively with androgen receptors and sex hormones binding globulin, these findings provide a mechanism by which chronic exposure to pesticides containing pyrethroids may result in disturbances in endocrine effects relating to androgen action (Brody et al., 1983 and Eil and Nisula, 1990).

Our results demonstrated that long term exposure to Matox® aerosols (pyrethroid) significantly decreased LH, FSH and testosterone levels. These findings might explain the significant depression in testicular, epididymal, seminal vesicle and prostate glands index weight and a significant reduction in sperm cell concentration. The reduction in sperm cell concentration could also be attributed to reduction in meiotic index of the testicular cells which might be due to the usage of the pyrethroid access the blood testis barrier (BTB) and gain access to the germ cells in seminiferous tubules. Dixon and Lee (1973) reported that the BTB appeared to represent an important aspect in the consideration of reproductive and mutagenic effects of environmental chemicals. Okumura et al. (1975) recorded that the permeability characteristics for the BTB are generally similar to those limit penetration of membranes of the central nervous system. Pyrethroids distributed through the brain and the principle action of them is the nervous system (Gosselin et al., 1984 and Kumar, 1984).

The present study revealed that Matox® aerosols have no influence on the rate of occurrence of chromosomal aberration including chromosomal fragments, ring chromosomes delation, translocations, gaps or abnormal number of chromosomes. However, point mutationa (change in the nucleotide of DNA) which can not be seen under the light microscope may occur. On the other hand, cattanach (1961, 1964 & 1955) has used one mutagenic compound to treat male mice subsequently used as breeders. He has recovered from among their progeny a number of translocations and autosomal trisomies. Many chemicals, pesticides and insecticides were found to have mutagenic effect (Regan et al., 1976 and Dean Belvins et al., 1977).

Concening the haematological changes, the anaemia induced by Matox® aerosols characterised by decreased RBCs counts, Hb content and PCV% could be resulted from inhibition of hematopoieses or defective hematopoiesis.

It could be concluded that Matox® (Pyrethroid) has a deleterious effect on reproductive organs

through decreasing the testosterone and gonadotrophins or through decreasing the meiotic division of the testicular cells. Also, it has an anaemic effect.

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