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PATHOLOGICAL STUDIES ON THE EFFECT OF PYROTHROIDS AEROSOL ON ALBINO RATS

BY

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SUMMARY

This study was performed on 50 albino rats with a body weight 150-200g. They were divided into five groups (A, B, C, D and E), The first four groups were exposed to daily aerosoling of pyrothroids for 15, 30, 45 and 60 days respectively while the fifth group served as control one.

Clinical signs, serum biochemical parametres , gross and histopathological findings were recorded.

The most striking lesions were congestion of all organs especially the lungs, peribronchitis and pneumonitis in addition to perivasculitis, degeneration of parenchymatous organs and some changes in the brain.

INTRODUCTION

The production of pyrethrum as an insecticide dates from about 1950 and unlike nicotine and derris, the use of pyrethrum has increased despite the large scale introduction of synthetic insecticides.

Griffith, 1977 reported that pyrethroids are widely used today in the control of agriculture pests and veterinary pests.

Feinberg, 1934 stated that some individuals showed hypersensitivity including sneezing and nasal discharge, while in laboratory animals Leonard, 1942 noticed high mortality due to respiratory failure. In mice Wallwork et al., 1974 recorded cases of spasm and convulsion.

Hend and Butterworth 1977 found that pyrethroids in rats result in changes in several haematological parametres and alkaline phosphatase activity. Coombs et al., 1976 reported that cypermethrin in rats result in increase in blood urea and Hb. concentration without any pathological alterations, on the other hand, Miyamoto, 1976 found that inhalation toxicity of allethrin in rats did not evoke any haematological or biochemical changes. Similar findings were observed in premethrin Clapp et al., 1977 and in D-phenothrin Martin et al., 1987.

Pham Huu Chanh et a l., 1984 reported that deltamethrin on rats and mice results in pulmonary hemorrhage and foci of necrosis among the hepatic cells. Further more Ahmed et al., 1989 noticed hyperplasia of spleen and glial cell proliferation. In dogs Gombe and Odourokelo, 1983 stated that Pyrethrum result in fibrosis and haemosidrosis in spleen and Liver. Kadry, 1981 studied fenopropathrin on sheep and dogs and he recorded leukocytic infiltration in lungs and liver, with bile duct hyperplasia and affection of the kidneys. Similar findings were observed by Nabila, 1990 and Shehata et al., 1991 in rats and mice in case of ezalo-Mat tablets.

Therefore the present study was designed to investigate the expected inhalation toxic effect of pyrothroids aerosol, which is widely used in Egypt, Through patholgoical, hematological and biochemical evaluations.

MATERIAL AND METHODS

I- toxic agent:

Kafrosol fort: The commercially used aerosale cans for control of house flies and Mosquitoes. It is produced by Kafr-El-Zayat pesticides chemical company.

Each can contains: New pynamine 0.1% pynamine fort 0.1% sumithrin 0.1%, perfumes and solvent agents till 400 cm^3.

II- Animals:

The present study was carried out on 80 mature male and female albino rats with a body weight range 150-200g. The rats were obtained from Faculty of Vet. Med. Cairo University. They were maintained in a balanced diet. They were divided into five groups (A, B, C, D and E). Experimental design as follow:

- Groups A,B,C and D: 10 rats of both sexes in each group were exposed daily to aerosoling in tightly closed chamber measuring 76 x 39 x 40 dimensions, for 30 mints/day. The time of exposure was 15,30,45 and 60 days respectively.
- Group E: 10 rats of both sexes served as control. The animals in all groups were clinically observed and the time of scarification was 24 hrs after the last exposure.

III- Sampling:

1. Blood and serum samples were collected from retroorbital venous plexus of rats. They were taken from different groups at 15, 30, 45 and 60 days for evaluation of haemogram and serum chemistry. The haemogram was performed following standard techniques described by Jain (1986). Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined colorimetrically according to Reitman and Frankel (1957). Serum urea and creatinine were estimated according to Kaplan (1965) and Husdan & Rapopart (1968) respectively. The haemogram and serum chemistry assays were statistically analysed according to Snedycor (1961).

2. Tissue Samples were collected from different organs (Lungs, heart, Liver, Kidneys, spleen, intestine and brain). They were fixed in 10% neutral formalin, dehydrated, cleared, embedded in paraffin, sectioned at 4-6 μ for H&E and stains for fat and hemosidrin were also performed (Carleton et al., 1967).

RESULTS

I- Clinical Signs:

The rats during exposure itch their nose with their hands and legs. Besides, some animals jumped upward, while others were rolling around themselves or walking on one side. Incoordination and restlessness were also noticed. Most of these signs continued even after exposure.

II- Serum biochemical changes:

Mean values of serum biochemical constituents in male and female rats inhaled Kafrosol 30 minutes /day for 15 /30, 40 and 60 days as well as those of the control rats are shown in table (1).

III- Haemogram:

Results of the changes in red blood cells count "R.B.Cs", packed cell volume "PCV", hemoglobin concentration "Hb". Mean corpuscular volume"MCV", Mean corpuscular hemoglobin concentration "MCHC" and, total and differential leukocytic counts in the control rats and those exposed to Kafrosol are shown in tables (2, 3).

IV- Gross findings:

The most frequent finding in the internal organs was congestion. Lungs and heart were more severely congested (Fig. 1) as well as the spleen appeared to be reduced in size when compared with control one (Fig. 2).

Table (1): Mean values ± standard errors of blood serum biochemical parameters in male and female rats exposed to Kafrosol.

Parameter	Groups	Days of exposure							
		15 days		30 days		45 days		60 days	
		Female	Male	Female	Male	Female	Male	Female	Male
ALT	Control	15.50±0.50	13.20±1.06	14.70±0.97	12.40±1.69	17.80±0.80	13.00±2.01	16.10±0.68	12.60±1.74
(IU/L)	Treated	9.50±0.94*	10.60±0.25*	14.60±1.02*	14.10±0.56*	20.30±3.74*	12.60±1.51*	14.60±0.40*	14.30±1.39
AST	Control	111.2±4.93	56.00±6.21	114.00±7.92	57.60±3.39	88.00±5.02	55.00±1.58	105.80±3.73	52.00±1.23
(IU/L)	Treated	105.20±5.45*	63.00±4.36*	101.00±5.12*	58.00±6.04	97.60±5.15*	60.50±2.00	119.20±5.23*	77.20±11.30*
Urea	Control	41.52±3.73	41.26±4.02	39.12±0.81	39.08±6.12	57.84±2.09	36.26±1.44	40.80±1.20	26.68±2.75
(mg.%)	Treated	44.64±2.19	44.44±6.13	58.56±3.84*	42.08±4.29	60.64±3.55	49.90±5.33*	49.40±4.13*	54.96±5.42*
Creatinine	Control	1.20±0.02	0.72±0.02	1.10±0.06	0.68±0.06	1.20±0.02	0.82±0.08	1.20±0.09	0.90±0.08
(mg/dl)	Treated	0.88±0.04	0.88±0.02*	1.10±0.17	0.98±0.04*	1.02±0.04	0.84±0.05	1.30±0.05*	0.70±0.06*

IU/L : One international unit per litre
ALT: Alanine Amino Transferase
AST: Asparate Amino Transferase
* P<0.05

Table (2): Mean values ± standard errors of blood erythrocytic parameters in male and female rats exposed to Kafrosol.

Parameter	Groups	Days of exposure							
		15 days		30 days		45 days		60 days	
		Female	Male	Female	Male	Female	Male	Female	Male
PCV	Control	47.80±0.37	47.40±1.62	35.90±2.21	40.50±1.41	35.10±1.66	39.80±1.09	42.50±3.10	36.40±1.25
(%)	Treated	52.00±0.63	42.70±2.20*	41.80±1.39*	42.00±2.72	37.50±2.88	39.00±3.11	45.40±1.07	40.90±1.31
HB	Control	13.00±0.55	11.60±0.49	12.30±0.52	18.80±1.80	14.10±0.46	12.90±0.84	14.50±1.07	11.40±0.49
(gm/dl)	Treated	12.90±0.37	13.30±0.77	16.40±1.24*	16.00±1.30	14.30±0.49	12.60±1.07	15.90±1.17	13.00±0.58
RBCs.	Control	6.71±0.24	7.41±0.33	6.57±0.48	6.80±0.28	7.18±0.32	7.29±0.44	6.27±0.83	67.30±0.69
($x10^6$ µl)	Treated	6.05±0.02	8.13±0.35	7.72±0.34	7.72±0.62	6.38±0.43*	7.29±0.50	7.97±0.38*	67.15±0.26
MCV	Control	71.76±2.10	48.66±1.84	55.06±2.83	60.56±3.48	54.32±3.38	55.26±3.80	59.66±2.76	51.70±3.92
(FL)	Treated	84.72±2.89*	52.700±2.85	46.56±2.86*	56.36±7.32	52.66±1.99	53.48±2.61	53.52±1.47	57.22±0.76
MeHc	Control	27.90±1.62	31.80±0.20	34.40±0.80	37.72±3.27	37.04±0.52	23.58±1.46	35.26±1.08	31.34±1.17
(%)	Treated	25.54±0.24	31.06±0.30	38.69±0.59	37.08±0.97	36.30±0.89	32.24±0.64	37.08±1.28	32.24±2.24

PCV : Packed Cell Volume
HB: Hemoglobin concentration
MeHc: Mean corpuscular hemoglobin concentration
MCV : Mean corpuscular volume
* P<0.05

Table (3): Mean values ± standard errors of total and differential leucocytic count in male and female rats exposed to Kafrosol.

Parameter	Groups	Days of exposure							
		15 days		30 days		45 days		60 days	
		Female	Male	Female	Male	Female	Male	Female	Male
WBCs.	Control	7.30±0.40	13.14±2.01	11.45±0.81	12.41±1.80	12.63±0.78	11.78±1.46	9.88±2.19	7.39±0.61
(x10^3/µl)	Treated	12.23±0.60*	17.32±2.65*	9.03±0.92	13.87±2.97	9.89±1.26*	10.38±0.86	13.21±1.72*	15.95±2.16
Lymphocyte (x10^3/µl)	Control	5.62±0.83	7.46±1.31	8.94±0.64	7.94±0.32	11.31±5.00	10.45±0.79	7.87±1.65	5.71±0.60
	Treated	8.56±0.58*	12.58±2.23*	7.10±0.85	12.29±2.52*	8.60±0.64*	9.14±0.78	7.96±1.39	12.70±1.21*
Neutrophil (x10^3/µl)	Control	1.30±0.59	4.33±0.73	2.37±0.49	2.55±0.91	1.41±0.26	0.71±1.16	1.39±0.11	1.58±0.35
	Treated	3.11±0.21	2.07±0.27	1.90±0.62	1.52±0.29	1.75±0.37	1.51±0.52*	3.79±0.63*	3.29±1.60*

* P<0.05

V- Histopathological findings:

The microscopical changes in all treated groups were nearly the same but widely differ in severity and extension, with the increase of exposure time.

Lungs: Some bronchi and bronchioles showed proliferation and folding of their epithelial lining (Fig.3), while others suffered from degeneration and destruction. Large foam cells were demonstrated in many alveoli (Fig. 4). These were associated with thickening of alveolar wall . Most of the pulmonary blood vesels were greatly congested and exhibited swelling of the endothelium, with areas of haemorrhage, consequemtly haemosidrin pigments were observed either free or trapped inside macrophages and this was confirmed by the prussian blue reaction (Fig. 5).

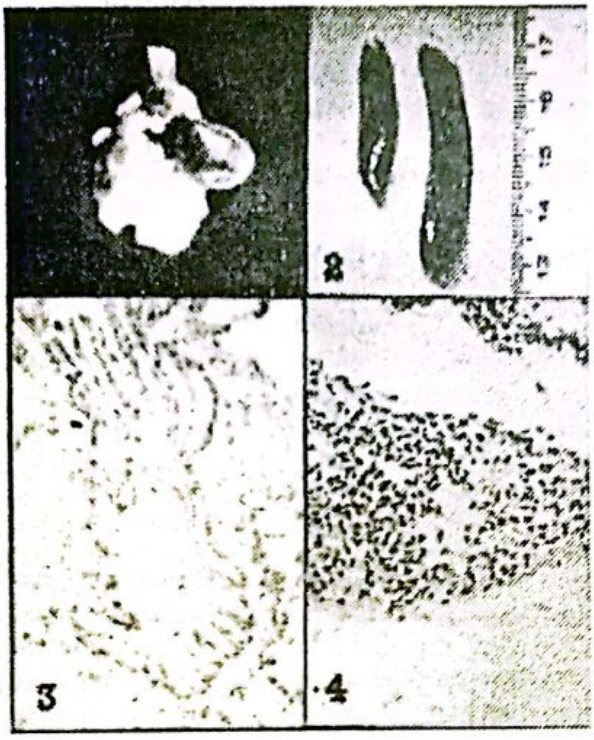
Liver: It showed disarrangement and distorsion of hepatocytes due to variable degenerative changes. Some hepatocytes were abnormally enlarged and became more eosinophilic while others suffered from vacuolation which was proved to be fat with sudan black B (Fig. 6). Furthermore small focal areas of mononuclear cells infiltration were demonstrated between hepatic parenchyma (Fig. 7). In the majority of cases, the central veins and hepatic sinusoids were dilated and engorged with blood with areas of haemorrhage between hepatic parenchyma.

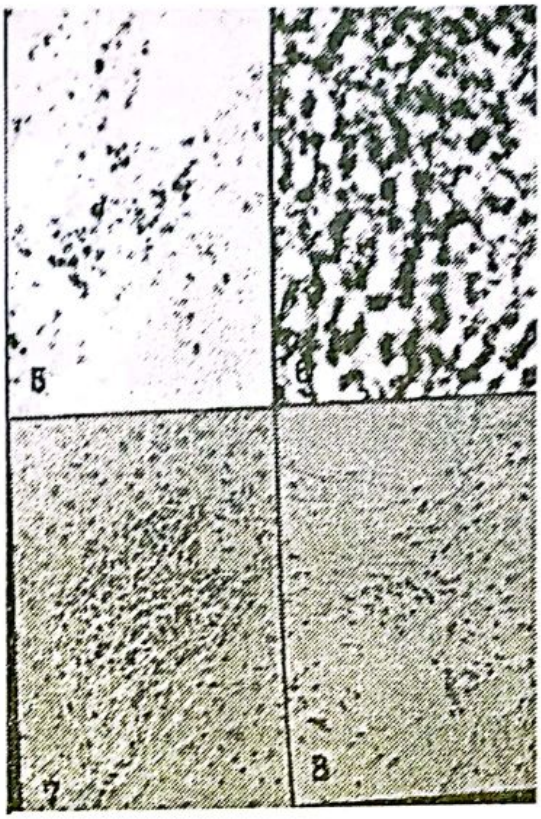
Heart: The myocardium suffered from several degenerative changes which ranged between slight swelling and granularity of cytoplasm, loss of striation, eosinophilia and to complete necrosis. These were in association with vasculitis and mononuclear cells infiltration mostly macrophages and lymphocytes (Fig. 8).

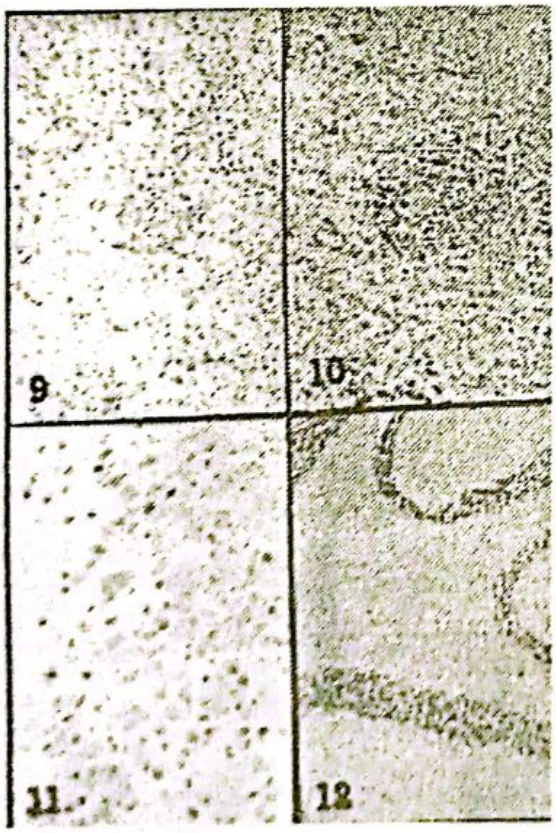
Kidney: The epithelial lining of some tubules suffered from coagulative necrosis specially the proximal convoluted tubules (Fig. 9), while other tubules contained hyaline casts. Moreover, the cortical and medullary blood vessels were congested.

Spleen: The splenic tissue was over loaded by hemosidrin pigments (Fig. 10). Few cases showed haemorrhagic areas within the white pulp, whereas some lymphoid follicles showed depletion.

Brain : The cerebral neurons particularly the large pyramidal cells suffered from necrobiotic changes (Fig. 11). The while metter of cerebellum showed demylination (Fig. 12) and degeneration of Purkinje cells. Focal and diffuse gliosis could also be seen.







DESCRIPTION OF FIGURES

Fig. 1: Heart. notice severe congestion
Fig. 2: Spleen. notice contracted spleen
Fig. 3: Bronchiole showing finger like projection with mononuclear cells and cellular debris. H & E x 250.
Fig. 4: Interstitial pneumonia. notice foam cells with faint pink vacuolated cytoplasm and eccentric nuclei. H & E x 400.
Fig 5: Lung showing macrophages loaded with haemosiderin pigment. Prussian blue. x400.
Fig. 6: Hepatocytes with numerous fat globules of variable sizes. Suddan black B.x 400.
Fig. 8: Higher magnification of degenerated cardiac muscles with round cell infiltration.. H & E x 400.
Fig. 9: Kidney tubules showing necrobiotic changes. H & E x 400.
Fig. 10: Spleen. notice depletion of white pulp with haemosidrosis. H & E x 250.
Fig 11: Cerebral cortex. notice ischemic neuronal degeneration. H & E x 400.
Fig. 12: Cerebellum. notice demylination of white matter. H & E. 100.

DISCUSSION

This study was performed to clarify the hazard effect of kafrosol fort on workers involved in handling and spraying these chemicals and also on animals which were accidentally exposed to aerosoling . There were no available literature could be obtained about the effects of kafrosol fort, so discussion of our results will depend on the effect of one or more of the ingredients that are present in the commercially used kafrosal cans, generaly the pyrthroids.

The values of ALT in treated male and female rats with pyrothroids showed fluctuated activity through out the experiment . Jess (1982) explained that ALT activity is elevated in serum only in case of extensive liver damage and acut hepatitis. Furthermore activity of serum AST was significantly increased in male rats throughout the experiment and during the peroid 45th, 60th of exposure days in female rats, Medway et al., 1969 attributed this increase due to liver, heart and kidney affection. The difference in results between males and females rats was due to differences in the quanitity and quality of enzyme system responsible for biotransformation of pyrethroids Testa and Jenner, (1976). The marked increase in the values of blood urea and serum creatinine specially at 60 days post exposure indicated reduction in glomerular filteration rate as well as impairment of renal blood flow. These results agreed with Syzuki et al., (1980).

The study of haemogram in treated rats revealed polycythemia, the results agreed with Coombs et al., (1976). The leucogram revealed leuckocytosis which was a direct consequence of neutrophilia, lymphocytosis or both. Neutrophilia was attributed to stress factor and increased muscular activity Duncan and Keith, (1986) while lymphocytosis could be attributed to the cytotoxic effect of the pyrethroids on immune system.

From the pathological point of view, it was noticed that the blood vessels and capillaries of the lungs were congested with swelling of their endothelial lining and subsequent irregular areas of haemorrhages. This could be attributed to the direct effect of the toxic material on the lung vasculature due to diffusion of small sized particles of the aerosol through the air blood barrier Shehata et al. (1991), whereas Miyamoto, (1976) didn't mention any of these finding and this difference might be due to chemical nature, concentration, route of inoculation and rate of absorption.

The presence of interstitial pneumonitis could be attributed to the diffusion of aerosol particles to alveoli causing its damage, and that initiate a sort of proliferation of alveolar cells on one hand and degeneration or chronic irritation on the other, and consequently proliferation of large number of mononuclear cells. These findings were coincided with Phillips et al., (1985).

The microscopical examination of parenchymatous organs (Liver, kidney and heart) indicated that they were greatly affected due to the direct effect of pyrothroids on them . these findings were in full aggreement with Nabila (1990). The spleen showed depletion of lymphoid follicles and these result, emphasized the bad toxic effects of the different ingredients of kafrosol on the lymphopiotic tissue as pyrethroids inhibited to mitogenic responsivness of murine splenic lymphocytes Stelzer and Gordon (1984). The effect of pyrethroids on the brain was quite obvious from the histopathological finding as congestions, neuronal degeneration and demylination. These results were greatly suggestive for the neurotoxic action of pyrethroids which act on central nervous system.

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