Research Paper

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Pyrethroid Inhalation Induced Histochemical Changes in the Liver of Albino Rats

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This investigation deals with the effect of inhalation of the pyrethroid, tetramethrine, on the histochemical components of the liver of albino rats. This pyrethroid is obtained from local market and is used as an insecticide for cockroaches and ants. Animals were kept individually in closed cages and 1ml of the pyrethroid was sprayed into each cage for 5 minutes daily for 15 days. The results showed that pyrethroid inhalation induced a significant decrease in the carbohydrate inclusions, total proteins and RNA content in the liver cells of treated animals. The magnitude of these changes was time-dependent.

Key words: Rat, liver, histochemistry, pyrethroid

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Introduction

Pollution of the environment by pesticides and their persistence in air, water, soil and food stuffs can cause serious health hazards (McEween and Stephenson, 1979). Pyrethroids have been knowvn as insecticides for many years. Their source is the flowvers of the pyrethrum plant *Chrysanthemum cineraria folium* (McLaughlin, 1973). Due to the persistence of these pesticides in the environment, structures similar to these pyrethroids have been synthesized (Casida, 1973). These insecticides are widely applied, in viewv of the fact that they have been proved to possess a highly insecticidal activity as vell as a broad spectrum of high initial toxicity of action on several types of pests (Narahashi, 1971).

The toxicity of pyrethroid insecticides to mammals has received much attention in recent years. Animals exposed to these insecticides exhibited changes in their physiological activities beside other histopathological changes (El-Dessouky *et al*., 1986; El-Elaimy, 1986; El-Banhavvy *et al*., 2000). Insecticides vere found to induce histochemical changes, and intoxicated tissues showed alterations in carbohydrates (El-Beih *et al*., 1991), total proteins (El-Beih *et al*., 1992; Abdeen *et al*., 1994) and other chemical components (El-Banhavvy and Ganzuri, 1983).

In a previous work, Sakr (1999) found that inhalation of tetramethrine induced many histopathological changes in the liver of rats. This work intends to study the effect of inhalation of the same pyrethroid (tetramethrine) on the histochemical contents of the liver of albino rats.

Materials and Methods

Eighty adult male albino rats (*Rattus norvegicus*), weighing 275±5gm, were used. Animals were kept in the laboratory under a constant temperature (24±2°C) for at least one week before and throughout the experimental work, being maintained on a standard diet with water available ad libitum. Animals were divided into two groups. Rats in the first group (50 animals) each inhaled 1 ml of the pyrethroid tetramethrine once per day for 15 days. Animals were kept in an individual closed cage and the pyrethroid was sprayed into each cage for 5minute (Sakr, 1999). This pyrethroid is obtained from local markets and is used as an insecticide for cockroaches and ants. It contains 0.20% tetramethrine, 1% propoxur and 98.8% solvents and propellants. Animals in the second group (30 animals) were used as controls. For histochemical study, treated animals and their controls were sacrificed by decapitation after 9, 12 and 15 days of treatment and their livers were removed, fixed in Carnoy's fluid. After fixation, the tissues were processed for embedding in paraffin wax. Using a rotary microtome, specimens were sectioned at 5 μm and sections were mounted on clean slides. Periodic acid Schiff's (PAS) reaction was used for detection of general carbohydrates (Hotchkiss, 1948), Mercury-bromophenol blue (Mazia *et al*., 1953) was applied for visualization of total proteins and the Feulgen-methylene blue method of Garvin *et al*. (1979) was used for the demonstration of nucleic acids (RNA, DNA).

Results

General carbohydrates: Liver sections of control rats stained by PAS method showed normal carbohydrate content and distribution in the form of deeply stained reddish granules in the cytoplasm of the hepatic cells, but the nuclei gave a negative reaction (Fig. 1). Table 1 showed the change in general carbohydrates content of the liver of treated animals. Liver cells of animals treated with tetramethrine for 9 days showed a moderate depletion of the carbohydrate inclusions.

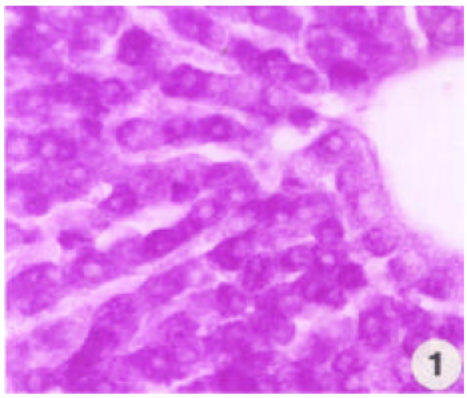

Fig. 1: Liver section of a control rat showing PAS-positive inclusions in the hepatocytes (X 300).

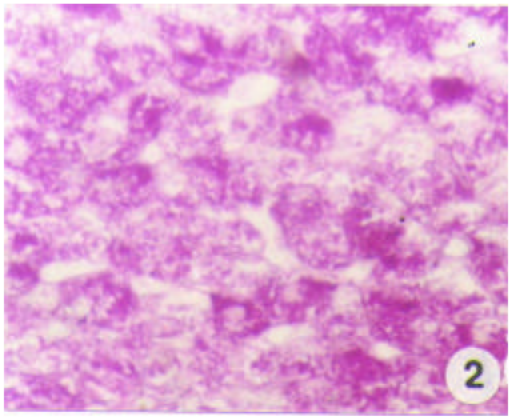

Fig. 2: Liver section of a rat treated with tetramethrine for 12 days showing a decrease of general carbohydrates in most of the cells (X 300).

After 12 days of treatment, most of the hepatic cells contained scarce amount of carbohydrate except few ones which showed moderate amount (Fig. 2). A marked reduction of carbohydrates was detected after 15 days of treatment (Fig. 3).

Total Protein: The protein materials in the liver cells of control rats were displayed in the form of small bluish irregular particles which were sometimes closely packed together making blue irregular dense bodies against a weakly to moderately stained ground cytoplasm (Fig. 4). The cells were limited by intensely stained cell borders. Each nucleus contained positively stained nucleoli together with some chromatine particles. Examination of rat liver after 9 days showed that some hepatocytes appeared with a slight decrease in the protein materials (Fig. 5). After 12 and 15 days-post tetramethrine treatment, there were obvious decrease in the protein contents. Some cells especially in 15 days treated animals were completely devoid of proteins and their remnants were mainly located at the peripheries of the cell which showed cytoplasmic vacuolation (Table 1, Fig. 6).

Ribonucleic acid (RNA): Using Feulgen methylene blue method, the RNA-containing particles appear in the hepatic cells of normal rats as small bluish-coloured particles distributed throughout the cytoplasm. A similar colouration was shown in the nucleoli denoting their content of RNA and the nuclei

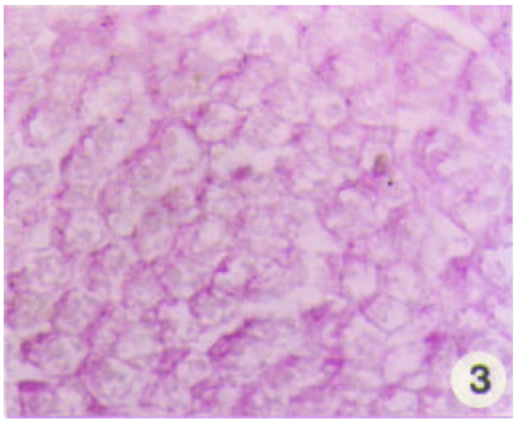

Fig. 3: Liver section of a rat treated with tetramethrine for 15 days showing marked decrease of carbohydrates (X 300).

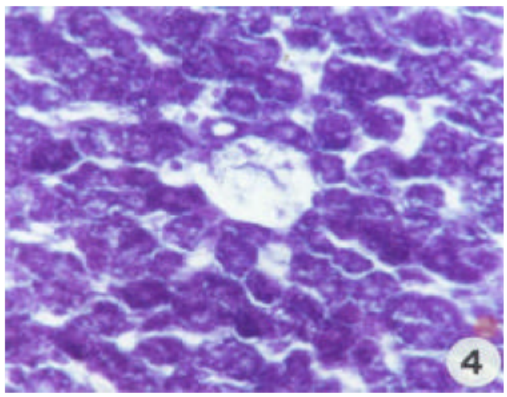

Fig. 4: Section in liver of a control rat stained by bromophenol blue showing strong protein staining in the cytoplasm and nucleolus (X 300).

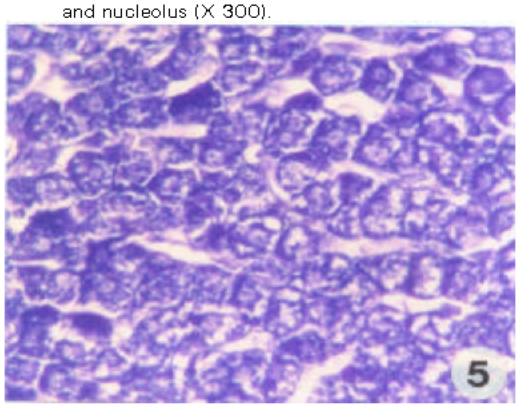

Fig. 5: Liver section of a rat treated with tetramethrine for 9 days showing moderate decrease of protein content in the hepatocytes (X 300).

exhibited a red colour indicating their DNA contents (Fig. 7). Hepatic cells of rats examined after 9 days of treatment showed slight decrease in RNA contents especially in the cytoplasm. A noticeable decrease in RNA particles was observed after 12 and 15 days of treatment (Table 1 and Fig. 9). The Fig. 9 also showed that DNA containing particles were decreased in the nuclei of the hepatic cells.

Table 1: Changes in general carbohydrates, total proteins and RNA content in the liver of tetramethrine-treated rats.

Days after treatment	General carbohydrates	Total proteins	RNA
Control	+ + +	+ + +	+ + +
9	+ +	+ +	+ + +
12	+	+	+ +
15	+ / -	+	+ / -

(+ + +): high, (+ +): moderate, (+): slight, (-): depleted

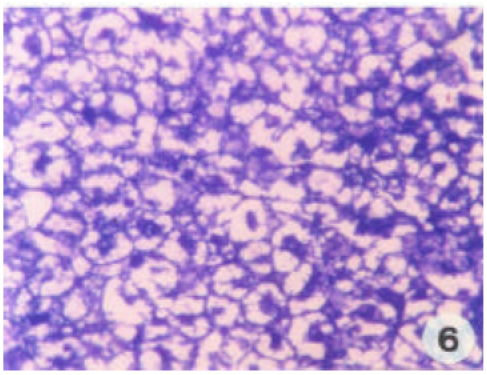

Fig. 6: Liver section of a rat treated with tetramethrine for 15 days showing obvious reduction in protein content (X 300).

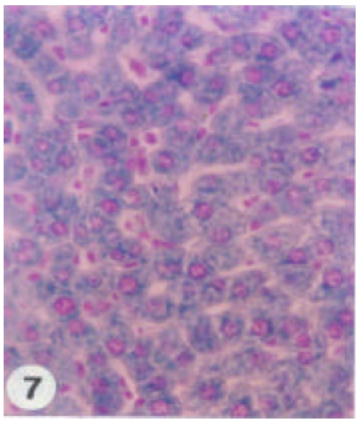

Fig. 7: Section in the liver of a normal rat stained with Feulgen methylene blue methods showing RNA inclusions in the form of small bluish coloured patches distributed in the cytoplasm. The nuclei exhibiting red colour stainability due to their DNA content (X 300).

Discussion

The results showed that inhalation of tetramethrine induced a reduction in general carbohydrates in the liver of rats. Such observation is in close agreement with that of Abdeen *et al.* (1994) who found that fenvalerate decreased both mucopolysaccharides and liver glycogen in mice. Liver glycogen was significantly decreased in chicken treated with cypermethrin (El-Saify and El-Shater; 1993). On the contrary, Abdel-Raheem *et al.* (1987) reported that glycogen increased in liver, but decreased in the brain and kidney of rats intoxicated with the pyrethroid, ripcord. Disturbances in carbohydrate metabolism were observed in a variety of animals under the effect of different insecticides and

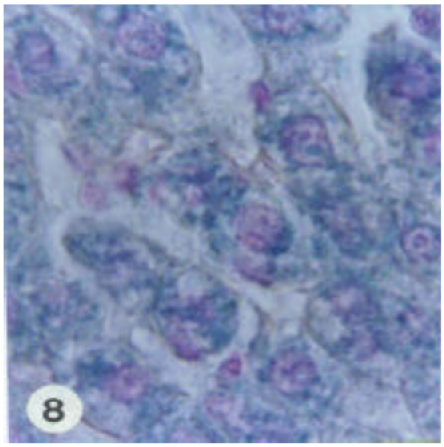

Fig. 8: Section in the liver of a rat treated with tetramethrine for 9 days showing a decrease in RNA content, (X 850)

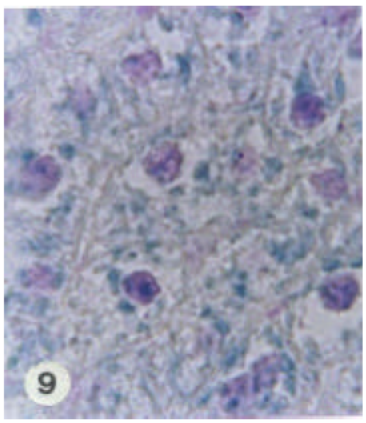

Fig. 9: Liver section in a rat treated with tetramethrine for 15 days showing noticeable decrease in RNA in the hepatocytes. (X 850)

were suggested to be achieved through modifying the activities of the enzymes of glycolytic pathway, TCA cycle, glucogenesis and the oxidative phosphorylation (Kacew and Singhal, 1973; Shakoori *et al*. 1988). It was also reported that some insecticides may affect the carbohydrate metabolism through their effects on the endocrine system, especially by modifying the secretion of glucocorticoids and insulin (Pilo and Mehan, 1987). However, one or more of such factors could be considered as the causal agent of carbohydrate reduction observed in the liver of tetramethrine-treated animals.

Results obtained in this work also revealed that rats treated with tetramethrine showed a marked decrease in liver proteins. Similarly, Abdeen *et al*. (1994) indicated that fenvalerate caused a decrease in protein content of mice hepatocytes. They added that protein decreased as a result of vacuolation and degeneration of the cells. Repeated administration of the pyrethroid ripcord induced a fluctuated reduction in the protein content, in liver, kidney and brain of rats (Abdel-Raheem *et al*., 1987).

The interference of insecticides in protein metabolism has been reported by many authors (Wilson *et al*., 1970; Surinder and Pawar, 1975; Mitjavila *et al*., 1981). Therefore, the reduction in protein content observed in this work may be attributed partially to the decreased level of hepatic protein synthesis in the cells suffering from pathological changes due to the hyperactivity of hydrolytic enzymes (Sivaprasada *et al*., 1983).

It has been noticed that nucleic acid, (RNA) exhibits a reduction in the hepatocytes of rats under the effect of tetramethrine. This result is in agreement with those of other studies which described such signs in the same organ as a consequence of treatment with pyrethroids. In this respect, Sanad (1989) reported a marked loss of RNA content in the liver cells of rats following treatment with permethrin. Abdel-Basset and Zaki (1990) observed a reduction of RNA content in hepatocytes of rats intoxicated with fenvalerate. Similar reduction in RNA was recorded in animals treated with different insecticides. Dieldrin and Sevin were noted by Riad (1971) to induce RNA reduction in liver cells of Guinea pigs. El-Ganzuri (1975) observed that the insecticides lindane, gusathion and tamaron exerted an obvious reducing effect on RNA inclusions in the rat liver cells. El-Elaimy and Hasab El-Nabi (1990) reported that the insecticide, roger induced marked loss of RNA in liver cells of mice. Treating guinea pigs with lannate caused a reduction of RNA in hepatocytes (El-Beih *et al*., 1992).

Many factors have been suggested to interfere in the reduction of RNA. One of the main factors is the lysosomal particles and their contents of hydrolytic enzymes. Among these enzymes, ribonuclease (RNAase) and deoxyribonuclease (DNAase). Once the lysosomal membranes are disruptured under any unusual conditions, such enzymes become free in the cytoplasm bringing about marked lysis and dissolution of the target materials, RNA and DNA. This concept confirms the findings of Anima (1968) who found that RNAase and DNAase increased after treatment with Sevin. Moreover, Awasthi *et al*. (1984) related the increased lysosomal enzymatic activity accompanied by a decrease in protein and RNA contents, to the adverse effects of organophosphate insecticide on the lysosomal membranes which release nucleases and proteases affecting RNA and protein metabolism.

From the foregoing consideration, it is worthy to mention that inhalation of the pyrethroid ,tetramethrine has a destructive effect on the investigated histochemical parameters. Therefore, the use of such insecticides must be under strict control.

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