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Histopathological Alterations in the Liver and Kidney of Toads *(Bufo regularis)* Intoxicated with a Pyrethroid Insecticide

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Abstract: The effect of the pyrethroid insecticide, fenvalerate, on the liver and kidney of toad (*Bufo regularis*) was studied. Feeding toads with a daily dose of fenvalerate (0.5 mg/kg body weight) for three weeks caused histopathological alterations in these organs. The normal structural organization of the hepatic acini was impaired, the hepatocytes showed cytoplasmic vacuolation, the blood vessels were congested and there was remarkable abundance of leucocytic infiltrations. In the kidney, the renal tubules were degenerated and the glomeruli were atrophied. Moreover, the transaminases enzymes GOT (glutamate-oxaloacetate transaminase)and GPT(glutamate-pyruvate transaminase) were elevated in the sera of treated animals. The magnitude of the changes was time-dependent being more prominent after the third week of treatment with fenvalerate.

Key words: Toads, liver, kidney, fenvalerate, transaminases

Introduction

Pyrethroids represent a new class of insecticides which showed excellent insecticidal properties with good biodegradability and they are highly active insecticides in considerable lower quantities compared to other insecticides (Souyri and Hoellinger, 1983). Toxicity of pyrethroids was studied in different animals and it was found that these insecticides have neurotoxic (Crafton *et al*., 1995) and genotoxic effects (Amer *et al*., 1993). He *et al*. (1989) reviewed 573 cases of acute pyrethroid poisoning, including 344 cases of accidental and 229 cases of occupational poisoning, reported in Chinese medical literature during 1983-1988. Most of the cases of poisoning were caused by deltamethrin (167 accidental, 158 occupational) followed by fenvalerate (133 accidental, 63 occupational) and cypermethrin (39 accidental, 6 occupational).

Some of the pyrethroids were found to induce histopathological alterations in the liver (Okuno *et al*., 1986; Abou-Zaid and El-Balshy, 1995; Luty *et al*., 1998; Sakr, 1999) and kidney (Parken *et al*., 1986, Abou-Zaid and El-Balshy, 1995, Sakr *et al*., 2001) of mammals. They also produced changes in enzyme levels (Breckenridge *et al*., 1982; El-Elaimy, 1986; Abu-El-Zahab *et al*., 1993). Little informations (Sakr and Al-Sahaf, 1996; Sakr and Hijji, 2000) are available on the effect of insecticides on amphibia. This experiment was planned to study the effect of pyrethroid insecticide, fenvalerate, on the liver and kidney of the toads *(Bufo regularis)* as a biological test animal.

Materials and Methods

Sexually mature male toads *(Bufo regularis)* (45 ± 5 g) were used in this experiment. They were transported to the laboratory of Zoology Department, Faculty of Science, Aswan, Egypt in January, 2002 and kept in large aquaria with small amounts of water which were changed twice daily. Toads were divided into two groups. Animals in the first group (25 toads) were enforced fed with the pyrethroid insecticide "fenvalerate" dissolved in tap water at a dose level of 0.5 mg kg^{-1}. body weight ($1/10LD_{50}$/4 days) once per day for 3 weeks. Toads of the second group (15 toads) were served as controls. Animals were killed and dissected after 1, 2 and 3 weeks treatment and their livers and kidneys were removed. For histological examination, tissues were fixed in Bouin's fluid, embedded in paraffin wax and sectioned at 5 microns thickness. The sections were stained with haematoxylin and counter stained with eosin. For enzyme study, sera were obtained by centrifugation of the blood samples and stored at -20 ^{0}C. GOT (glutamate-oxaloacetate transaminase) and GPT (glutamate-pyruvate transaminase) were measured using a fully automated Hitachi 911 analyzer (Tokyo, Japan). A commercial randox kits (randox Laboratories, Ltd, Ardomre, Crumlin, U.K.) were used in these analysis. The results were statistically analyzed using Student's "t" test (Snedecor and Cochran, 1980).

Results

Histological examination of the liver of control toad, showed that it is formed of numerous acini. Each acinus is composed of polygonal or rounded hepatocytes surrounding a bile canaliculie. The hepatic cell contains a relatively large nucleus and eosinophilic cytoplasm. The acini are separated from each other by blood sinusoids which are irregular narrow blood spaces. Among the acini there are pigment granules. The central veins have generally a circular outline and the portal veins are comparatively large in size being either empty or containing a few blood cells. The bile ductule appeared rounded and is bounded by a layer of cuboidal cells (Fig. 1).

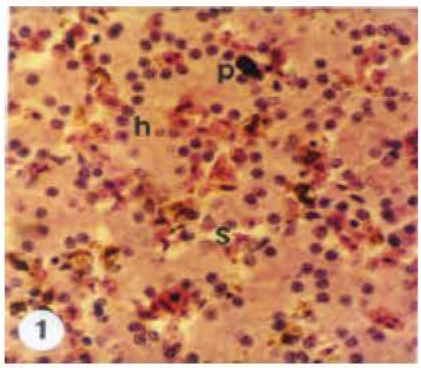

Fig. 1: Section in the liver of a control toad showing hepatic acini (h), pigments (p) and sinusoidal space (s), x400

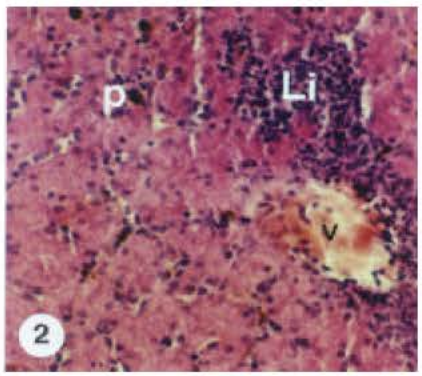

Fig. 2: Section in the liver of a toad treated with fenvalerate for one week showing mass of leucocytic infiltration (Li) and congested vein (v), x320.

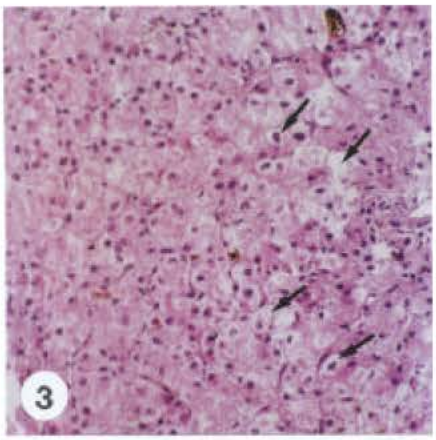

Fig. 3: Section in the liver of a toad treated with fenvalerate for 2 weeks showing cytoplasmic vacuolation in large number of the hepatocytes (arrows) with pyknotic nuclei, x320

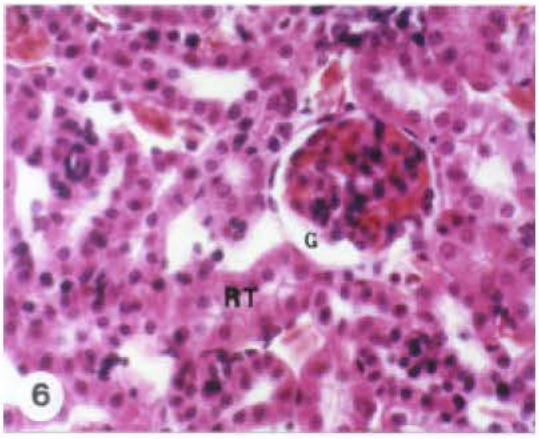

Fig. 6: T.S. in the kidney of a control toad showing a glomerulus (G) and renal tubules (RT), x400

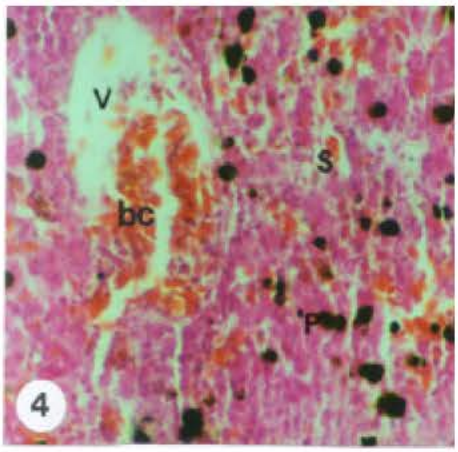

Fig. 4: Section in the liver of a toad treated with fenvalerate for 3 weeks showing impairment of normal organization of hepatic acini. The vein (v) appeared congested and filled with blood cells (bc), x320.

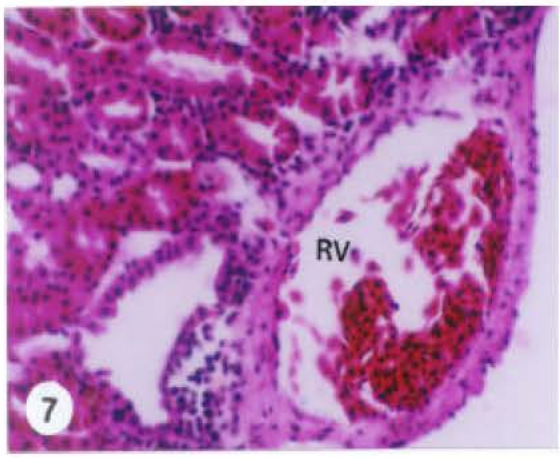

Fig. 7: T.S. in the kidney of a toad treated with fenvalerate for one week showing dilated renal vein (RV) and engorged with blood cells, x320.

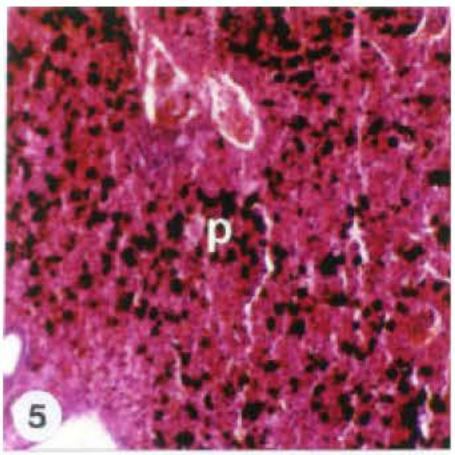

Fig. 5: Section in the liver of a treated toad showing a marked increase of pigment granules (p), x320.

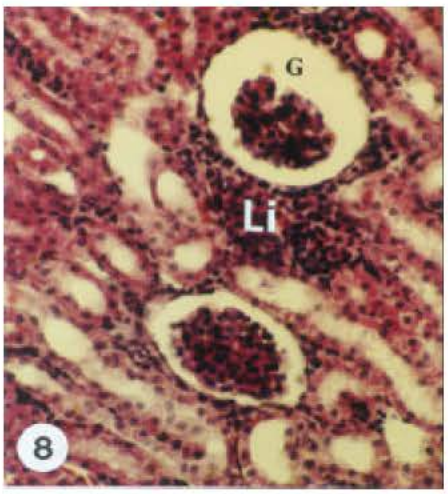

Fig. 8: T.S. in the kidney of a toad treated with fenvalerate for 2 weeks showing leucocytic infiltration (Li) and atrophied glomerulus (G), x320.

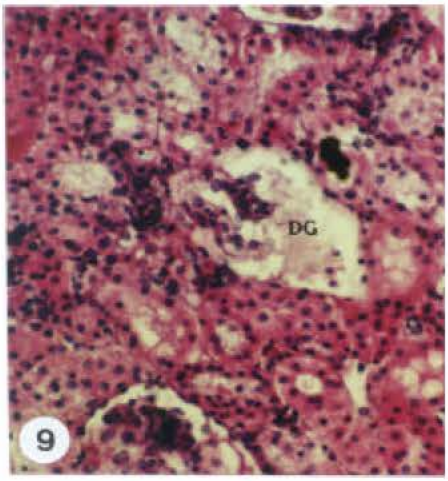

Fig. 9: T.S. in the kidney of a toad treated with fenvalerate for 3 weeks showing abnormal configuration of the renal tubules and degenerated glomerulus (DG), x320

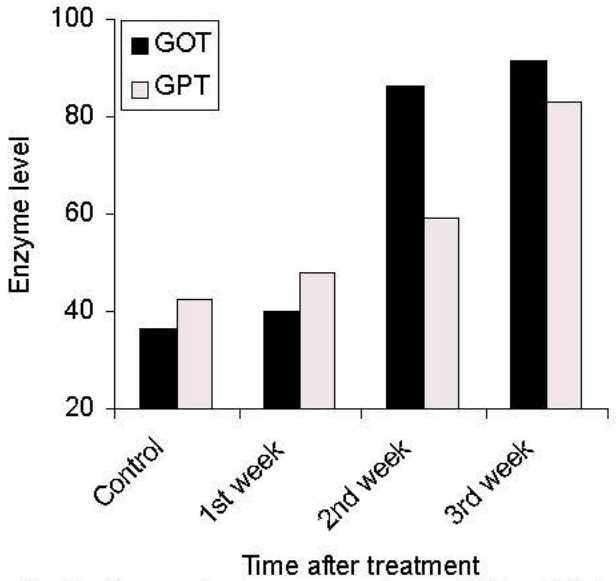

Fig. 10: Changes in serum transaminases (GOT, GPT) in experimental toads treated with fenvalerate

Examination of liver sections obtained after one week of treatment with fenvalerate revealed some histopathological changes. The blood vessels were congested and engorged with blood and their lining epithelium was eroded. Inflammatory leucocytic infiltrations comprised mainly of lymphocytes and sparse eosinophils was seen in several liver areas especially near the blood vessels (Fig. 2). After 2 weeks of treatment, the hepatic acini were destroyed and the hepatocytes showed cytoplasmic vacuolation with pyknotic nuclei (Fig. 3). The cell membrane of these cells were unrecognized and if some of them could exist they were ill-defined and ruptured. Liver sections examined after three weeks of treatment with fenvalerate showed an advanced degree of the previous histopathological changes. The normal organization of the hepatic acini was impaired and the hepatic cells were highly damaged. The blood vessels were enlarged and congested. The sinusoidal spaces were filled with blood which indicated clear phenomenon of internal haemorrhage (Fig. 4). Moreover, the pigment granules were markedly increased in comparison with that in control animals (Fig. 5).

Fig. 6 showed the histological structure of kidney of control toads. Kidney of animals examined after one week of treatment with fenvalerate showed that the renal vessels (veins and arteries) were dilated, congested and filled with blood (Fig. 7). After two weeks of treatment with fenvalerate, the intertubular spaces were infiltrated by masses of leucocytes. The epithelial lining cells of most of the renal tubules appeared with cloudy swelling of their cytoplasm. The glomeruli were shrunken and atrophied (Fig. 8). These histopathological changes were increased in kidney of toads inspected after three weeks of treatment. The renal tubules showed abnormal configurations with necrotic cells and some cells appeared with cytoplasmic vacuolation. The walls of Bowman's capsule were eroded and the glomeruli were markedly atrophied (Fig. 9).

Fig. 10 showed that treating toads with fenvalerate caused an elevation in serum GOT. This elevation was significant ($p<0.05$) after 2 and 3 weeks and the levels of GOT were 86.3 $\pm$ 3.4 and 91.7 $\pm$ 4.2 μl^{-1} after 2 and 3 weeks of treatment, respectively in comparison with 36.5 $\pm$ 3.6 μl^{-1} in controls. On the other hand, a significant increase in serum GPT was observed after 3 weeks of treatment and the mean value was 83 $\pm$ 5.3 μl^{-1} in comparison with 42.7 $\pm$ 2.3 μl^{-1} in controls.

Discussion

The results showed that fenvalerate induced many histopathological changes in the liver of toads. The most marked symptoms of hepatic tissue impairment were destruction of hepatic acini architecture, cytoplasmic vacuolation of the hepatocytes and remarkable abundance of leucocytic infiltrations. The magnitude of these alterations was time dependent being more prominent after three weeks of treatment. These findings receive good support from the observations reported by some investigators who studied the effect of pyrethroids in mammals. Abou-Zaid and El-Balshy (1995) observed necrosis, blood vessel congestion and leucocytic infiltration in the liver of newly born mice that inhaled "Ezalo", a commercial formulation of synthetic pyrethroid, for 15 days. In experimental animals exposed to pyrethrins, the lungs and liver showed considerable congestion and leucocytic infiltration (El-Dessouky *et al*., 1986). Okuno *et al*. (1986) and Kaneko *et al*. (1986) observed multifocal microgranulomas in livers of mice and rats treated with the pyrethroid, fenvalerate. Sakr (1999) reported that rats inhaled the pyrethroid, tetramethrin, showed destruction of liver architecture, cytoplasmic vacuolation of the hepatocytes and leucocytic infiltrations.

The obtained results revealed a significant increase in transaminases (GOT, GPT). Similarly, Foldstrom *et al*. (1988) showed that these enzymes elevated in serum of rats treated with fenvalerate. Transaminases also increased in rats inhaled mixed pyrethroids (tetramethrin and Sumithrin) (Abu-El Zahab *et al*., 1993) and after dermal application of baythroid (El-Elaimy, 1986). It was reported that hepatocellular damage could be correlated with the disturbed enzyme activities. Martin and Associates (1983) announced that liver tissues, which are known for their high content of transaminases (GOT, GPT) lose their enzymes in case of liver cell damage. This ultimately leads to their raised levels in the sera of those animals. Hence they suggested that the higher values of these enzymes, whenever they are detected in the blood sera, should be taken as an indicator of various causes of liver damage. Treating animals with fenvalerate in this experiment increased the levels of serum transaminases. This result, together with the histological observations, indicated that fenvalerate treatment caused liver injury in toads. Treating toads with fenvalerate induced many histopathological alterations in the kidney. The most marked symptoms of renal tissues impairment were destruction of renal tubules, congestion of blood vessels, degeneration of glomeruli and marked abundance of leucocytic infiltration. Although kidney is the second target organ in body for many toxic materials, relatively few studies have been done on the

effect of pyrethroids on such organ. Abu El-Zahab *et el.* (1993) observed congestion of blood vessels, hemorrhage, necrosis and inflammatory leucocytes in kidneys of rats inhaled pyrethroids. Abdeen *et al.* (1994) reported that treating mice with fenvalerate induced renal damage of the epithelial lining of the renal tubule, ruptured of the distal tubules and enlargement of the glomerulei with hydropic degeneration. Abou-Zeid and El-Balshy (1995) reported that inhalation of Ezalo (a synthetic pyrethroid) caused acute tubular necrosis and glomerulonephritis in kidneys of new born mice. Subchronic feeding of decaboxy fenvalerate was found to induce glomerulonephrosis in kidney of rats (Parken *et al.*, 1986). Sakr *et al.* (2001) observed that rats inhaled tetramethrin showed many histopathological changes in the kidney.

Thus, in this study it is speculated that one or more metabolites of fenvalerate may be responsible for histopathological alterations observed in the liver and kidney of the toads (*Bufo regularis*).

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