HISTOPATHOLOGICAL CHANGES IN THE LIVER AND KIDNEY TISSUES OF MARSH FROG (*Pelophylax ridibundus*) INDUCED BY THE ACTION OF TALSTAR 10EC INSECTICIDE

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Abstract. Histopathological biomarkers of toxicity in frog organs are a useful indicator of environmental pollution. The frogs were experimentally exposed to sub-lethal concentrations (0.5mg bifenthrin/g of body weight) of Talstar 10EC for 3 weeks. The present study proves its toxic potential in terms of the damages in liver and kidney level. Tissues were normal in the control group. Hepatic lesions in frog exposed to bifenthrin were characterized by vacuolation of hepatocytes and nuclear pycnosis, perisinusoidal and periportal fibrosis, dilatation of Disse space and sinusoid capillaries, presence of cellular infiltrates. Kidney lesions consisted of dilation of Bowman's space, tubular necrosis, and epithelial cells shows pycnotic nuclei, discrete degree of interstitial edema.

Keywords: bifenthrin, frog, kidney, liver, pesticides, pyrethroid.

INTRODUCTION

Surface water can be contaminated by an effluent pipe from an industrial plant or a sewage-treatment plant; a field from which pesticides and fertilizers are carried by rainwater into a river is another example of a pollution source of water. Since monocultural cultivations favor intensive reproduction of pest insects, application of insecticides is getting increasingly common and the amount of xenobiotics retain in the environment increases. The species most endangered by high doses of various kinds of pesticides are aquatic and terrestrial - aquatic animals living in waters located close to cultivated fields. High concentration of the insecticides is found in the bottom mud layer, which is the wintering place for many species of amphibians [24]. Therefore amphibians are among the animals most exposed to insecticides, not only because of their ecological niche, but mostly because of their reproduction biology [25]. In some regions, pesticides are associated with declining amphibians, but we have a poor understanding of the underlying mechanisms [20].

Talstar 10EC is part of pyrethroid pesticides, whose active ingredient is bifenthrin (100g/l) with $C_{23}H_{22}ClF_3O_2$ formula. It is a broad-spectrum insecticide - acaricide for vine crops, fruit trees, vegetables and ornamentals. Pyrethroid pesticides, synthetic analogues of pyrethrins natural substances produced by a species of *Chrysanthemum cinerariaefolium* ornamental plants have been used since the 1970's to protect grain and other agricultural products from pests and have been subsequently used to control ectoparasites bodies. Their use has increased significantly over the past two decades [2, 29]. These synthetic analogues have emerged as a necessity, since natural substances like pyrethrins were quickly photodegraded [9].

Except that this class of pesticides exhibits a low toxic action for birds and mammals [3], pyrethroids are high risk pesticides to aquatic organisms with high toxicity for fish [6, 26-27] and amphibians.

In the environment, pyrethroid insecticides are relatively rapidly degraded in soil and plants, by hydrolysis and oxidation. They have slight tendency to bioaccumulation in the body [7, 22, 30]. Based on chemical, neurophysiological properties of structure and toxicological action, pyrethroid insecticides have been divided into two types: type I and type II.

Bifenthrin is a contact insecticide which is part of the third generation of pyrethroid insecticides characterized by a strong persistence in the environment and a strong insecticidal action [15]. From a structural point of view, bifenthrin resembles cypermethrin, tetramethrin and permethrin but it shows a higher photostability [10, 12, 31]. Bifenthrin is a type I pyrethroid acting on the central and peripheral nervous system of invertebrates and vertebrates, namely the channels of Na^+ from nerve endings that closes and opens, resulting in presynaptic membrane depolarization and cell death [8]. It also affects the production of cellular ATP [21].

The aim of this study is to investigate the histopathological changes induced by the action of Talstar 10EC insecticide in liver and kidney tissues of marsh frog (*Pelophylax ridibundus*).

MATERIALS AND METHODS

The study was performed with the approval of the local Committee of Bioethics according to the Romanian law 205/2004 art.7, 18, 22 and regulation number 143/400/2002 for care and use of animals for research purposes. A total number of ten healthy adult frogs (male and female) were used in the study. The animals were captured in spring (April-May) from the surrounding areas of the city Piteşti (South Romania). The animals were kept in laboratory condition in aquaterrarios filled with tap water for five days to test their health and accommodate them for the experiment. The water was changed daily to avoid the accumulation

of toxic substances and the animals were fed "ad libitum".

After acclimatized for 1 week in the lab, the frogs were separated in two lots: (1) lot of control individuals, containing animals kept in laboratory which was subjected with saline solution 6.5‰, (2) a second lot containing animals which were subjected to Talstar 10EC in a dose of 0.5mg bifenthrin/g of body weight.

The toxic was administered by intraperitoneal injection, one injection in two days in a scheme for 3 weeks.

The frogs were killed by decapitation after chloroform anesthesia and fragments from the liver and kidney were quickly removed. Tissues samples were fixed in 8% neutral formalin for poikilothermes for 24h and were processed using a graded ethanol series and embedded in paraffin. Paraffin section were cut 5µm-thick slices using a rotary microtome (Slee Maintz Cut 5062) and stained with: hematoxylin (H) as a general screening method, Sirius red [11] for collagen stain (fibrosis) and Perl's method [17] for ferrous iron. The sections were viewed and photographed using an Olympus microscope with an attached camera.

RESULTS

No histopathological changes were observed in the liver of the control frogs. The structural details of the liver of control *Pelophylax ridibundus* are shown in Fig. 1.

Liver, the metabolic organ for xenobiotic substances, presents histological changes after the administration of Talstar 10EC in concentration of 0.5mg bifenthrin/g body weight.

In the liver parenchyma (Fig. 2a) there are fewer pigment disassimilation deposits reduced in size. Iron deposits, colored blue by Perl's reaction, resulting from hemolysis of erythrocytes are poorly represented and located in Kupffer cells. Sinusoid capillaries are dilated (Fig. 2a), presenting red blood cells in their lumen which agglutinate, together with cellular infiltrates, particularly lymphocytes, in response to the toxic action. Disse space is dilated and filled with collagen fibers with a slight perisinusoidal fibrosis (Fig. 2b-c).

Affected hepatocytes have small pycnotic nuclei and lipid inclusions in the cytoplasm which shows their steatotic degeneration (Fig. 2c). We observed degeneration of hepatocytes in periportal zones which may suggest the influence of toxic compounds in the digestive tract.

At the periphery of liver lobule there is a periportal fibrosis (Fig. 3a). Due to the fact that within the hepatic lobule, the sinusoid capillaries are blocked by gatherings of red blood cells, at the periphery of lobule, blood vessels dilate to form very thin-walled structures designed to ensure perfusion of hepatocytes (Fig. 3b). These structures were observed in the liver of 70% of treated animals.

No recognizable changes were observed in the kidney tissues of control frog (Fig. 4).

Talstar 10EC insecticide toxicity is manifested in the kidney. A concentration of 0.5mg bifenthrin/g body weight causes significant histological changes at both glomerular and proximal convoluted tubules level. Kidney impairment occurs in the renal glomerulus's, which presents an enlargement of the capillaries. Glomerular degeneration is marked by the existence of a much broader Bowman space, compared with the control (Fig. 5).

Nephron tubules are also affected by the toxic action, the greatest degree of damage being manifested in the proximal tubules. Some tubules are completely degenerated, showing cells different shaped nuclei, pushed to the periphery (Fig. 6b). Proximal tubules cells are in an early process of cytolysis, showing vacuoles in the cytoplasm (Fig. 6b). The toxic acts through a process of narrowing the tubules lumen; there are tubules with lumen completely obstructed by the deposit of hyaline material (Fig. 6a). The renal parenchyma has some tubular necrosis (Fig. 6a), another proximal convoluted tubules present early fibrosis (Fig. 6b) and a discrete degree of interstitial edema.

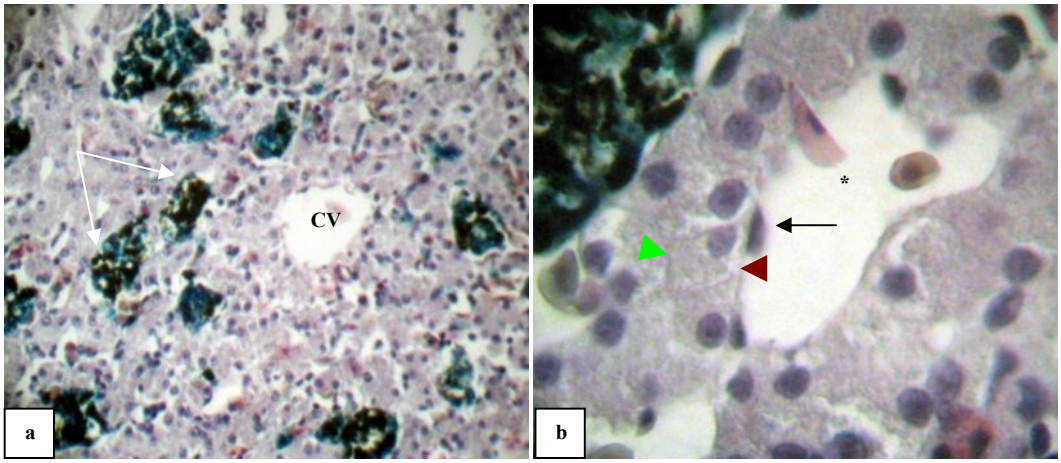

Figure 1. Liver of control animals had a typical parenchymatous appearance. a- centrolobular vein (CV); lipofuscin deposits (white arrows).100×. b- centrolobular vein (*), biliary pole (green arrow), vascular pole (red arrow), endothelial cell (black arrow). 400×. Perl's staining, H-Sirius red.

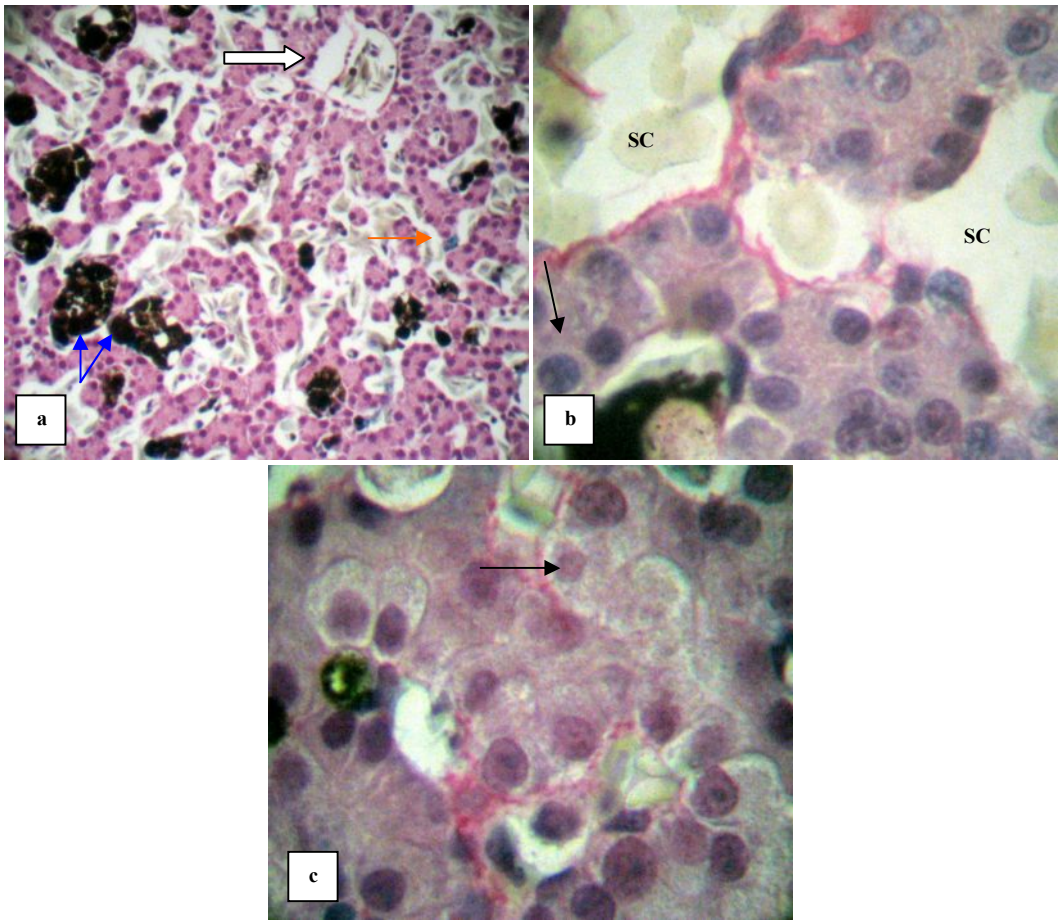

Figure 2. Liver in frogs treated with Talstar 10EC insecticide. **a** - extended sinusoid capillaries (white arrows), lipofuscine (blue arrows) and iron deposits (yellow arrow); centrolobular vein (white arrow). 100×. **b** - perisinusoidal fibrosis (black arrow), SC- sinusoid cappilaries with agglutinated erythrocytes. c- hepatocytes with smaller nuclei and accumulation of lipids in the cytoplasm (black arrow). 400×. Perl's staining, H-Sirius red.

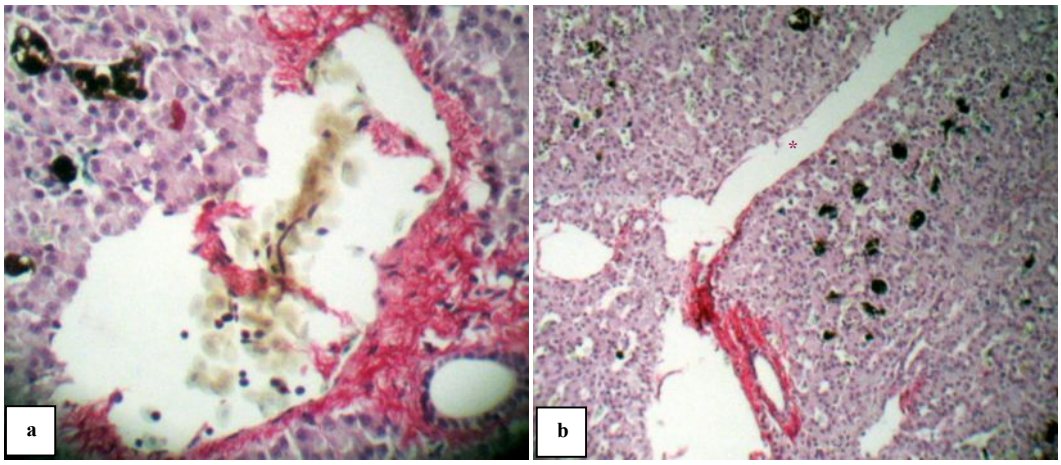

Figure 3. Liver of frogs from experimental group: **a** - periportal fibrosis. 100×; **b** - emergence of more dilated vascular structures at the periphery of the lobule (*). 40×. Perl's staining, H-Sirius red.

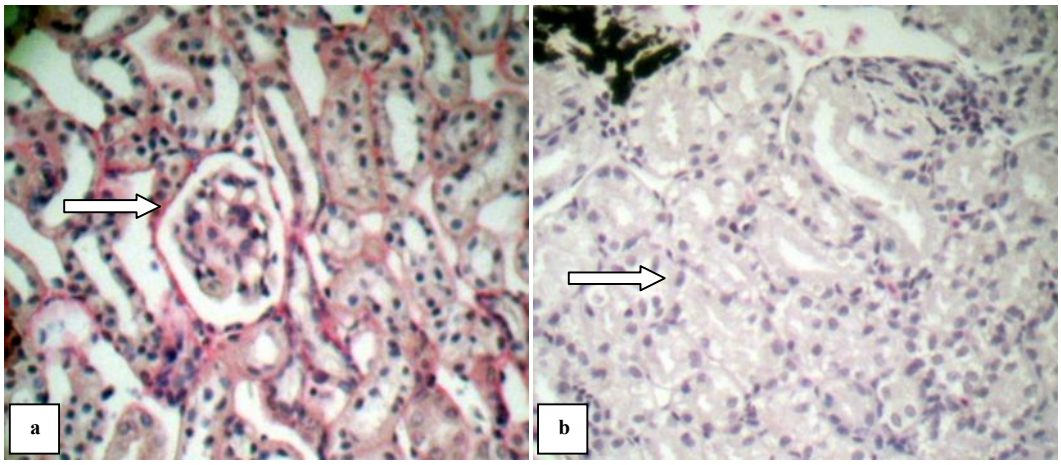

Figure 4. Control group showing normal appearance of glomerular (left) and proximal convoluted tubules epithelium (right). 100×; H-Sirius red staining.

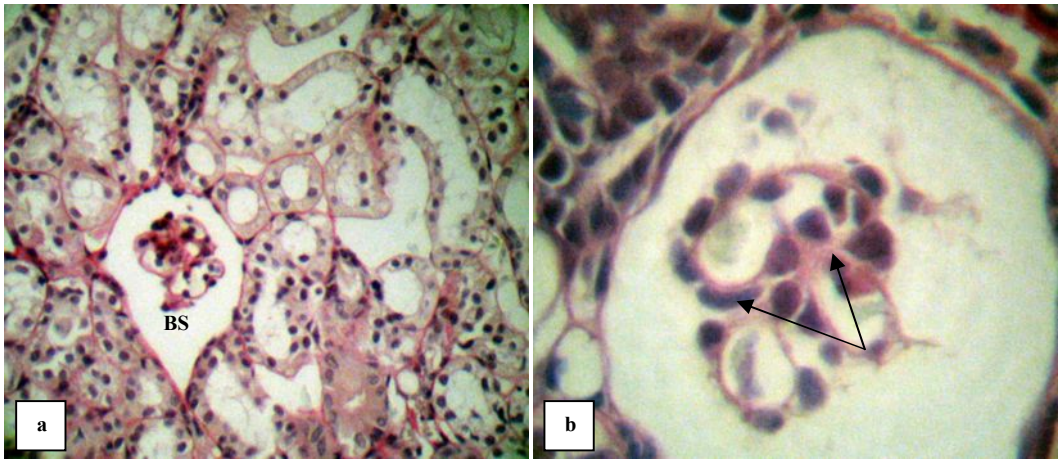

Figure 5. Renal glomerulus poisoned with Talstar 10EC. BS – enlarged Bowman space, dilated capillaries in renal glomerulus. 100× (left), 400× (right). H-Sirius red staining.

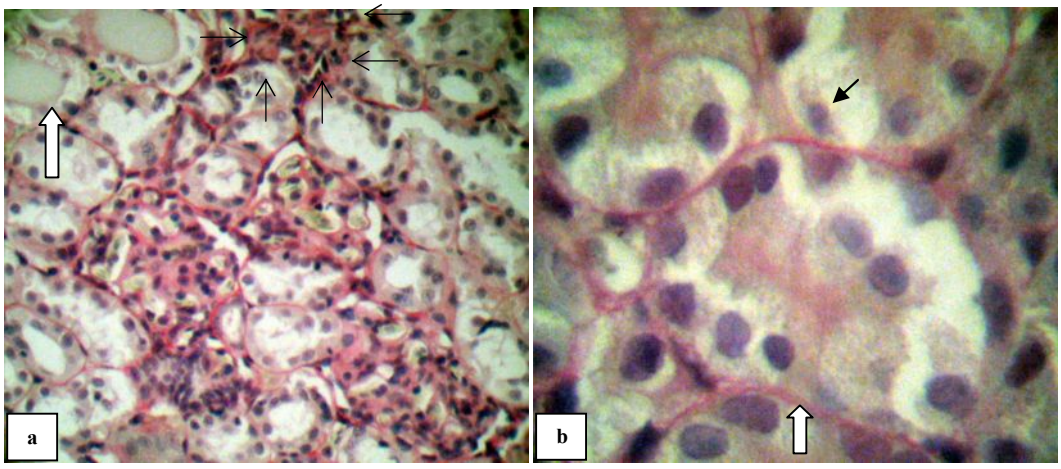

Figure 6. Kidney of *Pelophylax ridibundus* exposed to toxic insecticide Talstar 10EC. a- the presence of tubular necrosis (surrounded by arrows) and obstructed lumen of proximal convoluted tubules (white arrow). 100×. b- cells in the process of cytolysis (black arrow) with different shaped nuclei, peritubular fibrosis (white arrow). 400×. H-Sirius red staining.

DISCUSSIONS

Similar histological changes such as degeneration of the liver under the action of the insecticide Talstar 10EC was also observed by Velisek et al. at carp [28] and trout. In both species studied bifenthrin causes hepatocytes damage, the latter having pycnotic nuclei, lipid vacuoles in the cytoplasm and showing fatty degeneration of liver.

We appreciate that our results are similar to those obtained by Cengiz and Unlu [5] who studied histopathological changes induced by the action of deltamethrin (pyrethroid insecticide) on the liver of *Gambusia affinis*, recording hypertrophy of hepato-

cytes and Kupffer cells, necrosis, fatty degeneration of the liver. Cypermethrin, another pyrethroid insecticide, causes hyperplasia and necrosis of hepatocytes in *Labeo rohita* species [23]. Since pyrethroid insecticides are highly lipophilic they can be absorbed even when found in very small doses.

Loumbourdis and Vogiatzis [13] observed histological changes in melano-macrophages in cadmium poisoning of *Rana ridibunda* and Boncompagni et al., [1] in chromium and heptachlorepoxide poisoning of *Rana aesculenta.* These results show the role of melano-macrophages in the defense mechanisms by means of melanin synthesis (which has substantially increased in intoxicated animals) and by increasing the catalase activity.

Păunescu et al. [18] also observed histological and histochemical alterations in the liver of the frog *Rana ridibunda* induced by the action of the insecticide Reldan 40EC (chlorpyrifos-methil). These toxic induces an increase in the area occupied by the Kuppfer cells as well as an increase in their color intensity, mild karyomegalia and polyploidy together with accumulation of infiltrates, a fibrosis around the blood vessels and between hepatocytes. Extensive fibrosis is presumed to be attributed to carcinogenesis [14].

In amphibians, as in higher vertebrates, the kidney fulfills an important function in maintaining a stable internal environment in terms of a highly variable external environment. This balance can be disturbed by the presence of xenobiotic substances, which may cause histological changes in the kidney.

Similar renal histopathological lesions induced by the action of other pyrethroid insecticides have been obtained by other researchers in fish. Cengiz [4] describes the appearance of renal histopathological changes induced by the action of deltamethrin in carp. The lesion is characterized by a degeneration of renal tubular epithelium, dilation of glomerular capillaries and degeneration of the glomeruli, the presence of vacuoles in the cytoplasm of epithelial cells, cell hypertrophy and narrowing of the tubular lumen.

Osman et al., [16] describe the nephrotoxic effects in *Oreochromis niloticus* fish species after applying concentrations of 0.5, 1.0 respectively 2.5 mg Cu/l water. They refer to tubular degeneration, necrosis in the renal parenchyma, lymphocytic infiltrates and decrease in the number of melanomacrophages.

Under sublethal concentration of Champion 50WP fungicide, the renal tissue of the marsh frog (*Pelophylax ridibundus*) showed marked pathological changes such as: degeneration in epithelial cells of the renal tubule, vacuolization, Perl's stained material, interstitial edema, karyomegalia, and degeneration of glomerulus [19].

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Received: 28 October 2011
Accepted: 11 January 2012
Published Online: 14 January 2012
Analele Universităţii din Oradea – Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433