HISTOLOGICAL CHANGES IN DELTAMETHRIN-INDUCED INTOXICATION IN *Carassius auratus gibelio* (*Pisces-cyprinidae*)**

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Abstract: Deltamethrin is a pyrethroid insecticide harmful for poikilothermal vertebrates as fish are. During deltamethrin metabolism, overproduction of free radicals occurs and cellular oxidative stress appeared. In order to evaluate the deltamethrin toxicity in fish, we analyzed, by light microscopy, liver, kidney and gonads fragments prelevated from individuals of *Carassius auratus gibelio* exposed for 1, 2, 3, 7 and respectively 14 days to deltamethrin 2μg/L. Deltamethrin induced hepatic, gonadal and renal toxicity. The structure of liver and kidney was damaged. The decrease of spermatozoa number is a severe effect affecting the reproductive potential of the animals.

Key words: deltamethrin, *Carassius auratus*, histological changes

Introduction and literature review

Deltamethrin (S)-alpha-cyano-3-phenoxycarbonyl-(1R,cis)-2,2-dimethyl-3-(2,2-dibromvinyl)-cyclopropanecarboxylate belongs to the pyrethroid insecticides class, commonly used because of its high toxicity for insects, short biodegradation period and the lack of the tendency for accumulation in organism (Rozanski, 1985). Its toxicity depends on the vertebrate species, route of administration and substance in which the preparation was done. Pyrethroids exhibit a higher toxicity for poikilothermal organisms (Rozanski, 1985). Usually the fish exposure to the pyrethroid insecticides interferes with the process of neural transmission, blocking, in open position, the ionic channels and induce anatomopathological (Kumaraguru et al., 1982),
enzymatic activity and hormonal changes (Kozubek et al., 1992; Przybyska-Wojtyszyn et al., 1992) Long period exposure to lower concentrations of deltamethrin increased the fish enzymes activity for detoxification process in the liver, kidney and blood serum (Jungowska-Klin et al., 1992; Kozubek et al., 1992; Przybyska-Wojtyszyn et al., 1992). The aim of the present work was to evaluate the deltamethrin-induced, structural changes in one of the most common and pollutant-resistant species of Romanian freshwater, Carassius auratus gibelio.

Materials and methods

Two years old individuals of Carassius auratus gibelio obtained from Nucet Fishery Research Station were housed in 60 l glass tanks at 21°C. The fish standard length ranged from 14.8 to 19.5 cm, the body weight from 95 to 216 g, the liver weight from 3 to 10 g and the ovary weight from 1.8 to 10 g. Prior to deltamethrin exposure the fish were acclimated in tanks, at 21°C for 15 days and evaluated of overall fish health under laboratory conditions. Deltamethrin, dissolved in 1 ml of absolute ethylic alcohol, was added in a single dose before the commencement of the experiment in dechlorinated tap water until a final concentration of 2 μg/L was obtained. During the experiments, fish were exposed for one, two, three seven and respectively 14 days to the pollutant. Ten individual lots were used for every period of exposure. The control one was kept in a glass aquarium filled with dechlorinated tap water. During the experiment the fish were not fed. Specimens were collected for the microscopic investigation of the gills, liver, kidney, gonads both of control and treated fish. Tissue fragments were kept in Bouin fixative reagent three hours long, dehydrated, cleared, and embedded in paraffin wax, cut at 8-μm-thick sections, Hemalaun Meyer-Eosin stained and mounted for microscopically examination by Olympus light microscope.

Results of investigations and discussion

Recording the ovary weight for the individuals, the increase of the gonadosomatic index was noticed. The kidney exposed for 48 hr to deltamethrin 2μg/L (Fig. 1A) shows the cytoplasm vacuolization of the epithelial cells lining renal tubuli, the enlargement of the space between epithelial layer of the renal tubuli and the basal membrane. Nuclear
hypertrophy and pycnosis, lymphocytes infiltration, especially after 7 days exposure to the pollutant, were also noticed (Figure 1).

Figure 1 Carassius auratus gibelio kidney exposed for 48 hr to deltametrin 2μg /l (A) shows the enlargement of the space between epithelial layer and the basal membrane and nuclear hypertrophy (arrow). Lymphocytes infiltration (star) was noticed after 7 days exposure (B) H&E Stain, original magnification x80

Necrosis of tubular epithelium, pycnotic nuclei in the hematopoietic tissue, hypertrophied epithelial cells of renal tubules, narrowing of the tubular lumen, expansion of space inside the Bowman's capsule and contraction of the glomerulus were observed in kidney tissues of Cirrhinus mrigala after fish exposure to fenvalerate, un other pyrethroid insecticide, similar to deltamethrin (Velmurugan et al. 2007). The hipertrophy of Bowman’s capsules and hyaline deposits in renal tubuli of swiss mice exposed to deltamethrin were noticed by Tos-Lutry et al., (2001). The studies by Lukowicz-Ratajczak and Krechniak (1991) showed that deltamethrin did not exert a nephrotoxic effect. Rare hepatocytes exhibited pycnotic nuclei during the pollutant exposure, enlargement of synusoids and after 14 days exposure, large aggregates of macrophages (Figure 2). We frequently noticed both previtellogenic and vitellogenic follicle atresia in the fish ovary deltamethrin-intoxicated.(Figure 3). Both “cortical alveoli” follicle stage and previtellogenic follicles presented atresia. Zona pellucida folding of vitellogenic follicle and oocytes cytoplasm fractures are obvious. We noticed unusual enlargement of some nucleoli of previtellogenic follicles, large yolk globules containing basophilic bodies.

The testis were in an advanced maturation stage. Several germ cell types were found in the testis of Carassius auratus gibelio. Type A spermatogonia had round- to oval-shaped nuclei, two nucleoli associated with the nuclear envelope and heterochromatin granules. They are large, single cells,
distributed all along the germinal epithelium in between the cysts, adjacent to the lobule wall. Some spermatogonia nuclei were hypertrophied having a folded nuclear membrane. Spermatogonia B resulting from successive mitoses of spermatogonia A are found in small groups, close to the lobule boundary, whereas spermatocytes and spermatids are grouped within larger spermatocysts. The Sertoli “epithelium” presents a decreased number of cells in direct contact with the spermatogonia.

Figure 2 Liver exposed for 14 days to deltamethrin (H&E Stain, original magnification x 80). Aggregates of macrophages (arrow) and enlargement of the synusoids (star) are noticed.

Figure 3. Ovary exposed for 48 hr (A) and respectively 7 days (B) to the pollutant.; huge nucleolus in previtellogenic follicle; large yolk globules in vitellogenic follicle containing basophilic bodies (arrow) (H&E Stain, original magnification x 100)

Condensation of the nucleus starts with primary spermatocytes. The cysts containing late spermatids and spermatozoa lost, in some area, the particular alveolar appearance. Libido and sperm number also decreased in rabbits, the proportion of dead or abnormal mice sperm increased, rats plasma testosterone levels, testis weights, sperm cell concentration, live cell percentage, motility index reduced but total sperm anormalities increased.
Histological changes in Deltamethrin-induced intoxication in *Carassius auratus gibelio* during deltamethrin intoxication. (Salem et al. 1988).

![Image](image1.png)

**Figure 4 Testicle after 24 hr of deltamethrin exposure (A, B), (H&E Stain, original magnification x 100)**

Deltamethrin induces its effect by oxiradical production via activation of cytochrome P450 isozyme. Elevated oxiradical production as a consequence of induction of cytochrome P450 activities, may result in toxicological consequences as oxidative damage in the tissues. El Gohary et al., (1998) also revealed that deltamethrin induces testicular apoptosis in male albino rats, partly mediated by high levels of free radicals, including nitrous oxide. After chronic and subchronic deltamethrin intoxication in mice, lipid peroxidation increased, erythrocytes GSH-PX, Cu-Zn SOD and catalase activities were inhibited and malondialdehyde level elevated (Yarsan et al., 2002). Deltamethrin has an oxidative-stress-inducing potential in fish (Sayeed et al., 2003).

**Conclusion**

Histological changes of liver, kidney and gonads were recorded. We concluded that deltamethrin induced hepatic, gonadal and renal toxicity. The tissular damages are due to the overproduction of free radicals during deltamethrin metabolism which induce an oxidative stress state. The structure of the main organs involved in detoxification as liver and kidney was damaged. Among the analyzed organs, the kidney seemed to be the most sensitive to the pollutant action after 48 hr of exposure. The gonadal structural changes could seriously affect the reproductive potential of the animals. The decrease of spermatozoa number, noticed in other vertebrate species, is a severe consequence of deltamethrin pollution. Generally
nuclear changes were observed which means that nucleus could be an important cellular target.

**HISTOLOŠKE PROMENE KOD Carassius auratus gibelio (Pisces-cyprinidae) IZAZVANE INTOKSIKACIJOM DELTAMETRINOM**

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**Rezime**

Izloženost ribe deltametrinu je uticala na poremećaj procesa neuralne transmisije, anatomopatološku, enzimsku aktivnost i hormonalne promene. Dug period izloženosti nižim koncentracijama deltametrina uticao je na povećanje aktivnosti ribljih enzima u procesima detoksifikacije u jetri, bubrezima i krvnom serumu. Cilj ovog rada je ocena strukturalnih promena nastalih pod uticajem deltametrina kod *Carassius auratus gibelio*. Kontrola i ogledno jato *Carassius auratus gibelio* su bili izloženi dejstvu 2μg/L deltametrina u periodu od jednog, dva, tri i sedam i respektivno 14 dana. Svetlosni mikroskop je korišćen u analizi fragmenta jetre, bubrega i gonada. Bubreg izložen dejstvu 2μg/L deltametrina u trajanju od 48 sati pokazuje citoplazmatičku vakuolizaciju epitelnih celija koji se nalaze na obodu renalnih tubula (tubuli), povećanje prostora između epitelnog sloja renalnih tubula i osnovne/bazalne membrane. Hipertropija i piknoza nukleusa (*Nuclear hypertrophy and pycnosis*), infiltracija limfocita, pogotovo nakon 7 dana izloženosti dejstvu zagađivača, su takođe registrovani. Mali broj hepatocita je pokazao piknotske nukleuse tokom perioda izloženosti dejstvu zagađivača, sinusoidne su se povećale nakon 14 dana izloženosti, pojavili su se veliki agregati makrofaga. Često smo primećivali i atrezije previtelogenskih i vitelogenskih folikula (*previtellogenic and vitellogenic follicle atresia*) u jajnicama ribe intiksikovane deltametrinom. I stadijum folikula Both "cortical alveoli" i previtelogenske folikule su imale atreziju. U vitelogenskoj folikuli *zona pellucida* (folded) i frakture citoplazme oocita su bile očigledne. Kod previtelogenskih folikula, pojavile su se neobična uvećanja nekih nukleola i velike globule koje su sadržavale bazofilna tela. Testikularne ciste koje
sadržavaju kasne spermatide i izgubljene spermatozoide, u nekim delovima, primećena je i pojava posebnih alveola. Nepostojanje organizacije ciste, uvećanje prostora između spermatidnih cista i smanjenje broja spermatozoa je takođe zabeleženo. Zaključak je da je toksičnost hepatička, gonadalna i renalna izazvana deltametrinom preko povećane proizvodnje slobodnih radikala koji su izazvali stanje oksidativnog stresa

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