Research Article

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Histopathology of the Liver of Three-Spotted Gourami, *Trichogaster Trichopterus*, Exposed to Malachite Green, A Commonly Used Medicine in Aquaria

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Abstract

With the intensification of aquaculture and the heightened interest in ornamental fishes and fish keeping, use of medicaments has considerably increased. These agents are heavily bio accumulated by fish. In spite of this importance of drugs/therapeutic agents in fishery science, no serious attempt has been made to assess their effects on fishes, especially the histopathological changes brought about by them. Liver is the chief metabolic and detoxification organ in vertebrates and it is highly susceptible to metabolic disturbances and a variety of toxicants to which the animal is exposed. Results of controlled exposure of fishes in the laboratory to toxicants such as pesticides and related chemicals suggest that liver is the organ in which the highest residues of such toxicants accumulate and it is this organ that suffers the greatest damage and impairments following such episodes. The present study was, therefore, aimed at assessing the histopathological effects of therapeutic level of malachite green on the liver of a common aquarium fish, the three spotted (blue) gourami, Trichogaster trichopterus. The drug selected for the present study was malachite green, a chemical recommended for treatment of fishes against several infection, especially fungal infection of eggs. Malachite green is used at concentrations of 0.05-0.15 ppm in fish disease therapy. Healthy fishes of about 70.0±5 mm TL purchased from a local aquarist were used for the present study. Fish were exposed to malachite green for a period of 48 h in all-glass aquarium tanks. The exposure concentration of malachite green was 0.15 ppm. The procedures for histological studies were basically in accordance with the commonly practiced histological methods.

Keywords: Focal vacuolar degeneration, Histopathology, Malachite green, Three-spotted gourami, *Trichogaster tricho-pterus*

Introduction

That pathological changes occur in tissues and cells of fishes consequent on parasitic infection, diseases, neoplasia, altered nutritional conditions and the like is unequivocal [1-3]. Stresses of various kinds, both physical and chemical, also cause histological changes in fishes. Whereas water contaminants such as a variety of chemicals, biocides and metals have received attention from histologists, drugs/therapeutic agents used in aquaculture and aquaria have been neglected in this regard. Diseases and abnormalities could be characterized, and to some extent diagnosed, at the cellular/tissue level by studying the histology and histopathology of organ systems. With the intensification of aquaculture and the heightened interest in ornamental fishes and fish keeping, use of medicaments has considerably increased. These agents are heavily bio accumulated by fish. In spite of this importance of drugs/therapeutic agents in fishery science, no serious attempt has been made to assess their effects on fishes, especially the histopathological changes brought about by them. A major constraint in fish culture is disease which may be caused by viruses, bacteria, fungi or parasites. Most of the fish keepers use specific medicines prepared by reputable manufacturers for treating diseases. Malachite green is a medicine popularly used by fish keepers particularly against fungal infections. Though used as medicine, now it is banned in several countries since it is known to be toxic, malachite green is warned to be carcinogenic. INTERNET sites: www.thekrib.com/chemistry/malachitegreen.html; www.defra.gov.uk/news/2002/02061IIC.html) Liver is the chief metabolic and detoxification organ in vertebrates and it is highly susceptible to metabolic disturbances and a variety of toxicants to which the animal is exposed. Results of controlled exposure of fishes in the laboratory to toxicants such as pesticides and related chemicals suggest that liver is the organ in which the highest residues of such toxicants accumulate and it is this organ that suffers the greatest damage and impairments following such episodes. The present study was, therefore, aimed at assessing the histopathological effect of therapeutic level of malachite green on the liver of a common aquarium fish, the three-spotted (blue) gourami, *Trichogaster trichopterus*.

Materials and Methods

The experimental fish selected for the study, the three-spotted (blue) gourami, *Trichogaster trichopterus*, commonly called blue gourami or three-spotted gourami, is a very popular aquarium fish because of its attractiveness, generally peaceful nature and its breeding behavior. They may grow about 130-150 mm in length. Males have longer and generally more pointed fins; males make bubble nests for breeding.

They have accessory respiratory apparatus called the labyrinth organ, which enables them to extract oxygen from atmospheric air and thus to survive in oxygen poor waters [4]. For the present study, specimens of *T. trichopterus* were purchased from a local aquarist. The fish were transported to the laboratory under oxygen packing. Since the transportation was limited to 1 h, no mortality occurred.

In the laboratory, the fish were acclimatized in large aquarium tanks for one week. During this period they were given proper aeration and were fed with artificial feed. No mortality was observed during acclimatization period.

The drug selected for the present study was malachite green, a chemical recommended for treatment of fishes against several infection, especially fungal infection of eggs. Malachite green is used at concentrations of 0.05-0.15 ppm in fish disease therapy INTERNET sites:www.thekrib.com/ chemistry/malachitegreen.html; www.defra.gov.uk/news/ 2002/02061IIC.html) [5]. Healthy fishes of about 70.0±5 mm TL purchased from a local aquarist were used for the present study. Fish were exposed to malachite green for a period of 48 h in all-glass aquarium tanks. The exposure concentration of malachite green was 0.15 ppm.

The procedures for histological studies were basically in accordance with the commonly practiced histological methods [6]. In the present study, paraffin sectioning technique was employed. Livers, carefully excised from the control and exposed fish, were fixed in Helly's fixative, washed to remove mercury precipitate, dehydrated in alcohol series, cleared first in methyl benzoate and then in benzene and embedded with paraffin wax [6]. Sections cut at 7 µm thickness were stained with Harris' haematoxylin and alcoholic eosin (H & E) and mounted in Dextrene Plastisizer Xylene (DPX).

Results and Discussion

The liver of *Trichogaster trichopterus* is a large, tri-lobed, deep reddish-brown organ. In the liver of the control fish the hepatocytes are arranged in an anastomosing meshwork. Each hepatic cord surrounding the portal vein is two-cell thick. The hepatic portal vein draining blood from the stomach and intestine enters the liver, gradually branches off and divides into relatively wide blood spaces, called sinusoids, between the hepatic cell cords. The hepatocytes are polygonal cells with smooth margin and centrally located nuclei; the nucleolus is distinct. The exocrine pancreatic tissue is distributed around the portal veins in the form of tubulo-acinar glands. Near the gall bladder, adjoining the common bile duct, pancreatic tissue is organized into nodules with acini of exocrine pancreas and pancreatic islet; the acinar cells are almost cuboidal with deep basophilic cytoplasm and eosinophilic zymogen granules. The endocrine region of the islet constitutes a thickly packed mass of cells with larger nuclei; the mass stains dull pink with H & E (Figs. 1-5).

The gross anatomy of the liver of *T. trichopterus* exposed to malachite green was not much different from that of the control. However, the gall bladder in the exposed fish was very much enlarged and contained a dull, transparent, greenish fluid.

The most striking feature of the liver of the malachite-green exposed fish was the appearance of a large number of venules and sinuses/ sinusoids engorged with blood, in the liver parenchyma. The sinusoids were congested with blood; this change was so intense that the liver became highly hyperaemic. Red blood cells were found to infiltrate the liver parenchyma. Histopathological changes were noted in the parenchyma also, which were marked by focal, radiating, vacuolar degeneration of hepatocytes. All through the liver parenchyma, patches of large vacuolated hepatocytes, radiating from a central vein or blood engorged sinusoid, were observed. The vacuoles were of uneven size; usually larger vacuoles were found closer to the blood vessel/space (Figs. 6-8).

The pancreatic tissue of the malachite green-exposed fish also showed some changes inasmuch as the glandular cells were more basophilic than in the control. The glandular cells were atrophied with no or much less zymogen granules in them than in the control. The acinar nature of the pancreatic tissue was very much disturbed in the exposed fish. The number of pancreatic cells was fewer and the cells were very loosely packed in the exposed liver (Figs. 9-10).

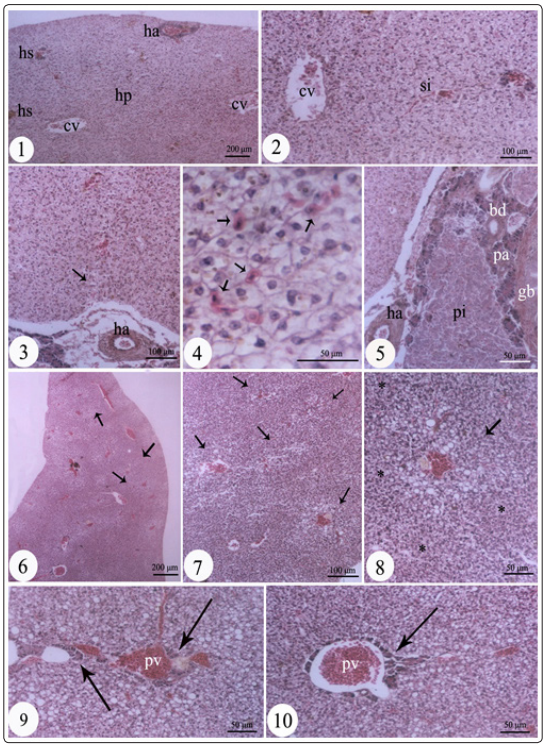

Figure 1: Low power view of the liver of control fish. **Figure 2:** Enlarged view of part of section in Figure 1. **Figure 3:** Enlarged view of the liver of control fish showing hepatic cords (arrow). **Figure 4:** T.S. of the liver of control fish showing hepatocytes; arrows indicate erythrocytes. **Figure 5:** T.S. of the liver of control fish showing arrangement of pancreatic tissue. **Figure 6:** Low power view of the liver of malachite green-exposed fish showing hyperaemia (arrows). **Figure 7:** Low power view of the liver of malachite green-exposed fish showing focal vacuolation of hepatocytes around blood filled sinuses (arrows). **Figure 8:** Enlarged view of part of Figure 7. Note vacuolar degeneration of hepatocytes around blood-filled sinus (arrow). Asterisks indicate normal hepatocytes. **Figure 9:** Enlarged view of T.S. of malachite green-exposed liver showing degeneration of pancreatic acinar tissue (arrow). **Figure 10:** Enlarged view of T.S. of malachite green-exposed liver showing degeneration of pancreatic acinar tissue--disruption of acinar nature, atrophy of acinar cells, loose packing of pancreatic cells (arrow). (All sections are stained with H & E) (**bd** - bile duct, **cv**- central vein, **gb**- gall bladder, **hp** - hepatic parenchyma, **hs** - haemosiderin deposits, **pa** - exocrine pancreas, **pi** - pancreatic islets, **pv** - portal vein, **si** - sinusoid)

From the point of view of liver pathology, several pesticides have been tested under controlled laboratory conditions. The overall picture coming forth from the results of such studies is that in the liver of fishes exposed to pesticides, a variety of histological damages occur: parenchymal cell vacuolation and degeneration, extensive lipid deposition in hepatocytes, hepatic cord disarray, intragonal and periportal inflammation, cellular shrinkage and loss of glycogen and fat depots, deposition of bile pigment in parenchymal cells, focal necrosis of hepatic cords, fibrosis, adenomatous changes, hepatocytic karyolysis, hepatocyte nuclear vacuolation, occurrence of abnormal PAS-positive material in the nuclei of hepatocytes, congestion of liver sinusoids, formation of large lytic cavities in the parenchyma, pleomorphism of parenchymal cells, degeneration of pancreatic acinar cells [1].

In the present study, exposure to malachite green induced vacuolar degeneration of hepatocytes. However, the noteworthy feature was the characteristic pattern of vacuolation of hepatocytes. Vacuolation was always localized around a venule or blood sinus and it appeared to radiate a short distance from the blood-filled sinus. Such a specific pattern of hepatocellular vacuolation is so far not reported in literature. Whereas generalized vacuolation is a non-specific histopathological change, the "focal vacuolar degeneration" of the liver parenchyma as noted during the present study could be a specific response to poisoning by malachite green. Further studies would be required to confirm this hypothesis.

To sum up, short-term exposure to malachite green has induced histopathological changes in the liver of *Trichogaster trichopterus* and the "focal vacuolar degeneration" of the hepatocytes of the liver of exposed fish appears to be a specific change induced by this toxicant.

References

1. Gurr E (1962) Staining Animal Tissues. Practical and Theoretical. Leonard Hill (Books) Ltd., London 681.
2. Ribelin WE and G Migaki (1975) The Pathology of Fishes. The Univ Wisconsin Press, Wisconsin, 1004.
3. Schaperclaus W (1991) Fish Diseases. Vol. 1 and 2. Amerind Publ Co Pvt Ltd, New Delhi. 1398.
4. Gilbert J (1970) Branchial pathogenesis in a freshwater fish, Puntius conchonius Ham., chronically exposed to sublethal concentrations of cadmium. Ecotoxicol Environ Saf, 15: 153-161.
5. Allison R (1958) New control methods for Ichthyophthirius in ponds. Proc. World Symp. Warm Water Pond Fish Culture FAO Fish Rep, 44: 389-392.
6. Roberts RJ (1978) Fish Pathology. Baillere-Tindall, London 318.