USING HISTOPATHOLOGY OF FISH AS A PROTOCOL IN THE ASSESSMENT OF AQUATIC POLLUTION

Reddy P.B.* and Waskale Kusum
Department of Zoology, Government Arts & Science P.G. College, Ratlam, Madhya Pradesh (INDIA)

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ABSTRACT

There is a need for sensitive bio-monitoring tools in toxicant impact assessment to indicate the effect of toxicants on fish health in polluted aquatic ecosystems. Histopathological assessment of fish tissue allows for early warning signs of disease and detection of long-term injury in cells, tissues, or organs. The aim of this study is to assess the degree of histopathological alterations of various organs in various fish species in different environmental conditions. Histopathological lesions in fish served as primary indicators of exposure to contaminants and certain diseases and lesion types have proven to be reliable biological indicators of toxic/carcinogenic effects resulting from such exposure. Use of histopathological techniques allows investigators to examine specific target organs and cells as they are affected by exposure to environmental chemicals. Moreover, it offers a means of detecting acute and chronic harmful effects of exposure in the tissues and organs of individual animals. The present study reviews the most important histocytopathological alterations in various organs of fish, which have been used as biomarkers in different international pollution monitoring programs. This review provides information concerning the exposure, effects and susceptibility of fish in the context of environmental monitoring and the biomarker approach. As such, it provides a powerful integrative tool for the assessment of biological effects of contaminants in these environments.

Key Words: Histopathology, Biomarker, Fish, Contamination, Environmental monitoring

INTRODUCTION

Xenobiotics derived from urban communities, agricultural operations and industrial effluents, finally released into the environment. In the context of pollution aquatic systems are highly at risk because of their trend for accumulation relatively high concentrations of chemicals entering from surrounding terrestrial systems, thus, regardless of their source of entry to the environment, water bodies are frequently stores for a large variety of stressor chemicals. Most biomarkers are narrow in their expression whereas histopathology is broad in its evaluation. Histopathological changes in animals tissues are powerful indicators of prior exposure to environment stressors and are net result of adverse biochemical and physiological changes in an organism. For filed assessment behavior studies and histopathology is often the easiest method assessing both short and long term toxic effects. Histopathological biomarkers can be indicators of the effects on organisms of various anthropogenic pollutants and are a reflection of the overall health of the entire population in the ecosystem. Well-documented lesions based on experimental data in liver, kidney, gill, ovary, skeleton system and skin have been used as biomarkers to date. Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. The mechanism of action of several xenobiotics could initiate the formation of a specific enzyme that causes changes in metabolism, further leading to cellular intoxication and death, at a cellular level, where as this manifest as necrosis, i.e. histopathological biomarker on a tissue level. Therefore, histopathology is the gold standard when defining toxicological effects, but it is
invasive, time consuming and expensive. Furthermore, as histological testing is often impractical in human subjects, using biomarkers with a known histological distribution may fill the need of localizing toxic injury to distinct organs or tissues. Therefore, histopathological evaluation remains an important part of the assessment of the adverse effects of xenobiotics on the whole organism.

AIMS AND OBJECTIVES

The present study reviews the most important histo-cytopathological alterations in various organs of fish, which have been used as biomarkers in different international pollution monitoring programs. This review also provides information concerning the exposure, effects and susceptibility of fish in the context of environmental monitoring and the biomarker approach.

DISCUSSION

Histopathological changes of gill

The gills of a fish comprise a multifunctional organ (respiration, ion regulation, acid-base balance and nitrogenous waste regulation, excretion) constitute over 50 percent of the total surface area of the animal that make it sensitive to chemicals in water. The fish gills play an important role in maintaining of whole animal ionic homeostasis in both freshwater and marine environment. Consequently, injury to gill epithelium is a common response observed in fish exposed to a variety of contaminants. The severity of damage to the gills depends on the concentration of the toxicants and the period of exposure.

The reviews of Wood and Au have provided extensive information on gill structural alterations in fish as a result of toxicants exposure. Lifting of the lamellar epithelial cells away from the basement membrane due to a penetration of fluid is the most common lesion, which could be give rise to reduce respiratory gas exchange by increasing diffusion distance and decreasing interlamellar distance. Fusion of neighboring lamellae and epithelial rupture are perhaps the direct results of pavement cell lifting and represent more severe gill damage. Lamellar fusion, hyperplasia, necrosis of different lamellar and filament cells like chloride and pavement cells is another most commonly reported responses, but is more common for metals than for organics or other pollutants, possibly since metals directly interact with ion transport proteins and inhibit their activity. Necrosis would be expected to increase diffusion of ions and water. In true necrosis, Transmission Electron Microscopy (TEM) shows that organelles and cytoplasmic volume swell and become more electrons dense in necrotic cells. Ultimately cell membranes would be ruptured and the contents possibly would be lost by swamping to the external water. Leukocyte infiltration should be also considered an adaptive response. Hypertrophy of the pavement cells is possibly an event associated with necrosis cell swelling. This lesion is also more commonly associated with metals. However, cell hypertrophy sometimes indicates the origin of pavement cells which occurs when they shrink back to expose increased chloride cell-surface area in return to acid-base and ionic interruptions. Proliferation of mucous cell, associated with excess mucus secretion, seems to occur more frequently in result of exposure to metals than to organic pollutants. Proliferation of pavement cells, mucous cells and chloride cells seem to be protective which limit the accesses of chemicals with the branchial surface, on other hand they may also block respiratory gas exchange and then lead to animal smothering.

Epithelial lifting, hyperplasia and hypertrophy of the epithelial cells with partial fusion of lamellae are defense mechanisms which result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants. These alterations, more commonly associated with chronic exposures than acutely lethal exposures, are greatly increase the blood-to-water diffusion distance, decrease interlamellar distance and lead to a total reduction in the diffusive conductance of the gills to respiratory gases. Lamellar aneurysms and blood congestion with dilation of marginal channels together with leukocyte infiltration could be considered part of an inflammatory response and occur when fishes suffer a more severe type of stress. These are the gill lesions in response to a wide range of contaminants, including organ chlorines,
petroleum compounds, organophosphates, carbonates, herbicides and heavy metals with a greater reported occurrence. The most of gill alterations reported in the literature, even though concentration dependent (i.e. more severe in acute lethal exposures than in chronic sub lethal exposures), are actually non-specific and are not correlated with the kind of toxicant, exposure level (acute or chronic), exposure medium (freshwater or seawater), or fish species. Epithelial lifting and lamellar fusion were observed in rainbow trout (Oncorhynchus mykiss) exposed to petroleum residues. The same changes have also been reported in the gills of the fishes exposed to organic toxicants and metals and industrial effluent. Definitively, the respiratory epithelium changes cooperates the host respiratory ability. Moderate changes don’t lead to mortality directly, but can harmfully affect the functioning of the fish. On the other hand, severe or extensive damage may directly cause death. Overall, though tissue preparation for histopathological study is time consuming, gill histopathology seems to be a promising biomarker for general environmental pollution monitoring.

**Histopathological changes of liver**

In general, liver is a target organ due to its large blood supply that causes noticeable toxicant exposure and accumulation and also its clearance function and its pronounced metabolic capacity. Numerous categories of liver pathology are present as reliable biomarkers of toxic damage. Therefore studies on liver histopathology in fish have increasingly been incorporated in national marine biological effects monitoring programs in both Europe and the USA. Myers et al. generally classified flatfish hepatic alterations into several distinct groups and it could be possible to rank them according to their relative importance as indicators of toxicant exposure:

1. Degenerative lesions such as biliary epithelial cells degeneration and polymorphism of hepatocytes and their nucleus;
2. Foci of Cellular Alteration (FCA), including basophilic, eosinophilic, clear cell and vacuolated foci;
3. Benign neoplasm, including hepatocyte adenoma, bile ducts cholangioma and blood vessels and capillaries hemangioma and 4. Malignant neoplasms, including hepatocyte carcinoma, cholangiocarcinoma and hemangiosarcoma.

Specific non-neoplastic proliferate lesions including hepatocytes regeneration, hyperplasia of bile duct and hepatic fibrosis in addition to general or non-specific degenerative alterations such as cellular necrosis, hyaline inclusion bodies comprise a second group of hepatic lesions. Ultimately, inflammatory changes consists a third group of liver alterations, which is considered as minimal significant indicator of pollutant exposure, although this group can offer more information on the general health status and condition of the fish. Overall, liver histopathological lesions are not specific to pollutants. For example, exposure to PAHs, PCBs, DDTs, chloranes and dieldrin increases the prevalence of liver lesions including neoplasm’s, Foci of Cellular Alteration (FCA), Megalocytic Hepatoes (MH), Hepatocellular Nuclear Polymorphism (NP), Hydropic Vacuolation (HV) in English sole (Pleuronectes vetulus); while in winter flounder (Pleuronectes americanus), exposed to PAHs, DDTs or chlordanes, non-neoplastic proliferative lesions and non-specific necrotic lesions significantly increased. Fanta et al. reported abnormalities such as irregular shaped hepatocytes, cytoplasmic vacuolation and nucleus in a lateral position in the siluriform Corydoras paleatus exposed to organophosphate pesticides. Reddy et al. and Reddy and Baghel observed signs of degeneration (cytoplasmic and nuclear degeneration and nuclear vacuolation) and the focal necrosis in the liver parenchyma of fishes exposed to the industrial effluent. These alterations have been reported as more severe changes, which are more commonly associated with the exposure of the fishes to contamination by various metals.

Lesions including hepatocellular cytoplasmic vacuolization, leucocytes infiltrations, blood congestion necrosis and fatty infiltrations were found in the liver of catfish Clarias gariepinus treated with fenvalerate. The same changes were reported by Teh et al. in the liver of 7-day-old larvae of the fish Sacramento spiltail. However, using of liver histopathology as a biomarker of contamination exposure may not be a highly cost-effective method for pollution
screening because it needs much time and effort to prepare liver samples and expected pathologists are also required to distinguish hepato pathological alterations.  

**CONCLUSION**

On the basis of the information presented in different studies, there is no doubt that the application of histopathological changes as a biomarker of organism exposure to contaminated sites, offers important information that can contribute to environmental monitoring programs designed for surveillance, hazard assessment or regulatory compliance. One of the most important benefits of the use of histopathological biomarkers in environmental screening is possibility of examining specific target organs, including gills, kidney and liver. However, the fish are responding to the direct effects of the pollutants as well as to the secondary effects caused by stress. This information verifies that histopathological changes are valuable biomarkers for field evaluation, especially in tropical regions that are naturally affected by variety of environmental variations. It should be highlighted that histopathology is able to assess the initial effects and reactions to acute exposure to chemical stressors.

**REFERENCES**


“Haldir had gone on and was now climbing to the high flet. As Frodo prepared to follow him, he laid his hand upon the tree beside the ladder: never before had he been so suddenly and so keenly aware of the feel and texture of a tree’s skin and of the life within it. He felt a delight in wood and the touch of it, neither as a forester nor as carpenter; it was the delight of the living tree itself.”

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