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HISTOPATHOLOGICAL STUDIES ON NORMAL AND INFECTED *Sardina pilchardus* FISH

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ABSTRACT

Histological studies may be conducted using tissue culture, where live human or animal cells are isolated and maintained in an artificial environment for various research projects. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of histological stains. Histology is an essential tool of biology and medicine. Histopathological changes have been used as production and a high diversity of habitat for wildlife biomarkers in fish exposed to contaminants. 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 infected, offers important information that can contribute to environmental monitoring programs designed for surveillance, hazard assessment or regulatory compliance. . In the present study was to examine the histopathological changes in liver, muscle, gill and kidney of normal and infected fish as *Sardina pilchardus.*

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INTRODUCTION

Histology is the study of the microscopic anatomy (microanatomy) of cells and tissues of plants and animals. It is commonly performed by examining cells and tissues under a light microscope or electron microscope, the specimen having been sectioned (cut into a thin cross section with a microtome), stained, and mounted on a microscope slide. Histological studies may be conducted using tissue culture, where live human or animal cells are isolated and maintained in an artificial environment for various research projects. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of histological stains. Histology is an essential tool of biology and medicine (Weiss, 2010).

Histopathology, the microscopic study of diseased tissue, is an important tool in anatomical pathology, since accurate diagnosis of cancer and other diseases usually requires histopathological examination of samples. Trained physicians, frequently licensed pathologists, are the personnel who perform histopathological examination and provide diagnostic information based on their observations (Coyne, 2012). The trained personnel who prepare histological specimens for examination are histotechnicians, histotechnologists, histology

technicians (HT), histology technologists (HTL), medical scientists, medical laboratory technicians, or biomedical scientists and Biomedical Science Support Workers. Their field of study is called histotechnology (Adelmann, Howard, 1966).

Food borne infections and illnesses have become a major international health problem with consequent reduction in economic growth. It is also identified as a major cause of illness and death worldwide. Recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety. In the developing world, foodborne infection leads to the death of many children, as well as resulting in diarrheal disease which can have long-term effects on children's growth as well as on their physical and noesis development and it also heavily affects the healthcare systems. In the present study was to examine the histopathological changes in liver, muscle, gill and kidney of normal and infected fish as *Sardina pilchardus*.

MATERIALS AND METHODS

Collection of Experimental Fishes

Normal and infected *Sardina pilchardus* fish (Fig 1 and Fig 2) were procured from Fish market, Kelavasal, Thanjavur Tamil Nadu, India.

Fig 1: Normal fish (*Sardina pilchardus*)

Fig 2: Infected fish (*Sardina pilchardus*)

Histological studies

The liver, kidney, gill and muscle tissue was fixed in 10% normal saline for 72 hr after which the tissues were sliced to a thickness of 2.1mm each. These were dehydrated using alcohol of graded concentration. They were further treated with paraffin wax and cast into blocks; sections of the tissues were cut on a microtome to 5 µm. These were later attached to a slide and dried. The samples slides were viewed on a photographic microscope to find out histological changes.

RESULTS

Histopathological analysis

The present study was carried out to examine the histological changes in normal and infected fish of liver, muscle, gill and kidney. The observations made on different subjects of fishes were compared as follows.

Histology is that branch of anatomy that studies tissues of animals and plants. This textbook, however, discusses only animal, and more specifically human, tissues. In its broader aspect, the word histology is used as if it were a synonym for microscopic anatomy, because its subject matter encompasses not only the microscopic structure of tissues but also that of the cell, organs, and organ systems (Kan and Bendayan, 1989).

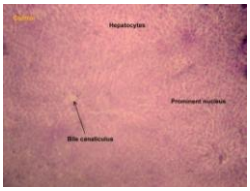
The body is composed of cells, intercellular matrix, and a fluid substance, extracellular fluid (tissue fluid), which bathes these components. Extracellular fluid, which is derived from plasma of blood, carries nutrients, oxygen, and signaling molecules to cells of the body. Conversely, signaling molecules, waste products, and carbon dioxide released by cells of the body reach blood and lymph vessels by way of the extracellular fluid. Extracellular fluid and much of the intercellular matrix are not visible in routine histological preparations, yet their invisible presence must be appreciated by the student of histology.

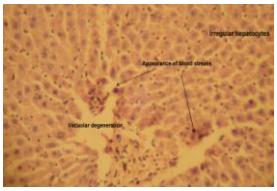
Histology of liver

The hepatic cells are polygonal in shape with distinct nuclei. Large numbers of blood sinusoids were also seen around the hepatocytes in normal fish (Fig 2). The infected fish showed the vacuolation, loose arrangement of hepatic cells, histolysis and disintegration of cell boundries. The infected fish shows damage as more severe and progressive than normal (Fig 2b).

Fig 2 Histology of liver in normal and infected fish

2a Normal fish **2b Infected fish**



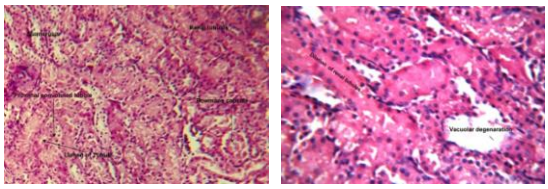


Histology of kidney

The normal fish kidneys are shows normal tubular cells, glomerulus and bowman's capsules (Fig 3a). The infected fish showed the renal tubules congestion, disintegrated bowman's capsules and aggregation of inflammated cells (Fig. 2b). The infected fish damage as more severe and progressive in infected fish with dilation of renal tubules and vacuolar degeneration.

Fig 3 Histology of kidney in normal and infected fish

Normal fish Infected fish

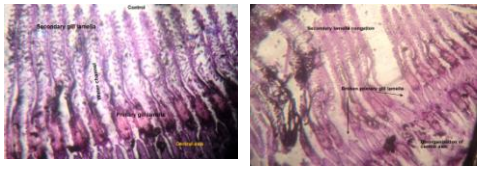


Histology of gill

In control fish, the secondary gill lamellae (SGL) appeared as finger –like structures. The SGL were thin, slender and attached on either side of the primary gill lamellae (PGL). The secondary gill lamellae are highly vascularised and surrounded by a thin layer of epithelial cells (Fig 4a). The overall observed results in the present investigation indicates that marked histopathological changes have been found in the gill of fish *Catla catla* infected. Fusion and shortening lamellae, hypertrophy, degeneration of epithelium and necrosis were found in the gills of infected fish (Fig 4b). Higher degree of hypertrophy and fusion of gill lamellae were prominent in the gills of infected fish.

Fig 4 Histology of gill in normal and infected fish

4a Normal fish 4b Infected fish

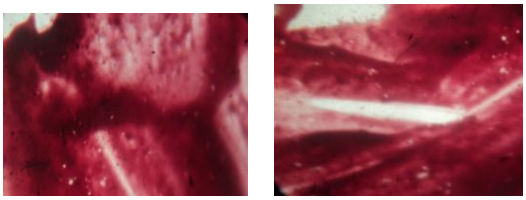


Histology of muscle

The photomicrograph of the muscle (Figure 5a) depicted the presence of normal myotomes with equally spaced muscle bundles. On infected fish shows marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed.

Fig 5 Histology of muscle in normal and infected fish

Normal fish infected fish



DISCUSSION

The subject of histology no longer merely deals with the structure of the body; it also concerns itself with the body's function. In fact, histology has a direct relationship to other disciplines and is essential for their understanding of organs. It is generally assumed that histopathological biomarkers are valuable indicators ofthe general health of fish and mirror the effects of exposure to a variety of anthropogenic pollutants (Hinton et al., 1992). These 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.

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer, Pawet, Schramm, Müller, & Triebskorn, 2001). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta et al., 2003) and serve as warning signs of damage to animal health (Hinton & Laurén, 1990).

Histopathological Changes of Liver

Liver is the site of metabolism. It plays a key role in biochemical transformations of pollutants under detoxification process. Due toxicant exposure and accumulation lesions and other histopathological alternations are common in liver. Contaminants also affect function and its pronounced metabolic capacity. Numerous workers used liver histopathology as reliable biomarkers of various contaminants (Mohamed et al., 2009). Therefore such studies have been incorporated in national marine biological effects monitoring programs in many countries including Europe and USA. Myers *et al*. (1993) generally classified fish hepatic alterations into several groups and ranked them according to

their relative importance as indicators of toxicant exposure. 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 (Reddy et al., 1992). Results of this study demonstrated that infected fish shows variety of histopathological changes in the Liver of exposed fish.

Histopathological Changes of Gill

The gills of a fish comprise a multifunctional organ and constitute over 50 percent of the total surface area of the animal that make it sensitive to chemicals in water. It is the site of gaseous exchange and osmoregulation. The fish gills play an important role in maintaining of whole animal ionic homeostasis. Consequently many pollutants come in contact with gill epithelium and causes injury (Reddy, 2010). However damage level depends on the concentration and period of exposure of the toxicants. The reviews of Wood (2001) and Au (2004) 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 (Butchiram et al., 2013). Results of this study demonstrated that infected fish shows variety of histopathological changes in the gill of exposed fish.

Histopathological Changes of Kidney

The kidney was composed of numerous renal corpuscles with well developed glomeruli and a system of tubules. The proximal segment was covered by tall columnar epithelial cells with basal nuclei and brush border located along the cell apices. The distal segment was lined with large, relatively clear columnar epithelial cells with central nuclei and the brush border was reduced or absent. The collecting duct or glomerulus was larger in diameter than the distal segment, containing columnar epithelial cells with basal nuclei and no brush border (Peebua *et al*., 2006). Histological study shows a typical structural organization of the kidneys in the untreated fish. Results of this study demonstrated that infected fish shows variety of histopathological changes in the kidneys of exposed fish. Gross changes included irregular diameters of renal tubules, glomerular expansion, renal corpuscle damage, severe degeneration in the tubules cells, in addition to the infiltration of edematous fluid.

Histopathological Changes of Muscle

Muscle is the tissue composed of elongated muscle fibers and muscle cells are held together by connective tissues. Histopathological study provides factual data concerning tissue changes prior to external manifestation. . Splitting of muscle fibers and vacuolar degeneration in muscle bundles were considered to be significant histopathological changes. Initial stimulus of pesticides and metals can induce hyperactivity and excitability in animals. All these changes were clearly evident as clinical signs at the initial stage of the experiment and were subsequently reflected through histopathological changes

One of the most important benefits of the use of histopathological biomarkers in environmental screening is possibility of examining specific target organs, including gills, muscle, 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 infections. It should be highlighted that histopathology is able to assess the microbial contaminations.

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