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Histopathological study of the kidney, liver and intestine tissues in goldfish (*Carassius auratus*) and angelfish (*Pterophyllum sp.*)

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Abstract. Histopathology is used for diagnosis of diseases in aquatic animals. This study was conducted to investigate histopathological lesions of kidney, liver and intestine of goldfish (*Carassius auratus*) and angelfish (*Pterophyllum sp.*) in some aquarium shops during a period between January-April 2010. Several histological alterations were observed in kidney which include glomerulonephritis, cell swelling of epithelial cells, tubular epithelium necrosis, hyaline droplets and hyaline cast in renal tubules and tubular dilation. Liver showed vacuolar degeneration of hepatocytes, karyolysis and karyorrhexis and focal areas of necrosis, haemorrhagia and hepatitis. In intestine, atrophy of epithelial cells and enteritis on the lamina propria and submucosal layer were seen. It was concluded that unfavourable environmental contamination of aquarium may induced several histopathological alterations in the tissues of goldfish and angelfishes.
Key Words: histopathology, kidney, liver, intestine, goldfish, angelfish.

Introduction. Ornamental fish industry is a very important aspect of aquaculture in many countries (Jha & Barat 2005a). Ornamental fish are very interesting as pet animals (Jha & Barat 2005b). Goldfish (*Carassius auratus* (Linnaeus, 1758)) is one of the most important pet animal and laboratory fishes in the world (Rylková et al 2010). Also, freshwater angelfishes (*Pterophyllum sp.*) belong to South American cichlids which are very popular among of all tropical aquarium fish (Axelrod 1985).

The health condition of fish depends on diagnosis, treatment and control of diseases and their environment (Snieszko 1983). Histological alterations of animal tissues provide a rapid method to show effects of irritants and pathogens in different tissues and organs (Bernet et al 1999). Generally, it has been supposed that histopathological biomarkers are valuable as indicators of environmental stress and exposure to chemical agents (Matthiessen et al 1993; Stentiford et al 2003).

The kidney receives the main part of the post-branchial blood and therefore, this organ is very important for investigation of renal lesions which occurre due to environmental stress and some pathogens (Kurtovic et al 2008). In fish, as in vertebrates, the liver is the principal organ associated with the detoxification and biotransformation processes and it is the potential site for lipid deposition (Camargo & Martinez 2007; Freeman et al 1983; Saleh 1982). The intestine involved important physiological functions, such as food digestion and ingestion (Caballero et al 2003). The objective of this study was to investigate the histopathological changes of the liver, kidney and intestine in two ornamental fish: goldfish (*C. auratus*) and angelfish (*Pterophyllum sp.*).

Material and Methods. Totally forty specimens of *C. auratus* (mean length 8.79±0.15 cm and mean weight 20.21±0.5 gr) and *Pterophyllum sp*. (mean length 7.81±0.11 cm and mean weight 8.46±0.45 gr) were selected during in a period from January to April

2010 from some aquarium shops of Abadan and Khorramshahr cities located in the south of Iran, and transferred to the fisheries laboratory, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology. They were kept in glass aquaria, supplied with aerated and filtered water before examination. The fish euthanized by blowing to the head. After necropsy, samples of the kidney, liver and intestine of fish were removed and immediately were fixed in 10% neutral buffered formalin (NBF). Tissue specimens were then prepared for histological analyses following routine laboratory procedures. Tissues were placed in tissue cassettes and processed (dehydration with increasing concentrations of ethanol followed by Xylol then embedding with paraffin wax) with an automatic tissue processor. Embedded tissues were then sectioned (5µm thickness), placed on microscope slides, deparaffinized with Xylol and stained with hematoxylin and eosin [H&E] and PAS and studied by light microscopy.

Results

Kidney. Based on the results, the kidney showed the highest rate of histopathological changes in goldfish and angelfish. A variety of histological changes in kidney were found in 19 (95%) of goldfish and 16 (80%) of angelfish (Table 1). The most common change in the kidney was glomerulonephritis in both fish species which has appeared by hypercellular vision in many of the glomeruli. Bowman's space was greatly diminished and partial to complete adhesions occurred between the parietal and visceral layers of renal corpuscles. The glomerular basement membrane was thickened (Figures 1 and 2) and stained intensely positive for the periodic acid-Schiff reaction. Cell swelling of epithelial cells, necrosis in the tubular epithelium, hyaline droplets and cast or a distinct dilation of the lumen with a homogenous, eosinophilic intraluminal mass in some proximal renal tubules and tubular dilation were observed in the kidney tissues.

Table 1

Summarized histopathological chenges in the kidney of goldfish and angelfish

Sample	*Goldfish*	*Angelfish*
Glomerulonephritis	14 (70%)	14 (70%)
Cell swelling of epithelial cells	8 (40%)	8 (40%)
Necrosis in tubular epithelium	8 (40%)	11 (55%)
Tubular dilation	4 (20%)	7 (35%)
Hyaline droplets and cast	10 (50%)	2 (10%)

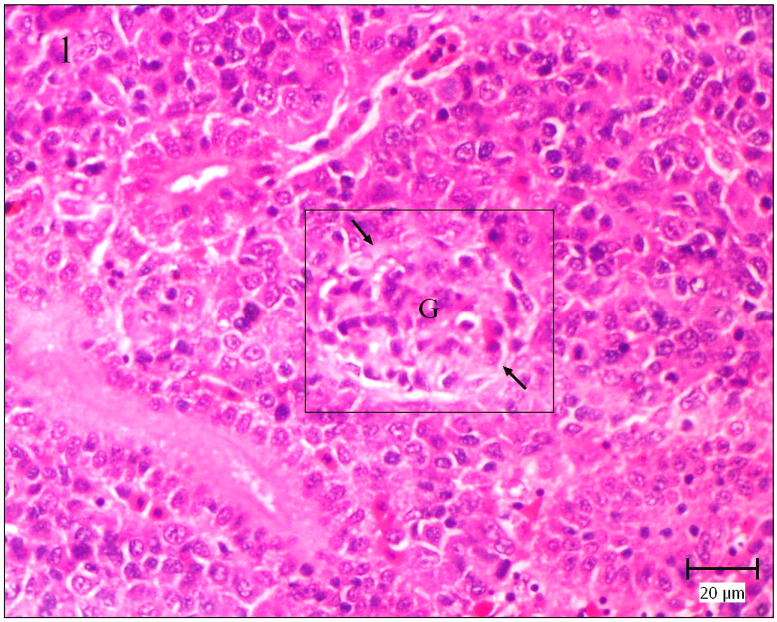

Figure 1. Kidney of angelfish: glomerulonephritis (square). Note the big tufts of glomerulus (G) and adhesion to parietal layer (arrows) (H&E staining, Bar 20µm).

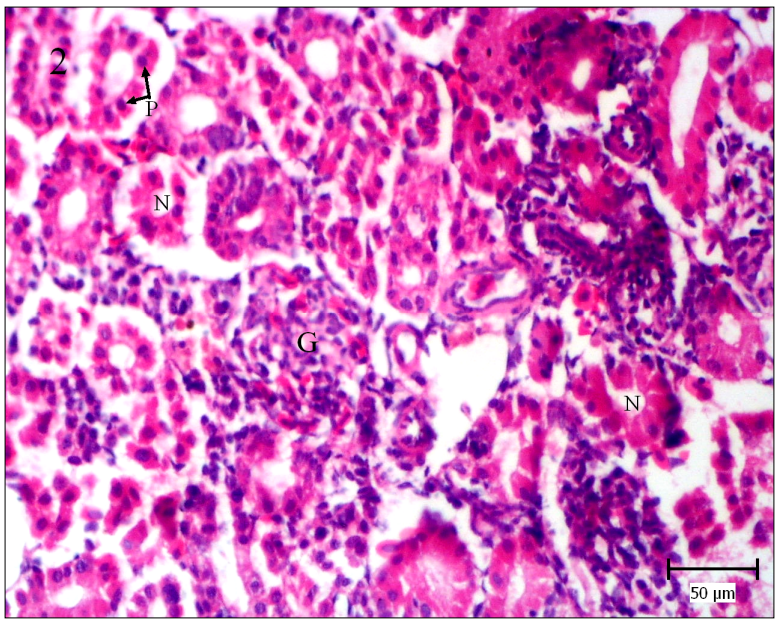

Figure 2. Kidney of goldfish: tubular necrosis (N), eosinophilic cytoplasm with dark and small nuclei as pyknotic nuclei (P) is obvious (H&E staining, Bar 50µm).

Liver. The noticeable histological lesions in the liver of goldfish and angelfish are shown in Table 2. Histopathological changes in the liver indicated by appearance of vacuolated cells, characterized the presence of large lipid vacuoles, which usually occupied the whole cell and displaced nuclei to the periphery and cystic degeneration that were more common than other injuries (Figure 3). Also, haemorrhagia, focal necrosis of hepatocytes (Figure 4) and hepatitis (aggregation of inflammatory cells between hepatocytes and around blood vessels) were seen.

Table 2

Summarized histopathological changes in the liver of Goldfish and Angelfish

Sample	*Goldfish*	*Angelfish*
Vacuolar degeneration	14 (70%)	17 (85%)
Focal necrosis	2 (10%)	2 (10%)
Haemorrhagia	2 (10%)	5 (25%)
Hepatitis	1 (5%)	2 (10%)

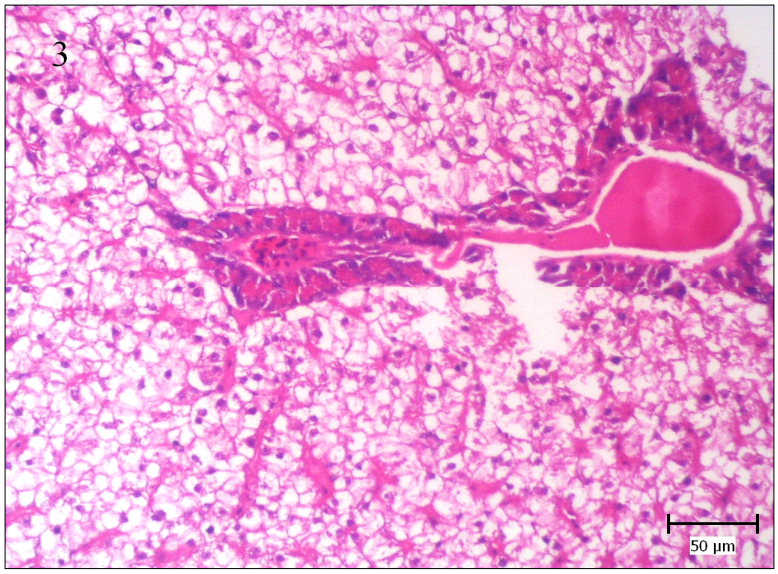

Figure 3. Liver of angelfish: vacuolar degeneration and fatty changes in most of hepatocytes (H&E staining, Bar 50µm).

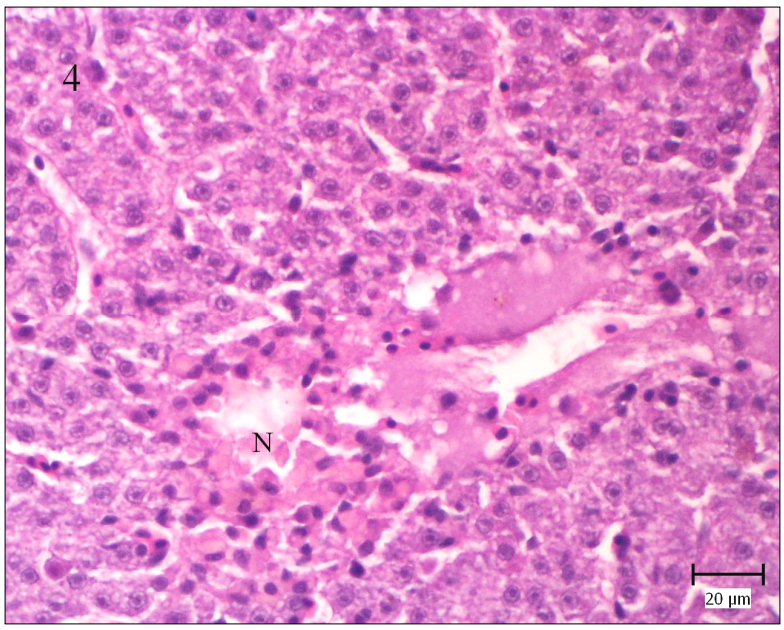

Figure 4. Liver of goldfish: focal necrosis. Note to eosinophilic cytoplasm with pyknotic nuclei (N) (H&E staining, Bar 20µm).

Intestine. Enteritis was the common lesion which was seen in intestine. This was characterized by accumulation of inflammatory cells in the lamina propria and submucosal layer in the intestine tissues of goldfish and angelfish.

Discussion. The investigation of tissue alterations of different body organs and tissues could be an important aid for diagnosis of many diseases in fish. Chandra (1987) reported that undesirable environmental conditions such as low dissolved oxygen level and high water temperature could cause a variety of diseases in fish. The high incidence of histological changes in the liver and kidney is a demonstration of the poor environmental quality (Lidia et al 2011).

In the current study, the kidney of 70% of fishes showed glomerulonephritis, which was characterized by glomerular hypercellularity with adhesion to parietal layer of Bowman's capsule and thickening of the basement membrane of glomerular tuft with excessive mesangial matrix. The hypercellularity of glomerular tufts resulted from proliferation of endothelial and mesangial cells accompanied by inflow of leukocytes leading to adhesion of tufts as well as degeneration of renal tubules may be due to viral, bacterial or protozoal diseases (Jones et al 1997). Hyaline droplets (accumulations of intracytoplasmic protein absorbed from the glomerular filtrate) and hyaline cast on some proximal tubules (Table 1) arise from glomerulonephritis (Kashgarian 1998). Laurén et al (1989) delineated the hyalinization of the renal tubular epithelium in rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) as a result of oral uptake of the antibiotic Fumagillin. These histological alterations on kidney have been observed by several authors in fish (Lidia et al 2011; Velmurugan et al 2007; Mohamed 2009).

Tubular cell necrosis usually is due to ischemic and nephrotoxic agents. Vasoconstriction and reduced blood flow causing ischemia resulted in tubular cell damage (Confer & Panciera 2001). Dilation of the lumen of the kidney tubules, necrotic changes and degeneration of glomerulus have been reported in *Labeo rohita* (Hamilton, 1822) exposed to hexachlorocyclohexane (Dass & Mukherjee 2000) and *Cyprinus carpio* Linnaeus, 1758 exposure to deltamethrin (Cengiz 2006). The necrosis of the renal tubules affected the metabolic activities and advertises metabolic irregularities in fish (Yokote 1982).

According to these results, glomerulonephritis is one of the most frequent lesions in kidney of ornamental fish; it mainly occurre in Ag-Ab complex on glomerulus affected by previous parasitic, bacterial or viral infestation.

Alterations in the liver may be useful as markers that show former exposure to environmental stressors (Velmurugan et al 2007) since it is the main organ for detoxification (Soufy et al 2007). In the present study, liver revealed various degenerative changes. The most demonstrated one was vacuolar degeneration (Table 2). The vacuolar degeneration observed in this study either in the form of hydropic degeneration in which the cells swollen with irregular vacuoles in the cytoplasm or fatty changes in which the cells showed circumscribed vacuoles in the cytoplasm.

Gingerich (1982) revealed that the vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulation system. On the other hand, Eder & Gedigk (1986) suggested that oxygen deficiency as a result of gill degeneration may be the most common cause of the cellular degeneration in the liver. The fatty degeneration changes in studied liver may be caused by decline in the rate of utilization of energy stock (Desai et al 1984). Vacuolar degeneration in fish fed by high doses of the antibiotic erythromycin was investigated by Hicks & Geraci (1984). They reported that the vacuolar epithelial degeneration was a result of lysosomal distension.

In previous studies, cell swelling, focal necrosis and hemorrhagia, pyknosis of nuclei and vacuolization of cytoplasm (Cengiz & Unlu 2006) have been reported, which is consistent with the current study (Table 2). In addition, hepatitis which was seen was characterized by increased numbers of inflammatory cells mainly lymphocytes and macrophages. The inflammation comprises a complicated series of homeostatic mechanisms involving the immune, nervous and circulatory systems in response to tissue damage or infection (Sharkey 1992).

Based on this fact that the liver is an important organ with detoxification role in body, any alteration in environmental conditions and nutrition may be resulted in liver lesions.

In fishes, the wall of the intestine is composed of an internal epithelium, lamina propria, stratum compactum, stratum granulosum, circular and longitudinal muscle layers and an external serosa, while the intestinal folds are created of columnar epithelium and a core of lamina propria (McDonough & Gleason 1981). In generally, serious damage to the intestinal folds will intensely reduce the available absorptive area for the digestive and absorptive functions of the animal (Taraschewski 1988).

The intestine of studied fish showed enteritis in the intestinal lamina propria and submucosal layers which is consistent with the observations of O'Brien et al (1993). They showed enteritis varying in severity from a mild, diffuse inflammation of the lamina propria and submucosa to a severe in angelfish infected with the *Spironucleus sp.* parasites. Mohamed (2009) has also found degenerative and necrotic changes in submucosa and mucosa with edema and atrophy in the muscularis and submucosal layer in the intestine of *Tilapia zillii* (Gervais, 1848) and *Solea solea* (Linnaeus, 1758). Histological analysis of intestine tissue of *Cirrhinus cirrhosus* (Bloch, 1795) after exposure to lambda-cyhalothrin showed infiltration of eosinophiles into the lamina propria and atrophy of epithelial cells (Velmurugan 2007).

Conclusion. Histological alterations indicated the response of aquarium fishes to undesirable environmental conditions. Histopathological patterns are sings of metabolic disorders, immunity suppression, compensatory reactions, development and functional abnormalities as well as some parasites in vital organs of studied fish. These conditions affect the normal tissues and direct to cell damage and physiological and pathological alteration that some of them may be pathogonomic. This revealed that histopathology may be an applicable and useful diagnostic tools in fish disease.

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