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Sub-Lethal Effects of Heavy Metals Toxicity on Pathological Lesions of Sea Bream

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Abstract

Histopathological indices have been largely used as biomarkers in the monitoring of fish health status during exposure to heavy metals, both in the experimental and environmental studies. The aims of the present study was to provide baseline data on the prevalence of histopathological liver lesions in marine fishes (case study of liver histopathology at mercury exposure) under experimental mercury exposure and to compare the sampling areas in terms of the types and prevalence of lesions present, for monitoring purposes. Experimental study was at seawater re-circulatory tanks. Mercury concentrations were determined using a standard cold vapor atomic absorption. Histopathological analyses were done in tissue processor and the slides were stained with haematoxylin and counter stained with eosin. There were many liver lesions in both area include enlarged and lateral nuclei, nuclear degeneration and vacuolation; oncotic, apoptic, focal, massive, centrilobular and periportal necrosis; atrophy, lipidosis, hydropic and cloudy swelling, oval cell proliferation; bile stagnation, dilation of sinusoid, intracellular edema and dark granules. In conclusion the present investigation indicated that mercury is a toxic substance in seabream and the sub-lethal mercury concentrations tested may cause several changes in the histological indices of the studied fish and we can use these changes as biomarkers of mercury detection.

Keywords: Cell, Fish; Histopathology; Mercury; Toxicology

INTRODUCTION

Histopathology is a useful tools for assessment the effects of toxicants, in cells, tissues and organs (Adams, 2002). Today histopathological biomarkers have been widely used in fish for detection and assessment on chemical effects of exposure to pollutants (Oliveira Ribeiro et al., 2006). Also histopathological indices have been largely used as biomarkers in the monitoring of fish health status during exposure to heavy metals, both in the experimental (Thophon et al., 2003) and environmental studies (Teh et al., 1997).

Quantitative histological studies are important to evaluate sub-acute and chronic effects of pollutants and to facilitate comparison of responses at the different condition. Histological changes are medium term responses to xenobiotics, and histology represents a rapid method for evaluation effects of chemicals in different organs (Jagoe et al., 1996).

Histopathological biomarkers allows examining specific target organs, including gills, gonad and liver, that are responsible for vital functions, and this fact is very important advantage of these category of biomarkers in monitoring programs of marine environments (Gernhofer et al., 2001). Moreover, the changes detect in these organs are normally easier to identify than functional ones, and serve as warning signs of damage to fish health (Fanta et al., 2003).

In recent years, due to the growing awareness of a cause effect relationship between marine pollutants and the occurrence of toxic liver lesions in fish, studies on fish liver histopathology have recently been incorporated (Oliveira Ribeiro et al., 2002a).

Fish tissues are sensitive indicators of marine pollutant and have a high mercury bioaccumulation capacity for both organic and inorganic forms solution in marine environment (Gochefeld, 2003). It is well confirmed links between exposure to pollutants and the development of hepatic lesions. For example toxicopathic liver lesions in fish species are suitable and sensitive signs of hepatit injury and have been used as biomarkers of chemicals in environmental risk assessments (Stehr et al., 2004).

However most studies on mercury are distribution and speciation and accumulation, open literature on mercury in tropical fish and its toxic effects on fish tissues and organs are rare (Oliveira Ribeiro et al., 2002b). The marine pollutant exposure can induce a number of lesions and injuries to different fish organs (Oliveira Ribeiro et al., 2006) but liver is a very important target organ suitable for histopathological monitoring program for detection of damages to tissues and cells (Oliveira Ribeiro et al., 2006).

In many studies the significance role of mercury for histopathological changes to various fish were confirmed (Oliveira Ribeiro et al., 2002a) and yet the damage of this heavy metal on the liver of yellowfin seabream is largely unknown, so the aims of the present study was to provide baseline data on the prevalence of histopathological liver lesions in A. Latus under environmental and experimental mercury exposure and to compare the sampling areas in terms of the types and prevalence of lesions present, for monitoring purposes.

MATERIALS AND METHODS

1. Experimental test

Yellow fine sea bream all immature male in same size (150 g final body weight average) were maintained in seawater re-circulatory system (300-L tanks) equipped with physical/chemical filters and with aeration to the Mariculture Research Station of the South Iranian Aquaculture Research Center, Mahshahr, Iran.

Seventy five fish were randomly divided into five equal groups (15 per group) and each tank was randomly assigned to one of five experimental treatments and allocated to

a 15 static cylindrical polyethylene tank filled with the appropriate concentration of an aqueous solution of Hg (standard solution for atomic absorbance spectrophotometer) in dechlorinated tap water (Safahieh et al., 2010).

Fish were exposed to mercury concentrations of 0 µg l, 10 µg l, 20 µg l, 40 µg 1, 80 µg l respectively, and maintained for three weeks with aeration. These sub-lethal doses were chosen on the basis of preliminary toxicity tests and determinations of LC50 96h for this species, suggestive of inducing toxic effects but not lethally so (Hedayati et al., 2010).

Conditions within each experimental tank were monitored daily with the temperature 25C ± 1, pH 7.8 ± 0.1 and salinity 46 ± 1 ppt under a natural photoperiod (12hL:12hD) in controlled room. Water was oxygen saturated through constant aeration in a static system. Voluntary feed intake was near to maintenance ration at the time of the maintenance, Fish were fed two times a day (08:30 and 17:30 h) but were starved for 48 h prior to the start of the experiment and throughout its duration. Fecal remains and food residues were removed by suction every other day. The food supply was provided to each predator fish with fresh prawn, collected from creeks without pollutants sources.

2. Mercury analysis

In laboratory water samples were filtered with Millipore strainer mesh size 0.45 µm, the filtrate was then acidified with 2mg/l of 20% K2Cr2O7 (w/v) prepared at nitric acid (Gochefeld, 2003) and soluble store at -4 ºC until mercury analyses.

For stabilize of weight, the sediments were freeze-dried (Shi et al., 2005), then were sieved through 63 µ mesh and were allowed to settle, the supernatant water decanted and homogenized, finely powdered sediment sub samples were dissolved in 60 ml container 4 ml of concentrated nitric acid and 2 ml of concentrated sulfuric acid. The mixture was digested at 90°C for l-2 h in hot plate. Upon cooling, 1 ml K2Cr2O7 or 0.5 ml BrCl was added. The solution was filtered using Whatman No.1 filter paper and diluted to 50 ml with deionized water (Gochefeld, 2003) and preserved prior to Hg analysis.

Mercury concentrations were determined by the Department of Marine Chemistry Laboratory, Khorramshahr University of Marine Science & Technology using a standard cold vapor atomic absorption (CV-AAS) method (Unicam 919) equipped with Hg cold vapor generator (VGA 77) (EPA, 1992).

3. Histopathological analysis

A small part of the right liver was removed and examined macroscopically. Liver samples were preserved by immersion in Bouin's fixative solution for 24 h. Dissected tissues were washed in ice cold 0.9% sodium chloride solution, and subsequently fixed in 10% formalin solution for 48 h. After 48 h, the tissues were transferred into 70% ethanol (Haschek, et al., 2010). After incubate, dehydration, xylene and lastly xylene: paraffin mixture in Tissue processor, (Triangle biomedical sciences USA), liver were embedded in paraffin and sectioned using an ultra microtome (Olympus CUT 4055E, USA) to obtain sections of 5µm in size. The sectioned tissues were fixed on the microscope slides and air-dried for 24 h. The slides were later stained with haematoxylin and counter stained with eosin (Haschek, et al., 2010).

In this study the understanding of morphological abnormalities was with data derived from fixed images of cells and tissues as seen through the light microscope using digital optical imaging techniques. The incidence of alterations was reported in a qualitative evaluation, plus a semi-quantitative scale scored in four categories according to the intensity of alterations: None (1), mild (2), moderate (3) and severe (4) (Di Giulio and Hinton, 2008).

RESULT AND DISCUSSION

1. Mercury analysis

Since the catch site was Zangi creek, the ranges of mercury in the Mahshahr creeks were determined. Water mercury concentrations ranged between 3 and 10 µg/l, with the highest concentrations found in Ghazaleh, the Petroshimi and Majidieh creeks had nearly same values while Zangi was the lowest one. Sediment mercury concentrations ranged between 0.3 and 1 µg/g, with the highest concentrations found in Petroshimi and Majidieh creeks, the other creeks had nearly same values while Jafari and Zangi was the lowest one.

Results concerning mercury accumulation in the liver of studied fish showed that there was bioaccumulation in the liver. Mercury concentrations in the liver ranged 2.4 to 140 µg/g in test treatments. The analytical data on mercury concentrations in the laboratory samples from the liver of Yellowfin seabream are summarized in Fig 5. Concentrations of liver mercury increased with increase of water mercury concentration. Results of Bioaccumulation Factor (BAF) strongly suggest that increase of mercury in the surrounded water will be accumulating much more in liver tissue, mercury bioaccumulation had same process with mercury concentration with the highest accumulation between 40 and 80 µg l treatments (table 5).

2. Histopathological analysis

The hepatic tissue of the control fish follows the standard that was described for teleost fish. It was constituted by hepatocytes, which was large in size, polygonal in shape with centrally located nuclei polyhedral form with a spherical nucleus with one or more nucleolus. There were also the portal vein and the central vein, which branches itself into sinusoids. A large number of blood sinusoids were observed and separates the hepatic cords one from another (Fig 3).

The bile ducts lined by cubic epithelial cells were distributed through the hepatic parenchyma and were usually associated with the port vein. There were bile canaliculi, surrounded by the plasmatic membrane of the hepatocytes. A very homogeneous hepatic parenchyma was found in the liver of fish from control group. Hepatocytes were arranged in cords, generally two cells thick between two contiguous sinusoids.

Changes to the histopathological abnormality of the hepatocytes elevated in severity with the increasing dose of mercury in test area. No mortality occurred during the experimental test but the morphological lesions observed in liver revealed important alterations throughout the course of the experiment. The tissues damages and injuries after mercury exposure are summarized in tables 1-5 and figures 4-8. No neoplastic features were observed.

Table 1. Summarized nuclear lesions in the liver of yellowfin seabream during experimental exposure to mercury.

Lesion	*Experimental* exposure (ppb)				
	0	10	20	40	80
Enlarged nuclei	-	-	+	++	++
Lateral nuclei	-	+	-	-	+
Nuclear degeneration	-	++	+	+++	+++
Nuclear vacuolation	-	+	++	++	++

None (−), mild (+), moderate (++) and severe (+++).

Table 2. Summarized necrosis lesions in the liver of yellowfin seabream during experimental exposure to mercury.

Lesion	*Experimental* exposure (ppb)				
	0	10	20	40	80
Oncotic necrosis	-	+	++	++	++
Apoptic necrosis	-	+	+	+	++
Focal necrosis	-	++	+++	+++	++
Massive necrosis	-	-	-	++	++
Centrilobular necrosis	-	++	+	++	+++
Periportal necrosis	-	-	-	++	++

None (−), mild (+), moderate (++) and severe (+++).

Table 3. Summarized other hepatocyte lesions in the liver of yellowfin seabream during experimental exposure to mercury.

Lesion	*Experimental* exposure (ppb)				
	0	10	20	40	80
Atrophy	-	+	-	+	++
Lipidosis	-	+++	+++	++	++
Megalocytosis	-	-	-	+	+
Hydropic swelling	-	+	++	++	++
Cloudy swelling	-	++	++	+++	+++
Oval cell proliferation	-	++	+	+	++

None (−), mild (+), moderate (++) and severe (+++).

Table 4. Summarized intracellular lesions in the liver of yellowfin seabream during experimental and environmental exposure to mercury.

Lesion	*Experimental* exposure (ppb)				
	0	10	20	40	80
Bile stagnation	-	+	++	+++	+++
Dilation of sinusoid	-	++	+++	+++	+++
Intracellular edema	-	++	+	+	++
Dark granules	-	-	+	-	+

None (−), mild (+), moderate (++) and severe (+++).

Table 5. Sub-lethal bioaccumulation factor (BAF) of mercury (µg/g) in the liver tissue of Yellowfin Seabream.

	Control	10 µg l	20 µg l	40 µg l	80 µg l
BAF	0.65± 0.03	1.23±0.11	1.14±0.09	0.96± 0.13	1.75±0.10

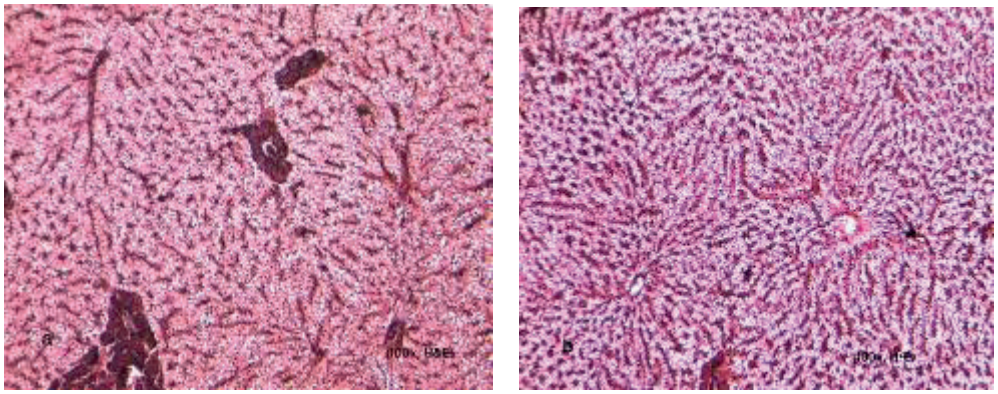


Fig 1. Light microscope features of hepatic parenchyma of yellowfin seabream during control treatment (a) hepatopancreas, (b) portal vein.

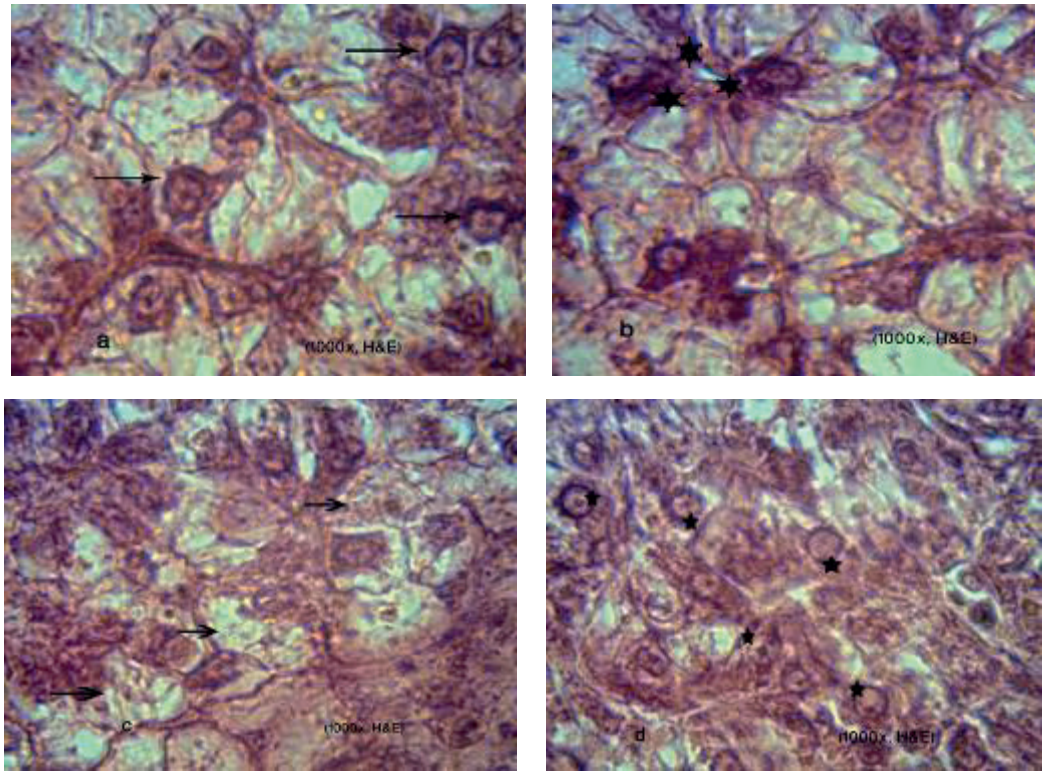
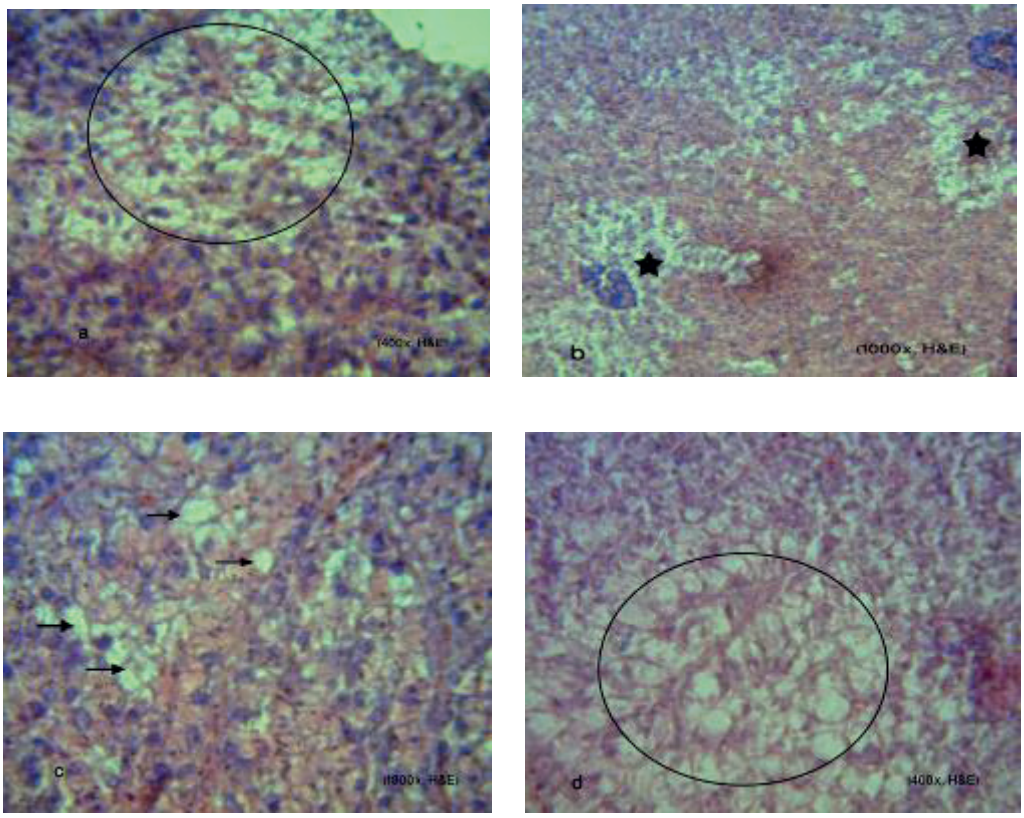


Fig 2. Light microscope features of nuclear lesions in the liver of yellowfin seabream during experimental exposure to mercury (a) Enlarged nuclei, (b) Lateral nuclei, (c) Nuclear degeneration, (d) Nuclear vacuolation.



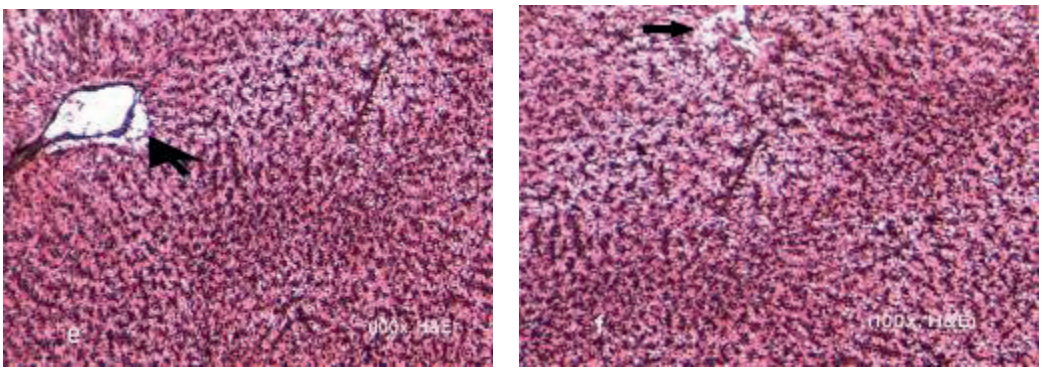


Fig 3. Light microscope features of necrosis lesions in the liver of yellowfin seabream during experimental and to mercury (a) oncotic necrosis, (b) apoptic necrosis, (c) focal necrosis, (d) massive necrosis,(e) centrilobular necrosis, (f) periportal necrosis.

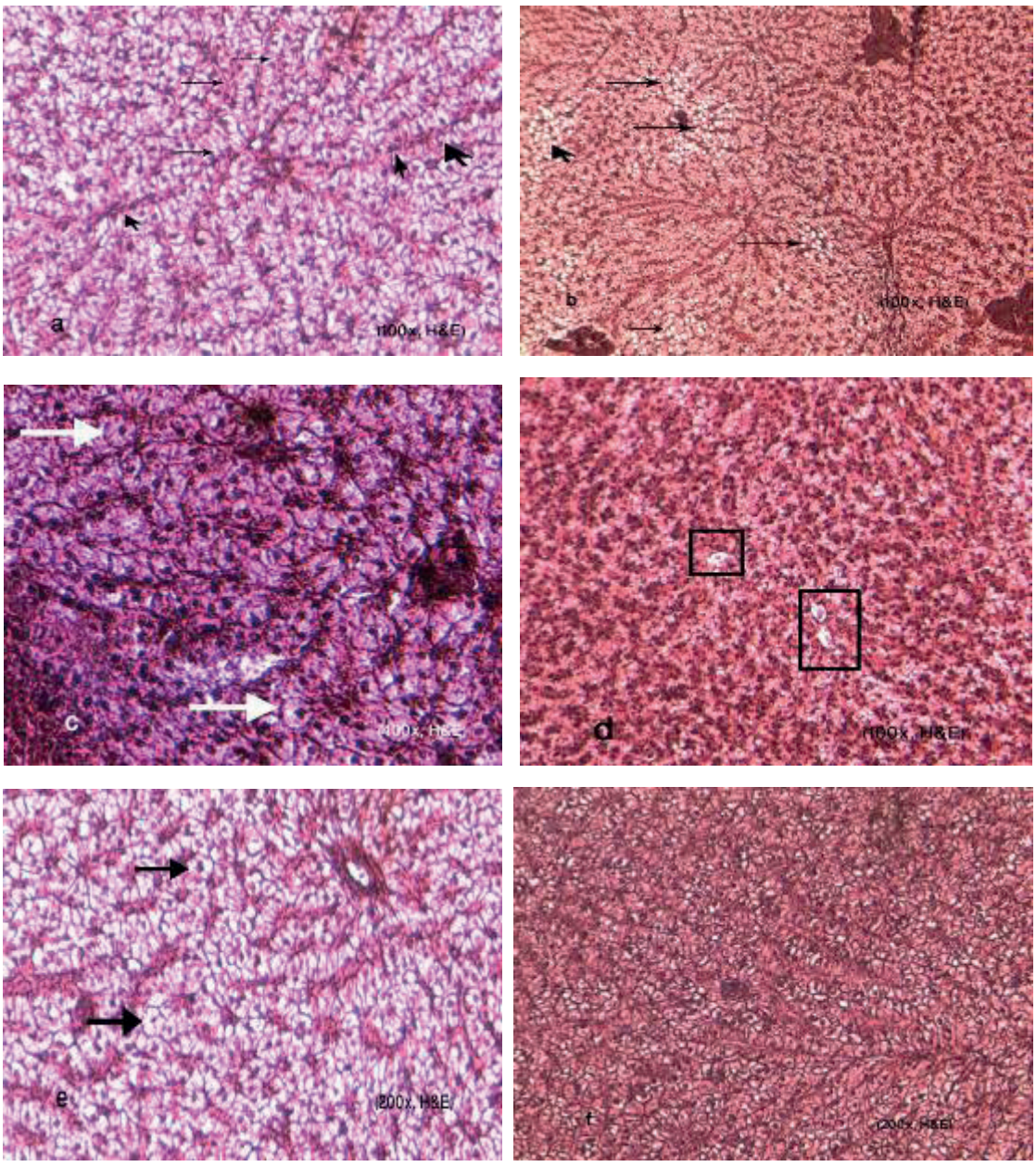


Fig 4. Light microscope features of other hepatocyte lesions in the liver of yellowfin seabream during experimental exposure to mercury (a) atrophy, (b) lipidosis, (c) megalocytosis, (d) hydropic swelling, (e) cloudy swelling, (f) oval cell proliferation.

The most frequent pathological modifications were the increase in the lipid droplets (lipidosis), nuclei change, necrosis, swelling, degeneration, cytoplasmic vacuolization of the hepatic cells, bile stagnation, and dilation of sinusoid, atrophy and pre-necrotic lesions within many hepatocytes with reference to the control treatment. Lipid bodies were also sporadically visible in the cytoplasm of hepatocytes. Also the presence of megalocytosis was significantly higher in the experimental group than in the control group. The Kupffer cells were absent in current fish. Cellular swelling was observed in a few livers of fish exposure. A sever increase of degenerated cells was observed in the exposed specimens compared to the control specimens. These degenerated nucleuses were characteristic of many exposed livers. In general, the degree of the histopathological findings was seemed to be related to the increasing concentrations.

The large vacuole in the cell forces the nuclei to the periphery of the hepatocyte and this condition may associated by nuclear atrophy. Vacuolation of hepatocytes are accompanied with the inhibition of protein synthesis, energy decrease, disaggregation of microtubules, or shifts in substrate utilization (Oliveira Ribeiro et al., 2002a).

Necrosis is irreversible injury that leads to death of the cell, but of course it should mentioned that the term necrosis encompasses not only the actual occurrence of cell death in the living organism, but also the degenerative alternation that follow the death process (Haschek, et al., 2010).

Oncotic necrosis may lead to the reduction in cell number, there will be some degree of inflammation, often with resultant scarring, however the reduction in tissue or organ size may be irregular and distorted (Di Giulio and Hinton, 2008).

Apoptosis is a controlled form of cell death that serves as a regulation point for biologic processes and may consider as the counterpoint of cell division by mitosis (Timbrell, 2009).

The swollen and necrotic hepatocytes appear to compress the vascular spaces, so it can conduct the reason of observed little blood in the sinusoids in the necrotic central lobular areas (Di Giulio and Hinton, 2008).

Periportal necrosis has also been used as peripheral lobular necrosis. Numerous oval cells may be found in the periportal area. Oval cells appear to be most numerous when hepatocyte regeneration is completely, or at least partially, blocked (Haschek, et al., 2010).

Massive necrosis is clearly evident on gross observation. In the sub-acute phase, the affected liver areas are abnormal in color (usually pale) and appear slightly swollen. In chronic exposure, the affected area is depressed below the surface of the adjacent tissue (Stehr et al., 2004). Focal necrosis may be observed grossly as small pale foci. The lesions are particularly evident when they include inflammatory cells (Haschek, et al., 2010).

The swelling hepatocytes in an affected liver were typically swollen, with compression or displacement of adjacent structures. Staining affinity was diminished, generally giving the cells a pale or cloudy appearance. Hydropic change was characterized in almost all treated by enlarged pale-staining cytoplasm.

A cell dealing with disrupted homeostasis can respond in a different ways to maintain itself short of death. This is called adaptation (Haschek, et al., 2010). Atrophy is simple adaptation. At the cellular level, atrophy is often a response to decreased demand for the specialized functions of a particular cell. Atrophy can lead to cell death, either accompanied by apoptotic necrosis or oncotic necrosis (Hinton et al., 2001). But in contrast, hypertrophy is a response to increased metabolic demand for a specialized function provided by the particular cell (Di Giulio and Hinton, 2008).

Lipidosis is often observed in cells that metabolize large quantities of lipids for energy (Haschek, et al., 2010).

Cell swelling is an early change that occurs in most types of liver injury, and which may be a prelude to more effective changes (Hinton et al., 2001). Hydropic swelling is a reversible injury with accumulation of water within the cytosolic matrix or rough endoplasmic reticulum of hepatocytes. This form of swelling can be attributed to a failure to maintain intracellular sodium ion balance (Mommsen and Moon, 2005). The swollen cells have the cytoplasm appeared cloudy and granular. Hinton et al. (2001) state that, although swelling is an integral part of adaptation to cell injury, the finding of hepatocyte swelling as the major indication of toxic injury is rare.

Liver toxicants are typically characterized as being cytotoxic or cholestatic. Cytotoxic mechanisms affect hepatocytes and are responsible for different types of liver injury. Cholestatic mechanisms affect the flow of bile. Intrahepatic cholestasis occurs when the flow of bile is blocked within the liver as it flows through canaliculi, as well as bile ductules (Mommsen and Moon, 2005).

The cholestatic mechanisms that lead to the blockage of bile are not understood very well. “Blockage” may result from blocked transport mechanisms in the cell membrane of hepatocytes (Hodgson, 2004).

Microscopic analysis of yellowfin seabream liver sections reveals a pattern of arrangement of hepatocytes different from that of mammals. A double row of hepatocytes was obvious. The bile preductular epithelial cells found between rows of hepatocytes in liver tubule, however nuclei of hepatocytes contrast with flattened and elongated nuclei of a different cell type. In 1000 timer feature, transects of hepatocyte arrays resemble tubules. Basal aspects of hepatocytes project toward sinusoids or adjacent hepatocytes of neighboring tubules, while cellular apices were directed toward the center of the tubule. It was noticeable that the high nuclear to cytoplasmic ratios were commonly encountered in the centers of tubules. Also larger elements of the bile passageways were present in the parenchyma of current fish liver.

The two-dimensional features of hepatic tubules suggest that individual tubules curve, anastomose and/or branch, thereby forming a complex continuum of parenchyma tunneled by an extensive microcirculation. This large blood supply no doubt leads to intensive mercury exposure and accumulation while hepatocytes and biliary epithelial cells.

The types of lesions to the liver depend on the type of toxicant, the severity of intoxication, and the type of exposure, whether acute or chronic (Hodgson, 2004). There have been numerous reports on histopathological changes in livers of fish exposed to a wide range of heavy metals in marine ecosystems (Rice, 2001).

Some of the indices observed in the hepatic cells in the present study, such as vacuolar degeneration and lipid droplet accumulation are consistent with those documented in specimens of D. labrax, Lates calcarifer and Carassius carassius acutely treated with other heavy metals (Giari et al., 2007).

The presence of necrosis is in fact one of the most visible damages in many tissues affected by heavy metals (Rabitto et al., 2005). In fish liver, the presence of necrosis area is also related with pollutant concentration during the detoxifying process.

Liver lesions such as irregular shaped hepatocytes, vacuolation and nucleus in a lateral position, were also described in the siluriform Corydoras paleatus affected by organophosphate pesticides (Fanta et al., 2003).

Pacheco and Santos (2002) described increased vacuolisation of the hepatocytes as a signal of degenerative process that suggests metabolic damage, due to exposure to marine pollutants. The liver parenchyma of fish exposed to

the environmental metals, such as mercury showed signs of degeneration (cytoplasmic and nuclear degeneration, and nuclear vacuolation) besides the focal necrosis (Oliveira Ribeiro et al., 2006). The changes induced by mercury in the liver hepatocytes such as vacuolization, necrosis and nuclear condensation were also reported for other heavy metals (Figueiredo-Fernandes et al., 2007).

Hypertrophy, vacuolization, nuclear and fatty degeneration of hepatocytes have also been seen by Gill and Epple (1993), who investigated the effects of different organic pollutant on liver of fish. In contrast, study to heavy metals by Cengiz et al. (2001) show that there were hepatic lesions including degeneration, hypertrophy, sinusoids enlargement, change position of nuclei, vacuolization, and infiltration of mononuclear lymphocyte.

The stagnant of bile indicates possible damage to the hepatic metabolism (Fanta et al., 2003). Our histological finds were considered not mercury specific but changes generally associated with the response of hepatocytes to many pollutants.

Degeneration of liver tissue and necrosis of central vein could be due to the accumulation of neutrophils and lymphocytes. Similar results have been found from studies of African catfish exposed to fuel oil for 14 days (Gabriel et al., 2007).

The histological changes observed in the present study indicate that the fish were responding to the sub-lethal effects of the mercury as much as to the secondary effects caused by stress. In conclusion the present investigation indicated that mercury is a toxic substance in yellowfin seabream and the sub-lethal mercury concentrations tested may cause several changes in the histological indices of the studied fish and we can use these changes as biomarkers of mercury detection.

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