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Gill Alterations in the Yellowfin Seabream, *Acanthopagrus latus*, Following Exposure to Chronic Sublethal Levels of Mercuric Chloride

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Abstract: In the present study, histomorphological alternations in gills were used to assess the physical condition of yellowfin seabream, *Acanthopagrus latus*, exposed to sublethal concentrations of $HgCl_2$ (10, 20, 40, 80 μgL^{-}) for 3 weeks. Treated fishes were erratic and showed respiratory distress. The most common morphological abnormalities included: filaments disorganization, increase of mucus secretion, debris, blood plaques on the filaments and losing or shortening of some filaments. The most frequent histopathological changes detected in the gills included extensive lifting of the lamellar epithelium and edema of the lamellae with enlarged sub-epithelial spaces, exfoliated epithelium of lamellae, telangiectasia, hypertrophy and hyperplasia of the epithelial cell resulted in partial fusion of the secondary lamellae and a reduction of the water space, club shaping of gill lamellae and congestion. Some severe alternations found in the gill of fishes exposed to higher levels of $HgCl_2$ (40, 80 μg L^{-1}) were lamellar aneurysms and hemorrhages with rupture of the lamellar epithelium.

Key words: Histopathology · Gill · Mercuric chloride · *Acanthopagrus latus* · Persian Gulf

INTRODUCTION

Mercury is a hazardous toxic metal that naturally exists in the earth's crust. Natural event such as erosion and volcanic eruptions, as well as anthropogenic activities such as industrial, municipal, or agricultural wastes, may lead to significant environmental contamination [1]. In the aquatic ecosystems, a part of the mercury can be converted into methylmercury by biological processes and readily taken up by aquatic organisms. Mercury finds its way up the trophic levels of the food chain, passing along from phyto- and zooplankton to larger organisms. In this way, its concentration increases in natural populations with the age and/or the trophic level of the organism (bio-accumulation) [2]. Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water [3]. Increasing of mercury in the aquatic environment lead to greater accumulation of this metal in the tissues of fish [4], which is considered the major source of mercury in humans [5]. These general features have been shown both in the natural environment and in the laboratory [6].

To reveal the biological effects of certain contaminants on aquatic bodies, a variety of biomarkers have been used, both in natural environments and/or experimental conditions [6]. Besides the measurement/evaluation of chemical and physical parameters, histopathological biomarkers can be used as indicators of the various pollutants effects on organisms [7, 8]. One of the most important benefits of using of histopathological biomarkers in environmental screening is possibility of examining specific target organs, including gills, kidney and liver, which are responsible for vital functions, such as respiration, excretion, accumulation and biotransformation of xenobiotics in the fish [9, 10]. Gills are multifunctional organs; besides of respiration, they are responsible for osmoregulation, acid–base balance and nitrogenous waste excretion, which also makes them extremely sensitive to water contamination [7, 11]. Irrespective of the accumulation path, the accumulation amount is partially determined by

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the rate of metal uptake at the interface between the organism and its environment. For aquatic animals, gill is one of typically biological barriers, the respective permeability and retention ability of which determines the potential uptake of mercury from water [6]. The exposure to chemical contaminants such as mercury compounds can cause number of damages and injuries to different fish organs [12] and gill represents important target organ suitable for histopathological assessment in searching for cells and tissue damages [12, 13].

In the present study, morphohistopathological features were examined in gill of *Acanthopagrus latus* after exposure to different concentrations of $HgCl_2$ over a period of 3 weeks. The aim of this investigation was to report lesions and damages in gill after an experimental dietary exposure to $HgCl_2$ in one of the most ecological and commercial species of Persian Gulf, yellowfin sea bream *Acanthopagrus latus*.

Our experimental method closely imitated the contamination conditions encountered in natural marine tropical ecosystems, increasing our biological knowledge about this tropical species and the toxicity of $HgCl_2$ in fishes inhabiting such polluted regions.

MATERIALS AND METHODS

Fish Maintenance: 90 *A.latus* (190±0.1 mm; 150±0.1g) were obtained randomly from Mahshahr Creeks and then acclimated for 2 weeks in Imam Khomeini Mari culture Research Station (Imam Khomeini Port, Iran) in fifteen 300L indoor tanks containing filtered aerated seawater treated with UV. Fish were fed daily with shrimp but were starved for 48h prior to initiation of the experiment and throughout it.

Experimental Design: Following acclimation, fish were randomly divided into 5 equal groups and allocated to a 300L tanks. Four concentrations of $HgCl_2$, 10 μg L^{-1} (group 1), 20 μg L^{-1} (group 2), 40 μg L^{-1} (group 3) and 80 μg L^{-1} (group 4) plus one Control, each with three replications, were used. The yellowfin sea breams were exposed to above mercuric chloride concentrations and maintained for 3 weeks.

Conditions within each experimental tank were monitored daily with the concentration of mercuric chloride being determined along with the other factors: The average temperature was 26.5°C, PH equal to7.8 and salinity concentration was 49 ppt. Water exchange in each tank was carried out every two weeks.

At the end of exposure time, fish from each tank were sampled and were killed by a knock to the head and right operculum is taken away and the gills (only the 2th and 3th pairs) were quickly and carefully removed to prevent damaging the tissues.

Tissue Processing: Tissue specimens of gill with diameter of 3–5 mm were fixed by immersion in Bouin's fluid for 48 h., the fixed samples, firstly were morphologically examined by stereo microscopy and then dehydrated in a graded series of ethanol and paraffin embedded. Sections were cut at 5–6 μm on a rotary microtome and resulting tissue sections were mounted onto glass slides before staining with haematoxylin and eosin (H&E) and Periodic Acid-Schiff (PAS). For histopathological investigations, stained sections were analyzed by light microscopy possess Dino Lite lens (with Dino capture software).

RESULTS

Morphological and histological analysis of the control and $HgCl_2$-exposed *A.latus* was conducted to assess whether the presence of concentrations of $HgCl_2$ caused changes in the structure of gills. It should be noted that no mortality occurred during the experiment. The histomorphological analysis of gills of *A. latus* showed important alterations all over the experiment course. Treated fishes were erratic and showed respiratory distress, though the control fish exhibited a normal swimming behavior. The morphological and histopathological alterations found in the gills of the treated fish are detailed in Table 1.

Stereo Microscopy Analysis

***Control fish*:** In control fish, gills had a normal morphological structure. Each gill arch supports perpendicularly many distinct and regular filaments arranged in two rows, without any lesions (Fig. 1).

Treated Fish: The gross structures of gills were conspicuously changed. The most common abnormalities found include: obvious filaments disorganization in groups treated with lower concentrations of $HgCl_2$ (group 1 and 2) and severe filaments disorganization in those treated with higher levels of $HgCl_2$ (group 3 and 4), increase of mucus secretion, debris and blood plaques on the filaments, fusion of some filaments, complete or partial losing or shortening of some filaments and swelling of blood vessels (plate 1 a-h).

Table 1: Histopathological changes in the gills of *A. latus* treated with chronic sublethal levels of $HgCl_2$, indicating stages of damage to the tissue. Stage I: do not alter the normal tissue function; Stage II: more severe and damage the normal function of the tissue

Groups	Morphological alternations	Histological alternations	Stage
Group 1 ($10\ \mu g\ L^{-1}\ HgCl_2$)	fusion of some filaments losing or shortening of some filaments increase of mucus	Increase of mucus cells. Epithelial lifting of lamellae. Edema of the lamellae with enlarged sub-Epithelial spaces Club shaping lamellae	I
Group 2 ($20\ \mu g\ L^{-1}\ HgCl_2$)	Fusion of some filaments Partial losing or shortening of some filaments More increase of mucus Filament disorganization	Epithelial lifting of lamellae Edema of the lamellae Club shaping lamellae Leucocytes infiltration Hyperplasia of the epithelial cells Lamellar fusion Hypertrophy of the lamellar epithelium Blood congestion	I
Group 3 ($40\ \mu g\ L^{-1}\ HgCl_2$)	Sever disorganization of filaments Severe increase of mucus partial losing or shortening of some filaments	Leucocytes infiltration Hyperplasia of the epithelial cells Lamellar fusion Hypertrophy of the lamellar epithelium Epithelial lifting of lamellae Lamellar aneurysm Lamellar disorganization	II
Group 4 ($80\ \mu g\ L^{-1}\ HgCl_2$)	Sever disorganization of filaments Severe increase of mucus debris and blood plaques on the filaments Fusion of some filaments complete or partial losing or shortening of some filaments	Leucocytes infiltration Lamellar aneurysm Lamellar disorganization Hyperplasia of the epithelial cells Lamellar fusion epithelium rupture Hypertrophy of the lamellar epithelium Epithelial lifting of lamellae	II

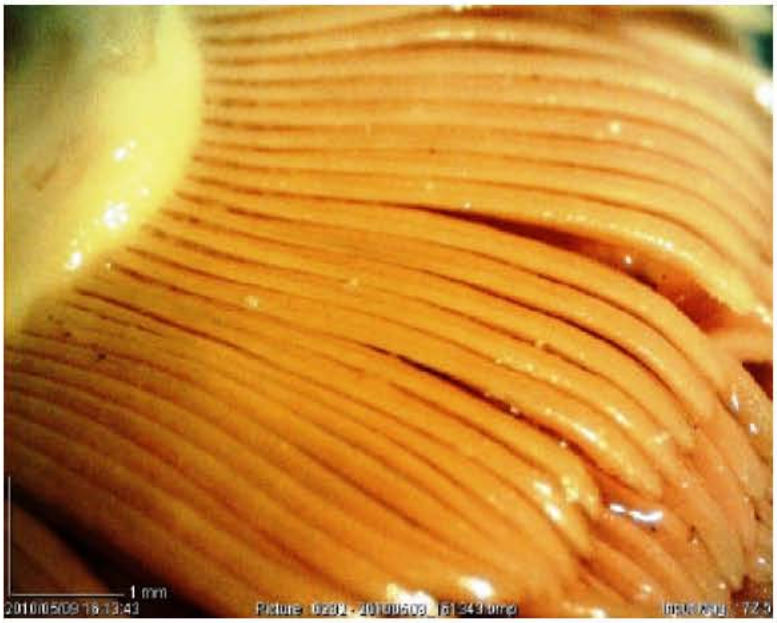

Fig. 1: Morphological structure of the gill within the control group of *Acanthopagrus latus* which possess distinct, regular filaments.

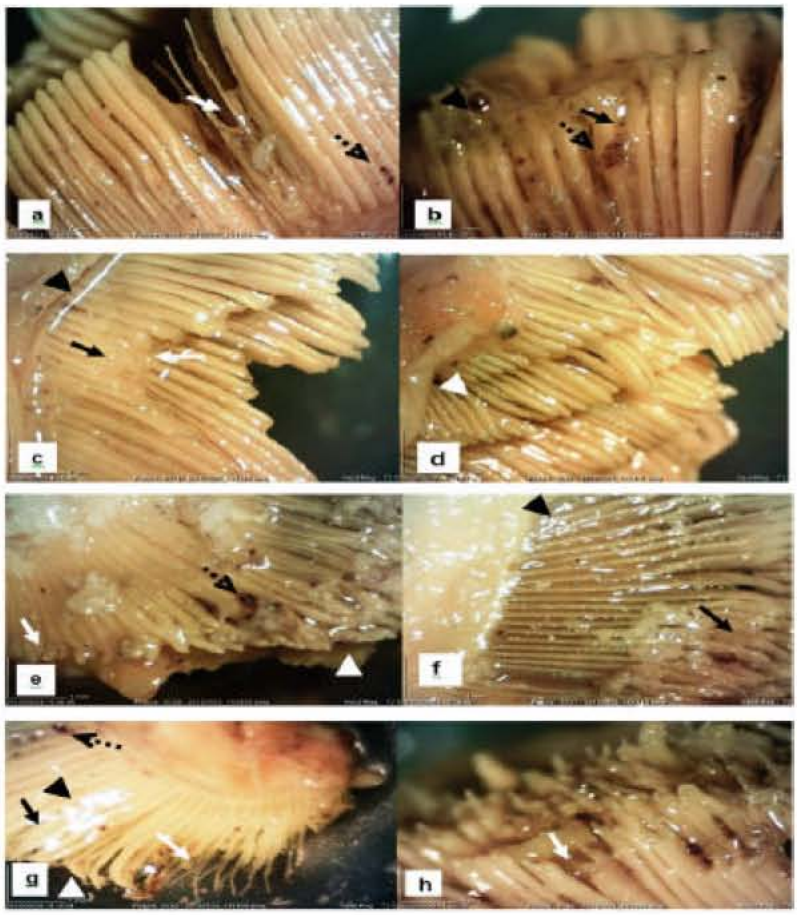

Plate 1a,b: Group 1; fusion of some filaments (black arrow), losing or shortening of some filaments (white arrow), increase of mucus (arrow head), debris and blood plaques on the filaments (dashed arrow); c,d) Group 2; fusion of some filaments (black arrow), partial losing or shortening of some filaments (white arrow), more increase of mucus (black arrow head); filament disorganization (white arrow head); e,f) Group 3; Sever disorganization of filaments (white arrow head), Severe increase of mucus (black arrow head), fusion of some filaments (black arrow), partial losing or shortening of some filaments (white arrow), debris and blood plaques on the filaments (dashed arrow); g,h) Group 4; Sever disorganization of filaments (white arrow head), Severe increase of mucus (black arrow head), debris and blood plaques on the filaments (dashed arrow), fusion of some filaments (black arrow), complete or partial losing or shortening of some filaments (white arrow); (×10).

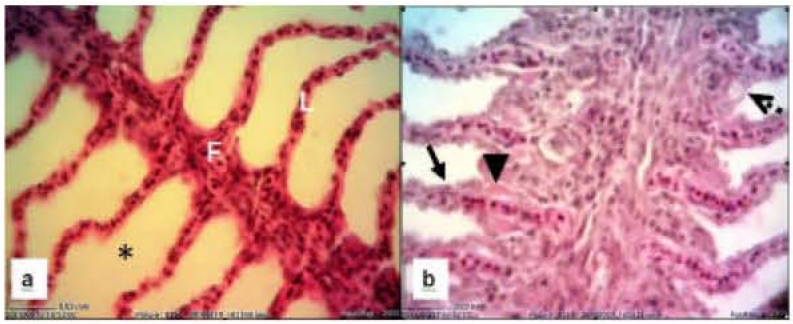

Fig. 2: Normal histological structure of gill within the control group of *Acanthopagrus latus*, a) Light micrograph showing the normal aspect of the filament which possess distinct regular lamellae: the filament (gray arrow), the lamellae (L)and the water channel (*). H&E (×40); b) Different cell types with common numbers and thickness, epithelial cell (black arrow), Chloride cell (black arrow head), mucous cell (dashed arrow). H&E (×40).

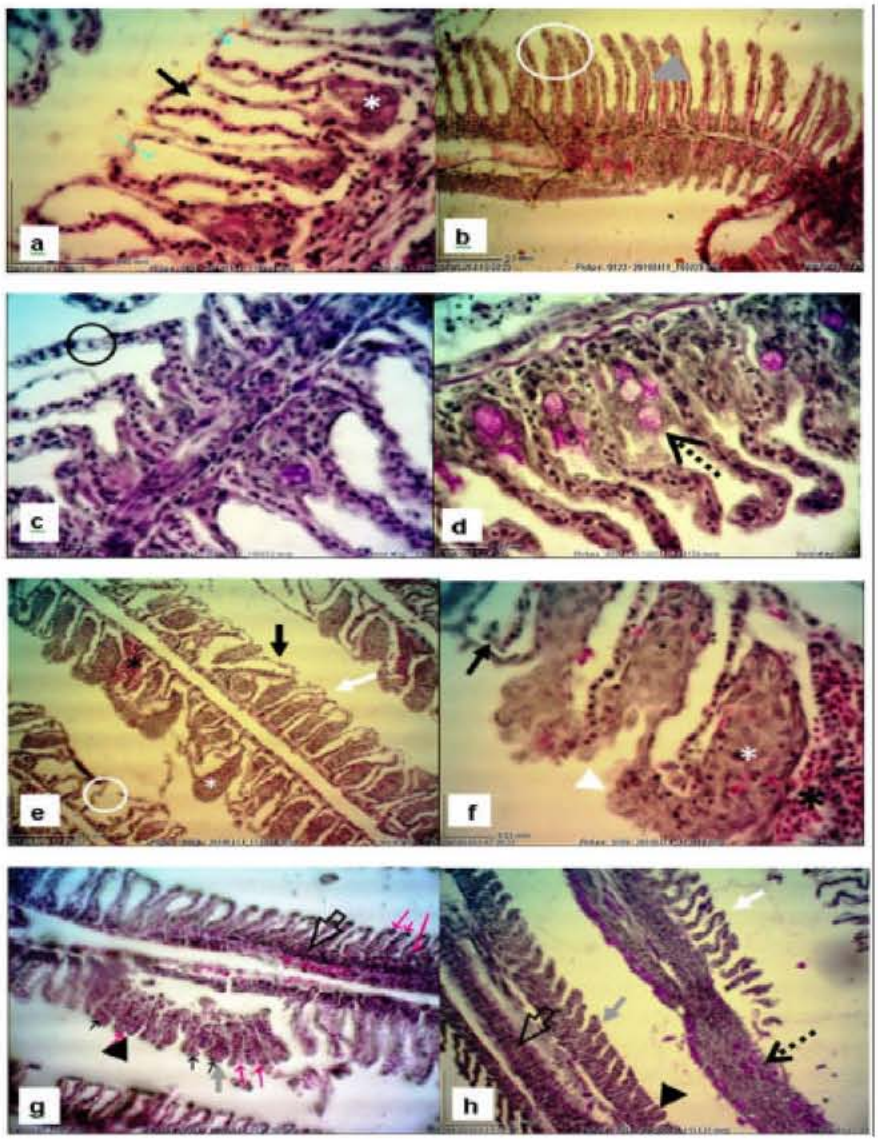

Plate 2: Photomicrographs of histopathological alternations of gills within the *A. latus* groups exposed to $HgCl_2$; extensive epithelial lifting and edema of the lamellae with enlarged sub-epithelial spaces (black arrows), hyperplasia of the epithelial cells (white *) with partial fusion of the lamellae (black arrowhead), club shaping of gill lamellae (gray arrow), lamellae with the marginal channel dilated (black marked circularly), blood congestion (gray arrowhead), lamellar aneurysm (black *) within the lamellae, lamellar disorganization (white arrow), hypertrophy of the lamellar epithelium (white arrowhead), leukocytes infiltration (hollow arrow), increase of mucosal cells (dashed arrow), epithelium rupture (white marked circularly); a,b,c) Sea breams treated with 10 μg L^{-1} $HgCl_2$ (group 1); d,e,f) Sea breams treated with 20 μg L^{-1} $HgCl_2$ (group 2), ; g,h) Sea breams treated with 40 μg L^{-1} $HgCl_2$ (group 3); and j,k) Sea breams treated with 80 μg L^{-1} $HgCl_2$ (group 4). (a,c,f,k: H&E;×40) (b,e,i: H&E;×10) (d:PAS;×40) (g,h:PAS;×10).

Light Microscopy Analysis

Control Fish: Gills didn't represent any abnormality in cell and tissue structure. Series of separate and regular lamellae, where gas exchanges and other physiological events occur, sited on the upper and lower surface of each filament (Fig. 3a). The entire length of the filaments and lamellae was lined by normal epithelial cells and chloride cells were large, scattered along the filaments (Fig. 2b). The mucous cells were sparsely distributed in the filament epithelium (Fig. 3b).

Treated Fish: The histopathological changes detected in the gills of the exposed fish, detailed in Table 1. The degree of tissue damages of gills within treated groups is determined as stages I and II. The most frequent abnormalities found include extensive lifting of the lamellar epithelium and edema of the lamellae with enlarged sub-epithelial spaces (plate 2a), blood congestion (plate 2b), exfoliated epithelium of lamellae (plate 2b), dilation of the marginal channel (plate 2c), increase of mucosal cells (plate 2d), hypertrophy and hyperplasia of the epithelial cell (plate 2e,f) resulted in partial fusion of the secondary lamellae (plate 2j) and a reduction of the water space, club shaping of gill lamellae (plate 2g), Lamellar disorganization (plate 2h). Some more severe alternations found in the gill (stage II) were telangiectasia (lamellar capillary aneurism) (plate 2k) and hemorrhages (plate 2j) with rupture of the lamellar epithelium (plate 2b).

DISCUSSION

Nowadays, histopathology is known as an important instrument to assess the effects of pollutants in vital processes, detecting early changes in cells, tissues and organs [14]. Histopathological biomarkers have been largely used in fish to identify and evaluate toxic effects of exposure to pollutants [12]. Due to the harmful effect of pollutants in aquatic environments, the histo-cytological responses of fishes to a variety of xenobiotics necessitated to be determined and characterized [15]. Gill structure provides a large surface area for direct and constant contact with water pollutants. Thus, this organ is too sensitive to chemicals in water [12] and is considered the primary target of the contaminants [10, 16]. Several types of gill impairment have been documented in fish experimentally exposed to contaminants or in those sampled from unhygienic ecosystems [17, 18].

It seems that, the biological characteristics of fish (such as sex and age) or seasonal factors don't affect the response of fish gill to stress/pollutant exposure. Generally, gill histopathology appears to be a promising biomarker for general environmental contamination, although tissue preparation for gill histopathological study is time consuming [12].

Mercuric chloride produces major histomorphological changes in the gill structure, decreasing its gas exchange capability. These changes ranged from mild to severe depending on the concentration of mercuric chloride and in some cases may result to death [19]. The death from exposure to $HgCl_2$ is possibly resulted from toxic actions on the biochemical processes associated with cellular metabolic pathways and other inclusions [20, 21].

The results showed that, although *A. latus* is one of the most resistant fish species, even the lower concentrations of $HgCl_2$ influenced the normal structure of gills. The most of the histopathological alterations of gill described in the present study were in agreement with those reported in other fish species under a broad range of exposure situations, then, it seems possible that these effects reveal physiological modification to stress rather than as special and restricted toxic responses to the concentrations of $HgCl_2$ considered here.

Changes such as edema with epithelial lifting and desquamation, telangiectasia, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some lamellae as recognized in the present investigation, are usual gill lacerations in response to many other chemicals like petroleum compounds, organophosphates, herbicides and other heavy metals [22, 23]. These are examples of protection mechanisms, which generally result in the increase of the distance between the water and the blood, to act as a barrier to the entry of contaminants [24].

The major alternations in gills of *A. latus* exposed to sublethal concentrations of $HgCl_2$ in the present experiment, were a thickening of the filament epithelium, which filled the space between lamellae in some instances, so lamellae appeared to shorten and disappear and detachment of the lamellar epithelium, or extensive edema, as have been reported upon exposure of mosquito fish *(Gambusia holbrooki)* to mercury (II) [25].

Camargo and Martinez (10), reported epithelial lifting and lamellar fusion in Neotropical fish species *Prochilodus lineatus*, subjected to *in situ* tests for 7 days in a disturbed urban stream. Similar alterations in the gills have also been reported in the fishes exposed to metals [19, 26] and organic contaminants [27].

Most of the gill damages resulted from sublethal exposures affected lamellar epithelium [28]; though, when fishes bear a more severe type of stress, some alterations in blood vessels may also occur. In situation like this, injured pillar cells can cause an increased blood flow inside the lamellae, leading to dilation of the marginal channel, blood congestion or even an aneurysm [29]. This is a severe type of lesion, recovery from which is possible, but more difficult than the epithelial changes [24]. The rupture of pillar cells in a result of bigger flow of blood or the direct effects of contaminants on these cells, lead to the formation of an aneurysm [26], a severe type of lesion, healing from which is possible, but more difficult than the epithelial changes [24].

In the present study, fishes treated with the higher concentrations of $HgCl_2$ (group 3 and group 4) showed vascular alterations, such as blood congestion and in some cases aneurysms which indicate the critical condition of the water in these groups.

The shortening and progressive disappearance of lamellae observed in the present study, are similar to the responses produced by other metals, including Al [30], Be [25] and Cd [31].

Winkaler *et al.* [32] described anomalies such as hyperplasia, hypertrophy, dilation of the marginal channel and aneurysms in Neotropical fish, *Astyanax altiparanae*, collected in Cambé stream, the water of which is really polluted and exposure to that causes structural damage to the gill of fish.

Khan *et al.* [33] reported hyperplasia of epithelial cells of gill filaments, fusion of secondary lamellae giving a club shaped appearance of filaments and contraction and sloughing of respiratory epithelium in fishes treated with $HgCl_2$ at sublethal dose 16 days. Shakoori *et al.* [34] reported the same results following 48 hours of exposure of chinek grass carp to sublethal doses of mercuric chloride. Similar histopathological changes in gills are also reported by many authers [45], [36] and [37].

Our findings exhibit that, structurally the gills of *A. latus* affect by exposure to $HgCl_2$, the concentration of which influenced the severity of the observed alterations to the tissue and the extent of damages. Also, the results of the present study suggest that morphological and histopathological changes of the gill provide helpful information about the environmental conditions and screening changes to them, as particular biomarkers, may provide imminent into evaluating the general health and stress status of fish.

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