Pathological alterations in response to water soluble fraction of Iranian crude oil in gill of yellow fin sea bream *Acanthopagrus latus* (Houttuyn, 1782)

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Abstract

Water soluble fraction (WSF) of crude oil can pollute water and cause histopathological alterations in fishes. Therefore, sea water pollution with crude oil could detrimentally impact the fish health. The aim of the present study was to evaluate the effect of different concentrations of WSF on gills of yellow fin sea bream. The fish were exposed to 0% (control), 2%, 4%, 8% and 16% of WSF for a period of 21-days. The histopathological alterations were examined in four categories of circulatory, degenerative, proliferative and inflammatory. Inclusion of WSF at concentrations of 4%, 8% and 16% led to significant elevation of total petroleum hydrocarbon concentration in water ($p < 0.05$). In the 8% group, the changes in histopathological lesions observed from day 16 compared with control group ($p < 0.05$). In the 16% group, increasing histopathological lesions was observed from day 8 compared with the control group ($p < 0.05$). However, no significant increase in histopathological lesions was observed in fish exposed to 2% and 4% WSF as compared with the control group ($p > 0.05$). In conclusion, the findings of the present study indicated concentration and period of exposure as determinant factors influencing the level of alterations in gills histopathological lesions following exposure to WSF in yellow fin sea bream.

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Introduction

It is well known that release of crude oil is a major threat to marine environment. At the previous studies, marine pollution was striking and toxic to fish and environment and might have a negative impact on marine ecosystem and fisheries resource (Jordi *et al*., 2006; Lockhart *et al*., 1996; Anderson *et al*., 1974).

Despite that water and oil are insoluble, crude oil included a very small soluble section, called water soluble fraction (WSF) (Edema, 2012). Fish have a very high capability to accumulate WSF (Collier *et al*., 1995). Water accommodated fractions could be the cause of the aquatic toxicity for three reasons: first, chemical toxicity associated with dissolved materials (from oil or dispersant), second, physical effects associated with contact with oil droplets, and third, effect associated with enhanced uptake resulting from direct animal oil contact (Singer *et al*., 1998).

WSF extracted from crude oil have damaging effects on water body population and decrease fish population and prepare the ground for establishing histopathological alteration and interacting with cellular and tissue processes in different ways to produce toxicity (Anderson *et al*., 1974; Solangi and Overstreet, 1982; Gundersen *et al*., 1996).

The evaluation of gills is useful for benchmarking in water pollution and in the meanwhile provides large surface area for contact with crude oil fraction and can be regarded as biomarker in environmental contamination (Oost *et al*., 2003; Torre *et al*., 2005; Milinkovitch *et al*., 2011; Sharifpour *et al*., 2011). During the last decade, structural changes in pollution in fish gills has been well studied and considering that efficiency of gills in the respiratory gas exchanges and nitrogenous waste excretion and ionic change and acid regulation, gills damage can lead to a threat to the fish health and after that observing histopathological lesion (Solangi and Overstreet, 1982; Agami, 2013; Askari Hesni *et al*., 2013; Dessouki *et al*., 2013; Khoshbavar and Yelghi, 2018).

Iran is the oldest middle East producer of crude oil and Khuzestan province accommodates the largest oil fields with huge petroleum production in Iran (Namdari *et al*., 2012). In Iran, yellow fin sea bream is frequent in Persian Gulf and Musa estuary and this estuary is located at the head of the Persian Gulf at the Khuzestan province (Savari *et al*., 2013). The yellow fin sea bream, on the one hand, is one of the economically important species through the world and on the other hand, it lives in estuaries and brackish water and one of the important food resources for local consumption (Gwo, 1994; Hu *et al*., 2007). Owing to contamination from the Persian Gulf transferred into the Musa estuary and the fact that this estuary is located around many small industries and the biggest Iranian petrochemical complex, this site developed the risk of marine ecosystem pollution (Scott, 1995).

The main objective of the present study was to evaluate the histopathological alterations in gills of

yellow fin sea bream after exposuring to WSF prepared from Iranian light crude oil.

Materials and methods

Fish and acclimatization conditions

The yellow fin sea bream specimens with 111.68±1.52 g body weight and 18.27±0.23 cm standard length were collected during 2013 from Musa estuary in the head of the Persian gulf using baited traps an acclimated to laboratory condition in south of Iran aquaculture research center for 20 days before the start the first experiment. During this period, fish were maintained in 5 numbers of 1000 (40 fish per tank) liter polystyrene tanks filled with seawater from the capture site and equipped whit filter and oxygenation system under a natural photoperiod and fed every two days with commercial food (Chineh). No mortality was seen during acclimatization period.

Chemicals

Light Iranian crude oil (API=36.70) was donated by petroleum industry from Abadan oil refining company. The crude oil barrels were kept and protected in a safe and convenient place before preparing the stock solution.

Preparation of water soluble fraction

The WSF was prepared according to the method described by Anderson *et al*. (1974). For preparation of WSF, a part of crude oil was added to nine part of water in container. The mixture container was capped and covered with aluminum foil to reduce the evaporation and avoid light. Then the solution was stirred by using a magnetic agitator with slow speed for 24 h at room temperature and allowed to settle 1 h to separate the water and oil phases. The solution below the oil phase siphoned off from the bottom of the mixing container and this stock solution was lenis to make up the nominal concentration for exposure. Only the liquid phase of the WSF of crude was used for the study. New solution was prepared daily.

Experimental treatment

For the entire duration of experiment a week before creation of the contamination after acclimation period fish were randomly transferred to stock tank to 15 number of 300 L polystyrene tanks (15 fish per tank), which had static system and continuous aeration and fish were held in temperature-controlled room on natural photoperiod with 13 h light and 11 h darkness at equable 22±1 °C during the three weeks of experimental period and water was continuously monitored for temperature, dissolved oxygen, pH, water salinity and conductivity.

Experimental design

The fish were divided into five groups. Four groups were then exposed to different concentrations of WSF and the control group was not exposed to the pollutant and any group with three replicate per exposure. In the present study each experimental tank contained 300 liters seawater to which 2 (2p), 4(4p), 8(8p) and 16(16p) % of WSF were added. In the control group, sea

water has been only used. Filtered untreated Seawater was obtained from the musa estuary.

Histopathological examination
The fish were quickly euthanized with (benzocaine, 60 mg L^{-1}) and measured for standard length and body weight. Then, the second gill from left side from individual fish of all groups was dissected and immediately fixed in 10% neutral buffered formalin for histopathological examination.

The tissues were later washed off and clean of formalin and passed through a series alcohol concentration to remove the water. The tissue were again passed through a chloroform/alcohol and pure alcohol therefore the gills sections, 5 µm thick, were cut with semi automate rotatory microtome RM2245 and then stained with hematoxylin and eosin and mounted on glass slide. Images were analyzed by light microscope and recorded with mioticcam 3000 photomicroscope.

Gills histopathological alterations were assessed semi-quantitatively by assortment the severity of alteration according to Bernet *et al*. (1999) into four significant reaction pattern (rp). In general, four major reaction patterns included circulatory disturbances, degenerative disturbances, proliferative changes and inflammatory changes.

Each reaction pattern divided into several alterations. Circulatory disturbance (rp1) included pathological condition of blood and tissue fluid that was considered in four major parts (aneurysm, vasodilation, haemorrhage, congestion of blood vessel).

Degenerative disturbance (rp2) included regressive alteration the lesion that can cause decreased gills function or loss of gills that was considered in six major parts (edema, rupture of pillar cells, epithelial lifting of lamellae, necrosis, shortening of lamellae, desquamation of lamellae). Proliferative changes (rp3) included alteration leads to an overactive cells or gills function that was considered in seven major parts (hypertrophy of epithelial cells, hypertrophy of chloride cells, hypertrophy of mucosa cells, fusion of the lamellae, cartilage proliferation). Inflammatory changes that related to other reaction pattern were considered in one major part (lymphocyte infiltration).

Each reaction pattern had importance factor (IF) that according to Bernet *et al*. (1999) was divided into three grades from grade one to grade three. Grade one minimal pathological importance that they are easily reversible as exposure to irritants end, grade two moderate pathological importance that these alterations are reversible in the most cases if the stressor is neutralized and grade three marked pathological importance that they are generally irreversible, leading partial or total loss of the organ function.

Every alteration was evaluated by using score value (SV) from grade 0 to grade 6 according to Bernet *et al*. (1999), based on the severity of the alterations in the tissues: grade (0) without alterations, grade (2) focal and mild alterations, grade (4) moderate alterations and grade (6) sever alterations. This rating was used to

collect an overall evaluation value of histopathological alterations for each gills of individual fish.

After determination of the SV and IF from the sum of these both

$K\,alt$ was obvious from following formula:

$$K\,alt = \sum alt$$

$$(cv_{org\ rt\ alt} \times mf_{org\ rt\ alt})$$

And from of the sum of $\sum$ alteration for specific alteration,

K rt characterized by the following formula:

$$K\,rt = \sum alt \sum alt$$

$$(cv_{org\ rt\ alt} \times mf_{org\ rt\ alt})$$

Eventually g_{org} was determined by the following formula:

$$g_{org} = \sum_{rt} \sum_{alt} (cv_{org\ rt\ alt} \times mf_{org\ rt\ alt})$$

Where (rt) is the reaction pattern, (alt) is the alteration, $(cv_{org\ rt\ alt})$ is the SV for specific alteration of reaction pattern, (org) is the organ that represents gills, $(mf_{org\ rt\ alt})$ is the IF and (g_{org}) is the gills index that represent degree of damage of the gills from individual fish.

The tables included comparison between groups exposed to 2p, 4p, 8p, 16p of WSF and control group that at days 0, 4, 8,12,16 and 20 were recorded and after calculation of organ index from comparison average value for each lesion tables for unraveling were complete.

Total petroleum hydrocarbon (TPH) seawater concentrations

The TPH concentration was measured based on the reference method 5520B according to EPA1664 (Lee and Neff, 2011). To the water samples were taken added 50 ml N-hexane and 2ml concentrated sulfuric acid to remove non-polar compounds from polar compounds in water and to acidification of the environment to prevent the growth of bacteria in oil-wet. All petroleum compounds were extracted from water samples and determined the gravimetric method. The water TPH concentration samples were taken at the beginning (T=day 0) and at mid time (T=day10) and at end of the experiment (T=day 20).

Statistical analysis

Data related to histopathological alterations were analyzed using MIXED procedure. In addition, LSMEANS statement was used to perform multiple comparisons. All analyses were conducted in SAS (SAS, 2008). Data are presented as mean± SD. Differences with $p<0.05$ were considered significant.

Results

Mortality

No fish died during the acclimatization period and no obvious mortality rate in fish in 2p ,4p and 8p groups and control group except in 2 cases from the 16p group on 8 and 16 days point of experiment was observed.

Total petroleum hydrocarbons (TPH)

In TPH concentration, there was no difference between 2p and 4p groups at Day 0. TPH concentration in 2p group was higher than that in control group ($p<0.05$) and slightly lower than 4p

group and lower than 8p and 16p groups ($p<0.05$) on days 10 and 20. TPH concentration in 4p group was lower than that in 8p and 16p groups ($p<0.05$) at three time point of experiment. TPH concentration in 8p group was lower than that in 16p group at three time point of experiment .There was no difference in TPH concentration in control group at three time point of experiment but in 2p, 4p, 8p and 16p groups on day 10, it was higher than that on day 0 ($p<0.05$). TPH concentration in 2p,4p,8 p and 16p groups on day 20 was higher than that on days 0 and 10 ($p<0.05$; Table 1).

Table 1: Water TPH concentration (ppm) in different groups on Days 0, 10 and 20 of experiment.

TPH	Group				
Day	0	2	4	8	16
0	0.37 ± 0.15^a	1.00 ± 0.10^{Aa}	1.87 ± 0.06^{Ab}	3.57 ± 0.15^{Ac}	6.00 ± 0.66^{Ad}
10	0.53 ± 0.21^a	2.23 ± 0.2^{Bb}	5.37 ± 0.15^{Bb}	8.27 ± 0.32^{Bc}	15.10 ± 0.36^{Bd}
20	0.70 ± 0.25^a	3.37 ± 0.15^{Cb}	6.50 ± 0.20^{Cb}	12.30 ± 0.46^{Cc}	25.20 ± 0.26^{Cd}

^{ABC}Values with different superscripts within columns differ ($p<0.05$).
^{abcde}Values with different superscripts within rows differ ($p<0.05$).

Histopathological findings

The results of histopathological examination showed in four major types of reaction pattern in Tables 2, 3, 4 and 5. In addition, in Table 6 organ index represent the damages of the gills.

Table 2: Index of circulatory disturbance in different groups on Days 0, 4, 8, 12, 16 and 20 of experiment.

Circulatory disturbance	Day of experiment					
Group	0	4	8	12	16	20
2p	5.17 ± 1.51	7.67 ± 2.34	10.33 ± 1.51	14.00 ± 1.26	15.33 ± 1.21	16.50 ± 1.38
4p	5.67 ± 1.51	10.00 ± 1.26	11.67 ± 0.82	14.50 ± 1.38	16.83 ± 1.60	15.83 ± 1.47
8p	5.33 ± 1.03	9.67 ± 0.82	13.00 ± 1.10	18.17 ± 1.17	20.50 ± 0.84	18.83 ± 1.83
16p	6.00 ± 1.26	11.33 ± 1.63	13.00 ± 1.26	18.67 ± 0.82	26.17 ± 1.17	27.33 ± 2.07
control	5.67 ± 0.82	5.33 ± 1.03	5.33 ± 1.63	8.33 ± 1.97	7.67 ± 1.51	8.00 ± 1.79

Table 3: Index of degenerative changes in different groups on Days 0, 4, 8, 12, 16 and 20 of experiment.

Degenerative changes	Day of experiment					
Group	0	4	8	12	16	20
1	6.00 ± 0.00	9.00 ± 1.10	14.00 ± 1.10	17.50 ± 0.84	22.83 ± 1.94	21.00 ± 2.19
2	7.00 ± 2.45	10.67 ± 1.63	15.33 ± 1.63	22.00 ± 2.53	24.83 ± 1.83	22.50 ± 1.64
3	7.33 ± 1.03	13.67 ± 1.51	16.17 ± 1.83	23.83 ± 0.98	31.33 ± 2.07	27.33 ± 2.66
4	6.67 ± 1.03	14.00 ± 2.53	21.00 ± 2.45	24.83 ± 1.17	38.50 ± 1.76	34.33 ± 1.86
5	7.00 ± 1.10	6.67 ± 1.03	7.37 ± 1.03	8.67 ± 1.03	9.33 ± 1.63	10.17 ± 2.04

Table 4: the index of proliferative changes after 6 time point of experiment.

Proliferative changes	Day of experiment					
Group	0	4	8	12	16	20
2p	9.00 ± 1.26	13.00 ± 2.19	17.33 ± 1.37	25.17 ± 1.72	27.67 ± 1.51	24.17 ± 1.17
4p	11.17 ± 2.04	15.00 ± 2.19	20.33 ± 2.07	27.17 ± 1.60	32.83 ± 1.72	29.67 ± 2.07
8p	9.33 ± 1.97	15.00 ± 2.53	21.00 ± 2.28	29.17 ± 1.94	40.17 ± 2.04	32.33 ± 1.63
16p	10.33 ± 1.03	16.00 ± 2.45	25.00 ± 0.63	33.33 ± 2.16	49.67 ± 1.86	47.67 ± 3.44
Control	8.33 ± 1.03	10.00 ± 2.68	10.50 ± 1.97	12.33 ± 2.07	13.33 ± 2.94	12.50 ± 2.66

Table 5: Index of inflammatory changes in different groups on Days 0, 4, 8, 12, 16 and 20 of experiment.

Inflammatory changes	Day of experiment					
Group	0	4	8	12	16	20
2p	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.33 ± 0.82
4p	1.00 ± 0.00	1.33 ± 0.82	1.00 ± 0.00	1.33 ± 0.82	3.00 ± 0.00	1.67 ± 1.03
8p	1.33 ± 0.82	1.00 ± 0.00	2.33 ± 1.03	3.00 ± 0.00	3.33 ± 0.82	3.00 ± 0.00
16p	1.00 ± 0.00	1.33 ± 0.82	3.00 ± 0.00	2.67 ± 0.82	5.17 ± 0.41	4.00 ± 0.00
contorl	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00

Table 6: Index of gill in different groups on Days 0, 4, 8, 12, 16 and 20 of experiment.

Gill index	Day of experiment					
Group	0	4	8	12	16	20
2p	21.67 ± 2.34	30.67 ± 2.07	42.67 ± 1.63	57.67 ± 1.63	66.83 ± 2.71^{AB}	63.00 ± 3.90^{AB}
4p	24.83 ± 2.99	37.00 ± 3.74	48.33 ± 2.94	65.00 ± 4.05	77.50 ± 3.89^{AB}	69.67 ± 4.13^{AB}
8p	23.33 ± 1.67^{a}	39.33 ± 3.01^{ab}	52.50 ± 1.52^{ab}	74.17 ± 1.72^{ab}	95.33 ± 2.34^{ABb}	81.50 ± 2.59^{ABb}
16p	24.00 ± 2.19^{a}	42.67 ± 3.72^{ab}	62.00 ± 2.28^{ab}	79.50 ± 2.35^{b}	119.50 ± 2.88^{Ab}	113.33 ± 3.08^{Ab}
control	22.00 ± 1.26	23.00 ± 2.57	24.17 ± 2.23	30.33 ± 4.08	31.23 ± 2.07^{B}	31.67 ± 4.32^{B}

^{AB}Values with different superscripts within columns differ ($p<0.05$).

^{a,b,c}Values with different superscripts within rows differ ($p<0.05$).

Circulatory disturbance (Table 2) in the control group (Fig. 1) was lower than that in 8p and 16p groups ($p<0.05$). Moreover, it was lower in the 2p group as compared with 16p group ($p=0.003$). Circulatory disturbance was higher on day 16 than 0 ($p=0.003$; Fig. 2).
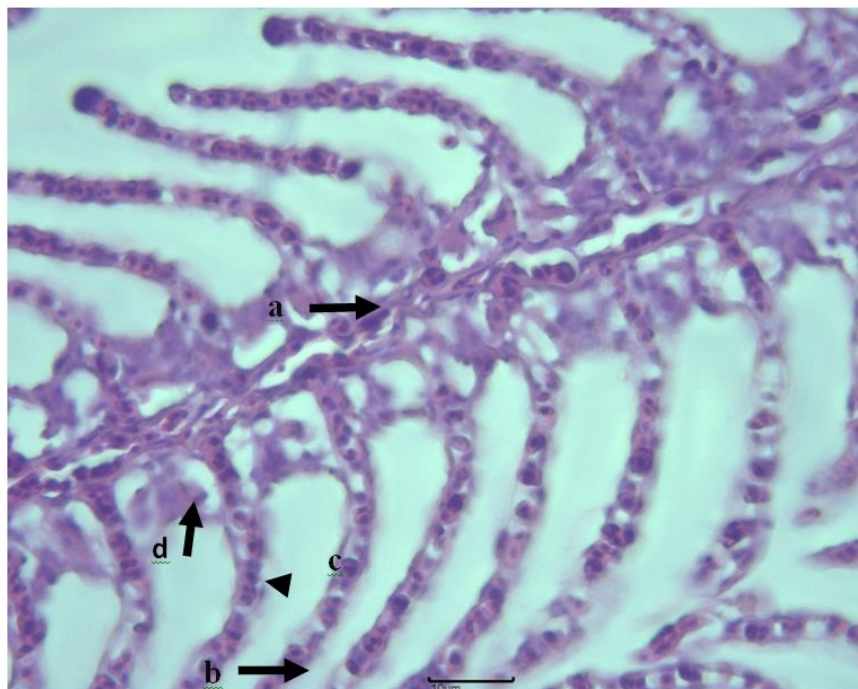

Figure 1: Showing gill of control fish that represents central venous sinus (a) is covered by filamentary epithelium and epithelial cells with higher magnification and regular secondarylamellae (b) and pillar cells (c) and chloride cells (d) are located in the intra lamellar pits on the gill filaments (H&E, Bar: 10μm).
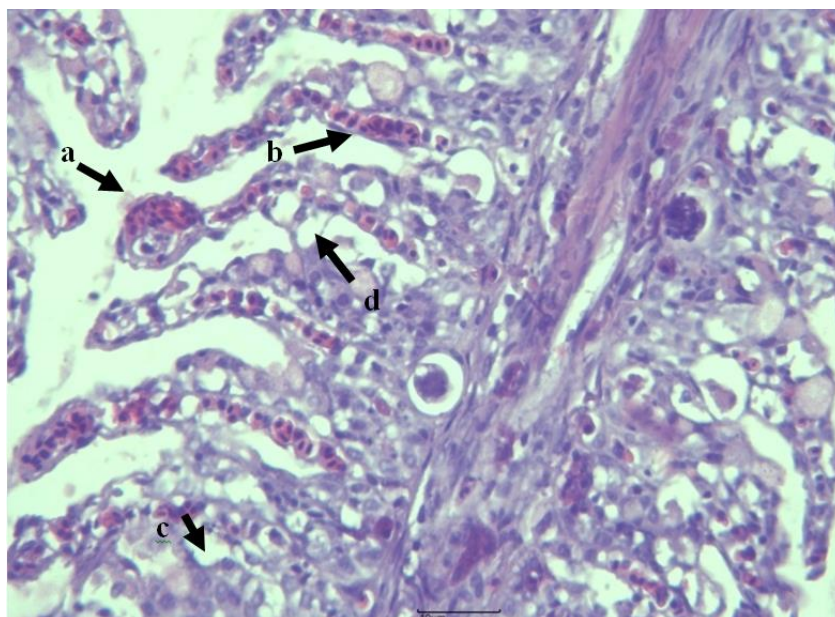
Figure 2: Showing gill of fish exposed to 16% WSF after 12 days of experiment that represents circulatory disturbance such as moderate telangiecstasis (a), congestion (b), moderate hyperplasia proliferation of mucous cells (c) and separation of secondary lamellar epithelium (d) (H&E, Bar:10µm).

Degenerative changes (Table 3) in the control group were lower than those in 8p and 16p groups ($p<0.05$). Moreover, it was lower in the 2p group as compared with 16p group ($p=0.020$).

Degenerative changes on days 16 and 20 were higher than those on day 0 ($p<0.05$; Fig. 3).
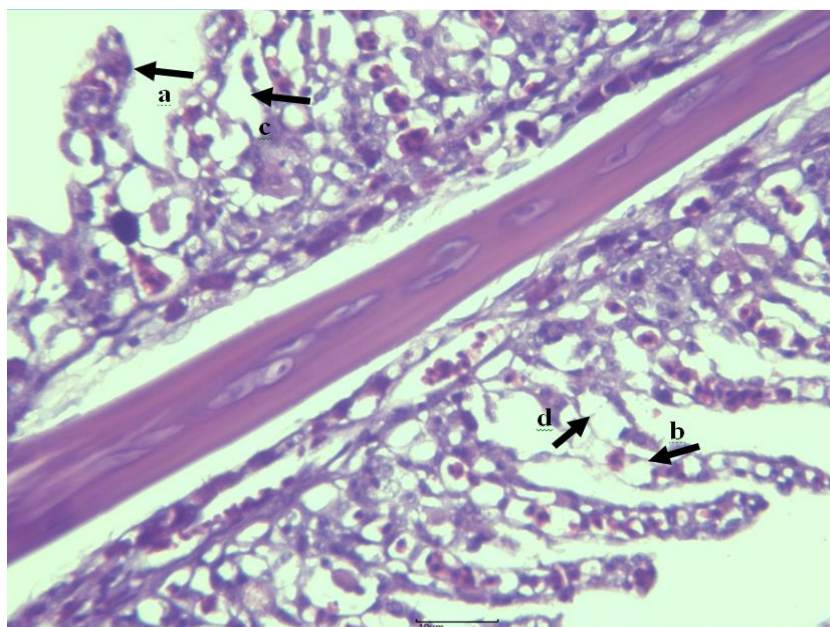
Figure 3: Showing gill of fish exposed to 16% WSF after 16 days of experiment that represents degenerative changes and shortening lamellae (a), mild to severe lifting of secondary lamellae and desquamation of secondary lamellae (b) and edematous suburb of lamellae (c) and necrosis of secondary lamellae cells (d) (H&E, Bar: 10µm).

Proliferative changes (Table4) in the control group were lower than those in 8p and 16p groups ($p<0.05$). Moreover, it was lower in the 2p group as compared with 16p group ($p=0.025$). Proliferative disturbance on days 12, 16 and 20 was higher than that on day 0 ($p<0.05$). In addition, proliferative changes on days 16 and 20 were higher than that on day 4 ($p<0.05$) (Fig. 4).
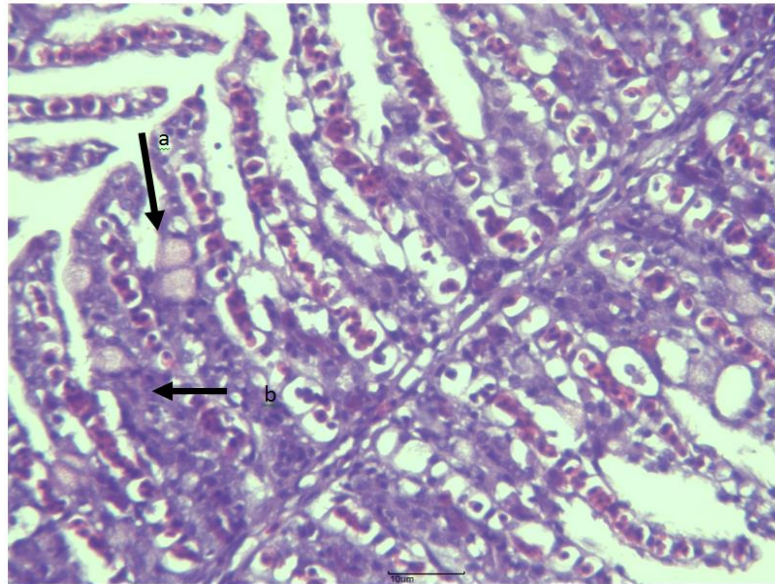

Figure 4: Showing gill of fish exposed to 16% WSF after 16 days of experiment that represents proliferative changes such as hypertrophy and hyperplasia in mucus cell in intra lamellar epithelial cells (a) and fused hyperplasic lamella (b) (H&E, Bar:10μm).

There was no effect of group, time and interaction group by time on the inflammatory changes ($p>0.05$) (Table 5).

Organ index in the control group was lower than that in 4p, 8p and 16p groups ($p<0.05$). Organ index in group 16p was higher than that in 2p and 4p groups ($p<0.05$). In addition, organ index was higher in 8p group than 2p group ($p=0.011$). In 8p group, organ index on days 16 and 20 was higher than that on day 0 ($p<0.05$). In16p group, organ index on days 12, 16 and 20 was higher than that on day 0 ($p<0.05$). On Days 16 and 20, organ index in 16p group was higher than that in the control group ($p<0.05$) (Table 6).

Discussion

Pollution by crude oil in fish especially in the oil producing countries has been consistently examined (Anderson *et al*., 1974; Solangi and Overstreet, 1982; Prasad, 1988; Brand *et al*., 2001; Milinkovitch *et al*., 2011; Fasulo *et al*., 2012; Agami, 2013; Askari Hesni *et al*., 2013). However, in Iran, less has been studied on native species experimentally. Classification of damages to gills can be one of the important assessment ways to diagnose contamination with pollutants. There are different methods and assessment scales for evaluation of histological changes (Bernet *et al*., 1999; Simonato *et al*., 2008; Reddy and Rawat, 2013).

The aim of this study was to evaluate the histopathological alteration in gills of yellow fin sea bream after exposure to water soluble fraction prepared from light Iranian crude oil and to classify this alteration to for major approach for easy evaluation damages to gills of fish. Considering that elegant structure of gills and contact with any irritant the gills, as Roberts (2001) asserted, are sensitive to the invasion of pathogens and any chemical and physical changes. In this study circulatory disturbance increasing has been observed after more than 4 percent of WSF exposed to fish, this matter may be reflected the fact that the amount of release of WSF in aquatic environment could be considered as an alarm to initiate the damages in blood and tissue fluid flow. The circulatory disturbance in this study demonstrated four major changes aneurism, vasodilation, congestion of blood vessel, and haemorrhage according to Agami (2013) who surveys that observed circulatory disturbances with twenty-four hours difference with nine days of exposure to water accommodated fraction. Several other studies have shown similar effects in environmental pollution as Van *et al.* (2009) studies that in 24% of the cases exposed to urban have observed circulatory disturbances. Preserving the epithelial layers together appears consequently blood flow through the lamellae around pillar cells and on the other hand pillar cells have supportive role in circulatory disturbance and following rupture of pillar cells, aneurysm will be seen (Martinez *et al.*, 2004; Simonato *et al.*, 2008). In this

study, following circulatory disturbance, degenerative disturbance have been observed.

In this study, the most degenerative changes have seen on days 16 and 20, which increased corresponding to the presence of WSF, while after 16 days point of experiment has a slight decline. In Agami (2013) studies degenerative lesion observed after 12 days point of experiment whereas this alteration declined after 16 days point of experiment. Some of this degenerative alteration compatible with this alteration can be protecting the fish from admittance xenobiotics investigated this alteration and observed compatible alteration and defence mechanism in fish (Akaiashi *et al.*, 2004; Martinez *et al.*, 2004; Agami, 2013).

The result of proliferative changes showed this alteration could be considered as defence mechanism in exposed fish after this time point of exposure to WSF. Many studies reported about the impact of pollutant and increasing some of histopathological proliferative alteration (Martinez *et al.*, 2004; Yasser and naser, 2011; Agami, 2013). Brand *et al.* (2001) noted that after exposure to sub lethal concentration of the WSF of north slope crude oil in salt water acclimated pink salmon fry gills observed abnormalities such as fusion of lamellae and mucous cell hyperplasia. Proliferative alterations similar to the one described in this study have been observed in fish living in polluted Huelva estuary (Olive *et al.*, 2013).

The gills index result showed that the period of exposure and presence of WSF could play a role in the resultant gills lesions while gill index showed major role to WSF toxicity to fish and could be considered as biomarker in water born pollutant and could help in diagnosis of alterations. However, gill index data in this study enhance the ability to evaluation of histopathological effect on gills of exposed fish. Gills have been appropriate tools for evaluating environmental health after sub chronic exposure to light Arabian crude oil (Agami, 2013). Akaiashi *et al.* (2004) supported the importance of histhopathology as biomarker to evaluate contaminant toxicity. Some other studies that have shown exposure of the fish to crude oil contamination could lead to appearance of histopathological lesions in gills and emphasize on to tracing role of gills after development environmental pollutant (Mallat, 1985; Yasser and Naser, 2011; Olive *et al.*, 2013).

In conclusion, with regard to the important role of gills for tracing the effect of environmental pollution this study investigated the histopathological alteration in gills with classified statistical analysis method. We have raised the gills lesions as an indicator for direct and indirect WSF contamination. However in this study, data showed the gills may pose decisive role for warning to start development of pollutant in aquatic ecosystems. Iran has one of the largest harvest crude oil in Persian Gulf; therefore, monitoring adverse histopathological effect of exposure to WSF could help to keep yellow fin sea bream from waterborne pollutant and prevent the serious threat for this fishe.

Acknowledgements

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