Histopathological and bacterial study of Persian sturgeon fry, *Acipenser persicus* (Borodin, 1897) exposed to copper sulfate and potassium permanganate

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Abstract Persian sturgeon fries were exposed to different concentrations of copper sulfate and potassium permanganate in order to the evaluation of their impacts on bacterial load of skin, gill and surrounding water and also the histopathological alternations of gill tissue. For this purpose, the sublethal doses were determined after a pre-test and then the experiment was done in 4 (for copper sulfate: 0.07, 0.14, 0.26 and 0.5 mg/l) and 5 (for potassium permanganate: 0.07, 0.14, 0.26, 0.5 and 1 mg/l) treatments with three replicates inside the glass aquaria. Also, one group without disinfecting drug was considered as control for each experiment. The microbial and histopathological investigations were done after 96 h exposure. According to our results, a range of histopathological alternations were observed in gills tissue including mucus coagulation and secretion, hyperplasia, lamellar necrosis, hyperplasia, lamellar adhesion, haemorrhage, thickening of secondary lamellae, hypertrophy of supporter cartilage, clubbing of gill lamellae and sliming of primary lamellae. The severity of these alternations increased with increasing of the doses of the copper sulfate and potassium permanganate. The bacterial load (CFU/g) of gill, skin and surrounding water was lower in 0.07 mg/l copper sulfate treatment and 1 mg/l

potassium permanganate treatment ($P < 0.05$) than in other treatments. In conclusion, our results showed that the certain doses of the copper sulfate and potassium permanganate have disinfecting effects on bacterial load of gill, skin and surrounding water, although this is along with some histopathological alternations. Also, it seems that the copper sulfate has higher disinfecting power than potassium permanganate.

Keywords Copper sulfate · Potassium permanganate · Histopathology · Gill · Persian sturgeon

Introduction

Aquaculture is in period of rapid development and growth, and the intensification of fish farming often leads to the emergence of numerous infectious diseases. Aquaculture needs the availability of disinfecting drugs to avoid severe economic losses, and disinfection will remain one of the main means of controlling diseases in the future (Lafont 1992). At now, various disinfecting chemicals are commonly used for the control of bacterial and fungal pollution in cultured fish and surrounding water (reviewed by Torgersen and Hastein 1995). The disinfecting chemicals such as malachite green, formalin, copper sulfate, potassium permanganate, chloramines, chlorine dioxide and quaternary ammonium compounds. Irrespective of disinfecting role, these chemicals are subsequently released to the aquatic environment and may have impacts on other aquatic organisms and their habitat (Boyd and Massut 1999; Nash 2003; Erondur and Anyanwu 2005). Therefore, to modify the undesired impacts of disinfecting chemicals on fish and aquatic environment, the doses of these chemicals should be optimized at the time of usage. In fact,

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in addition to have a disinfecting role on fish and water, a disinfecting chemical should be safe for fish and natural environment. The Persian sturgeon, *Acipenser persicus* is a critically endangered anadromous species that has been considered for restocking programme in the southern part of the Caspian Sea (Kiabi et al. 1999; Niksirat and Abdoli 2009). In this respect, numerous fry are produced annually by means of the artificial propagation and then released to nature. At now, some disinfecting chemicals such as malachite green, formalin, copper sulfate and potassium permanganate are used routinely for disinfection of fish and water in sturgeon hatcheries of Iran. Nevertheless, there is very little information on the toxicity and histopathological impacts of these chemicals on sturgeons. Thus, in the present study, we investigated the impacts of two disinfecting drugs i.e. copper sulfate and potassium permanganate on histopathological status of gill and on bacterial load in skin, gill and surrounding water of Persian sturgeon fry.

Materials and methods

The broodfish

The experiment was carried out in International Sturgeon Research Institute, Rasht, Iran. The Persian sturgeon fry (1–3 g) were used for the experiment. Before the experiment, a pre-test was done to determine the sublethal doses of copper sulphate and potassium permanganate. In this regard, the values of LC₁₀, LC₅₀ and LC₉₀ were measured after 24, 48, 72 and 96 h exposure of fry with various concentrations of copper sulphate and potassium permanganate. After pre-test, a range of 0.07–1 mg/l was considered as sublethal range and used for the experiment. The experiment was conducted in 36 glass aquaria (18 aquaria for copper sulphate treatment and 18 aquaria for potassium permanganate treatment) containing 20 l of dechlorinated and gentle aerated water in each of them. For each disinfecting drug, 5 treatments and one control group with three replicate were considered. The treatments were: 0.07, 0.14, 0.26, 0.5 and 1 mg/l copper sulphate or potassium permanganate and control group was water without disinfecting drug. To each experimental aquarium, a set of ten Persian sturgeon fry were added. After 96 h, the histopathological status of gill and also the bacterial load in gill, skin and surrounding water were investigated.

Sample preparation for bacterial study

From each experimental aquarium, one fish was sampled for bacterial study of gill and skin tissue according Ampofo and Clerk (2010). To remove the bacterial flora of water of culture, fish was washed with 0.9 % physiological serum.

After that, small pieces (1 cm) of skin or gill tissues were separated by a surgical knife. Then, each tissue piece was diluted nine times its weight with 0.9 % physiological serum. After homogenization, 1 ml of the diluents was added into sterile Petri dishes containing Tryptic Soy Agar (TSA) and then incubated at 25 °C for 24–48 h. To measure the bacterial load of aquaria water, the water samples were plated into TSA and incubated for 24–48 h at 25 °C. After incubation, the bacterial load (Colony Forming Units/g (CFU/g)) was counted from each bacterial culture plates.

Gill histopathology

The gill samples were fixed in Bouin's solution, embedded in paraffin wax, sectioned at 5–7 mm thick, and stained with hematoxylin and eosin. The slides of gill tissue samples were investigated under a light microscope at 200× magnification.

Statistical analysis

The SPSS software (Version 16) was used for data analysis. All data had normal distribution according to Kolmogorov–Smirnov test. One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which means were different.

Results

After 96 h expose of Persian sturgeon fry to various doses of copper sulfate and potassium permanganate, a range of histopathological alternations were observed in gills tissue including mucus coagulation and secretion, hyperplasia, lamellar necrosis, hyperplasia, lamellar adhesion, haemorrhage, thickening of secondary lamellae and sliming of primary lamellae were observed (Figs. 1 and 2). The severity of these alternations increased with increasing of the doses of the copper sulfate and potassium permanganate so that the highest histopathological alternations were observed in doses of 0.5 and 1 mg/l copper sulfate and potassium respectively. The bacterial load (CFU/g) of gill, skin and surrounding water was lower in 0.07 mg/l copper sulfate treatment and 1 mg/l potassium permanganate treatment ($P < 0.05$) than in other treatments (Tables 1 and 2).

Discussion

In the intensive systems of aquaculture, fish are always in danger of various infections that if uncontrolled, the

Fig. 1 Cross section of gill of Persian sturgeon fry (*Acipenser persicus*) exposed to various doses of copper sulphate (**a** 0.07 mg/l; **b** 0.14 mg/l; **c** 0.26 mg/l; **d**: 0.5 mg/l). (1, haemorrhage; 2, hypertrophy of supporter cartilage; 3, clubbing of gill lamellae). H.E. ($\times 200$)

infections may become systemic, resulting in death of the fish. Correct use of disinfecting drugs can effectively control many bacterial, parasitic and fungal agents before systemic infections become established, often eliminating the need for antibiotic therapy. In addition to this, the aquaculturists can save money because use of expensive antibiotics is decreased, thereby decreasing the incidence of resistant bacterial strains. In the present study, the effects of sublethal doses of two disinfecting drugs including copper sulfate and potassium permanganate were investigated on bacterial load in gill, skin and surrounding water of Persian sturgeon fry. Potassium permanganate (KMnO_4) is an oxidizing agent that has been used for many years in aquaculture. Also, the copper sulfate is a chemical compound used to control bacterial and fungal diseases of fish and surrounding water. Several studies demonstrated the disinfecting role of potassium permanganate and copper sulfate in aquaculture (Plumb Plumb 1992; Phillips 1996; Hasan and Ahmed 2002; Faruk et al. 2005; Mitchell et al. 2008; Farmer et al. 2012). Nevertheless, the disinfecting action of these chemicals is dependent to the water alkalinity, water hardness, pH,

dosage and duration of treatment (Palmer 1962; Tucker and Robinson 1990; Peres and Pihan 1991; Straus and Tucker 1993; Erickson et al. 1996; Straus 2004; Carvalho and Fernandes 2006). In our study, the increase of copper sulfate doses from 0.07 to 0.5 mg/l did not have obvious impact on the bacterial load of gill, skin and fish surrounding water compared to control. Even, it is interest that the lowest bacterial load was observed in Persian sturgeon fry treated with 0.07 mg/l. This shows that the doses more than 0.07 mg/l have not additional effects on bacterial load, indicating a threshold level of effectiveness for copper sulfate. In contrast to copper sulfate, the good impacts of potassium permanganate on bacterial load were found in higher dosage i.e. 1 mg/l. This may be due to their different chemical nature and influencing power. Generally, information about the influencing doses of disinfecting drugs can prevent from their adverse environmental harmful impacts arising from excessive use of them.

In the present study, a wide range of lesions including hyperplasia, lamellar necrosis, hyperplasia, lamellar adhesion, haemorrhage, thickening of secondary lamellae and sliming of primary lamellae were observed in gills tissue

Fig. 2 Cross section of gill of Persian sturgeon fry (*Acipenser persicus*) exposed to various doses of potassium permanganate (**a** 0.07 mg/l; **b** 0.14 mg/l; **c** 0.26 mg/l; **d** 0.5 mg/l; **e**: 1 mg/l).

(1, haemorrhage; 2, hypertrophy of supporter cartilage; 3, clubbing of gill lamellae; 4 lamellar adhesion). H.E. ($\times 200$)

after treatment of Persian sturgeon fry with copper sulfate and potassium permanganate. The severity of these lesions increased with increasing of the doses of copper sulfate and potassium permanganate. Similar histopathological alterations have been reported for various fish species after expose to disinfecting chemicals (Peyghan et al. 2007; Wani et al. 2011). These alterations may play a defensive role against disinfecting chemicals. However, these modifications can produce adverse effects on fish health, and

may increase their susceptibility to secondary infectious diseases and even death (Hawkins et al. 1984). Therefore, these histopathological alternations should be in normal limit. This gains when the disinfecting chemicals are used in safe doses.

In conclusion, our results showed that the copper sulfate and potassium permanganate with different doses have disinfecting effects on bacterial load of gill, skin and surrounding water, although this is along with some

Table 1 Bacterial load (CFU/g) in gill, skin and water of Persian sturgeon fry after exposure to various doses of copper sulphate. Bars (Mean $\pm$ SD) with different letters are significantly different ($P < 0.05$)

Treatments (copper sulphate)	Gill	Skin	Water
Control	4.16 $\pm$ 0.58 ^a	4.43 $\pm$ 1.45 ^{ab}	5.06 $\pm$ 0.10 ^a
0.07 mg/l	3.67 $\pm$ 0.33 ^a	2.46 $\pm$ 0.15 ^c	4.85 $\pm$ 0.05 ^c
0.14 mg/l	4.17 $\pm$ 0.36 ^a	3.66 $\pm$ 0.85 ^a	4.97 $\pm$ 0.07 ^a
0.26 mg/l	2.63 $\pm$ 0.30 ^b	3.49 $\pm$ 0.69 ^a	5.26 $\pm$ 0.15 ^b
0.5 mg/l	4.73 $\pm$ 0.65 ^a	4.71 $\pm$ 0.14 ^b	5.18 $\pm$ 0.17 ^b

Values with the different alphabetic letters are significantly different ($P < 0.05$)

Table 2 Bacterial load (CFU/g) in gill, skin and water of Persian sturgeon fry after exposure to various doses of potassium permanganate. Bars (Mean $\pm$ SD) with different letters are significantly different ($P < 0.05$)

Treatments (potassium permanganate)	Gill	Skin	Water
Control	3.63 $\pm$ 1.15	5.77 $\pm$ 0.25 ^a	5.06 $\pm$ 0.10 ^a
0.07 mg/l	3.12 $\pm$ 0.78	3.79 $\pm$ 1.15 ^b	5.20 $\pm$ 0.17 ^a
0.14 mg/l	3.05 $\pm$ 0.48	4.66 $\pm$ 0.85 ^{abc}	5.29 $\pm$ 0.32 ^a
0.26 mg/l	3.43 $\pm$ 0.23	3.75 $\pm$ 1.60 ^{abc}	4.68 $\pm$ 0.43 ^{ab}
0.5 mg/l	3.49 $\pm$ 0.71	3.55 $\pm$ 0.67 ^{abc}	4.48 $\pm$ 0.23 ^b
1 mg/l	3.41 $\pm$ 0.79	3.05 $\pm$ 0.89 ^c	4.23 $\pm$ 0.82 ^{ab}

Values with the different alphabetic letters are significantly different ($P < 0.05$)

histopathological alternations. It seems that the copper sulfate has higher disinfecting power than potassium permanganate since the copper sulfate had good disinfecting role in lower dosage than potassium permanganate (0.07 mg/l copper sulfate Vs. 1 mg/l potassium permanganate).

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