

Full Length Research Paper

Acute effects of $Zn(NO_3)_2$ on gill and liver tissues of Silver Carp (*Hypophthalmichthys molitrix*)

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Zinc is one of an environmental water pollutant, added to diet and water as a micronutrient for increase of plankton production and fish growth. Therefore, this research was carried out with the aims of studying on histopathology of $Zn(NO_3)_2$ toxicity on gill and liver tissues of silver carp (*Hypophthalmichthys molitrix*) within the period of 96 h. Totally, 135 fishes with mean weight 50 ± 10 g were stocked in 12 aquariums with capacity of 200 L water and divided in to 3 trails including control, 5 ppm and 10 ppm of Zn with 3 replicates. Tissue samples were fixed by buin's solution and sectioned in 7 μ m based on histological regular method and stained with Hematoxylin and Eosin (H & E) method for microscopic study within the period of 96 h. Results showed some damaged such as hyperplasia, telangiectasis and edema, necrosis of second filaments, jerky movement, aneurism, hyperemia and fusion of second filaments in gills; and cell atrophy, necrosis, fatty degeneration, hyperemia and bile stagnation at different treatments in comparison with control. Gill and liver tissue damages were severed with the increase of Zn concentration and days. Therefore, Zn had acute toxicity effects on gill and liver tissues in Silver Carp at 5 and 10 ppm concentrations.

Key words: Silver carp, Zn, gill, liver and histopathology.

INTRODUCTION

Heavy metals are serious danger for the earth ecosystem. Accumulation of heavy metals in water, air and soil is a major environmental problem. A little amount of zinc is produced from destroying of soils and rocks (Kasraiee, 2009). Naturally, heavy metals formed lower than 1% of body weight and their concentration fluctuations lead to environmental impermanent and disaster in animals (Clark, 1986). Industrial development and environment chemical pollution threatens aquatic animal life. Environmental pollutions with heavy metals were increased in the world and it may be bioaccumulated in fish tissues (Mansour and Sidky, 2002). Therefore, heavy metals are the most important pollutant of aquatic ecosystem that is the cause of major problems for human (Karan et al., 2002). Some metals

such as zinc and ferric are needed in little amount for natural development, but some of them such as Cd, Pb and Hg are toxicant at lower concentrations. Therefore, discharge of industrial, mineral, agricultural, house sewage and fuel (Swarup et al., 2006; Patra et al., 2005; Woodling et al., 2001), algacides and fungicides used in aquaculture (Onwumere and Oladimeji, 1990) are considered as aquatic ecosystem pollutants so that industrial wastewaters are the main source of pollution (Kaviraj and Das, 1995) that include various kinds of toxic pollutants such as suspended solid, minerals, poisons and pesticides (Kumar and Singh, 2010). Therefore, heavy metal pollutions have harmful effects on environmental equivalent and animal diversity (Vinodhini and Narayanan, 2008).

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Zinc is one of these pollutants and also added to ponds as a micronutrient for increasing in production of planktons and fish (Adhikari and Ayyappan, 2004). Silver carp is a warm water fish that feed on planktons. Zinc may be transferred by plankton to fish and human finally. Higher amount of zinc leads to pathologic disasters in tissues and causes fish death. Some studies were done in Iran that showed the effects of Zn on different tissues of *Cyprinus carpio* (Mohammad Khanlo Ashaieri, 2003; Rostami et al., 2000; Rostami and Soltani, 2009) in rainbow trout (*Oncorhynchus mykiss*) (Farangi and hajimoradloo, 2007) and in fingerlings of *Acipenser persicus* (Moshtaghi et al., 2009; Fathollahi et al., 2010). Therefore, the aim of this study was investigating histopathological effects of Zn acute toxicity on gill and liver tissues in *Hypophthalmichthys molitrix*.

MATERIALS AND METHODS

135 fishes with mean weight 50 ± 10 g in 3 treatments (0, 5 and 10 ppm) with 3 replicates were stocked in aquarium with capacity of 200 L water in spring 2011. Firstly, the fishes were adapted to clinical conditions and then introduced to detected concentrations. Desired concentrations of Zn were measured by the Germ/volume method and using $C_2V_2 = C_1V_1$ formula. Firstly, the total required Zn was measured and then dissolved in a specific volume of the whole water for providing stock solution. Then, specific volumes of the stock were poured into aquariums. Samples of gill and liver tissues were collected at 24, 48, 72 and 96 h, fixed by Buin's solution and dehydrated based on standard method, clarified, embedded, sectioned with $7 \mu\text{m}$ diameters by using microtome set (model Letiz 1512, Germany), stained based on Hematoxylin and Eosin (H & E) method (Hallajian, 2010) and were studied by light microscope.

RESULTS

Clinical studies results showed some apparent signs such as fast opening and closing of operculum and mouth, fidgety and air swallowing.

(A) Microscopic study of gill

Some damages including hyperplasia, telangiectasis, and edema, necrosis of second filaments, jerky movement, aneurism, hyperemia and fusion of second filaments in gills at 5 and 10 ppm concentrations than control and severe with increase of concentration and days were observed (Figures 1 to 3).

(B) Microscopic study of liver

Microscopic studies of liver samples showed some damages such as cell atrophy, cell necrosis, fatty degeneration, hyperemia, bile stagnation and melanomacrophage at 5 and 10 ppm than control (Figures 4 to 6). Liver damages were lower at 5 ppm than 10 ppm of Zn. Therefore, with the increase of zinc nitrate

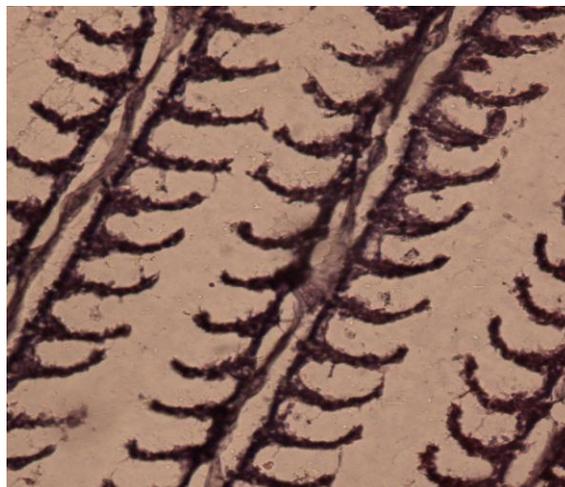


Figure 1. Gill at control (H&E, 245X).

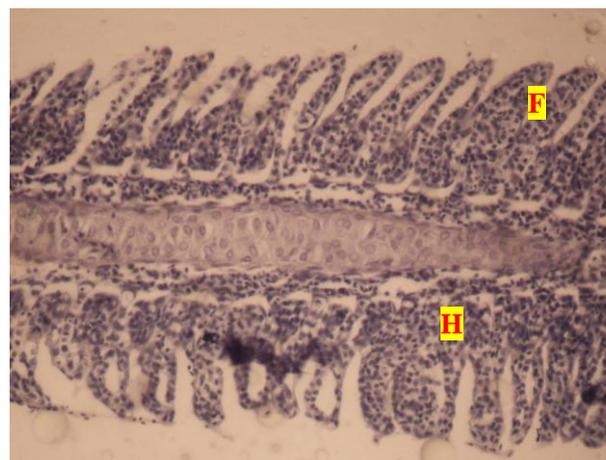


Figure 2. Hyperplasia (H), fusion of gill filaments (F), at 5 ppm of Zn after 12 h. (H & E, 245X).

concentration and days up to 72 h liver damages were severe but at 72 and 96 h.

DISCUSSION

Heavy metals have an important physiological effects and causes of many diseases if decreased or increased. Zinc is a mineral ingredient and its toxicity has bad effects on the body of fishes (Donmez et al., 1993).

Gill structure in Silver Carp includes primary and second lamella. Secondary lamellas of this fish are long and free from the end of distal. A normal secondary lamella complex was from two thin layers of epithelial cells, blood vessels and row of cells (Athikesavan et al., 2006). Zn has effects on chloride cells, gill morphology and physiology such as pH reduction in blood vessel, decrease in oxygen absorption and heart beat and delay

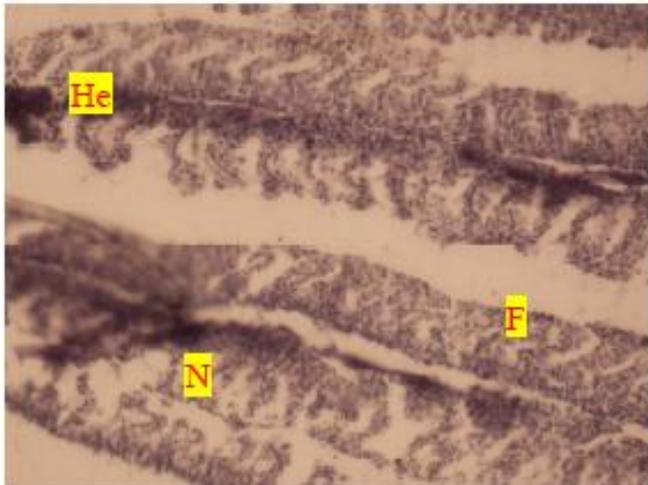


Figure 3. Hyperplasia, haemosiderin (He), fusion of filaments (F) and necrosis of cells (N) at 10 ppm at 96 hrs. H & E, 245X.

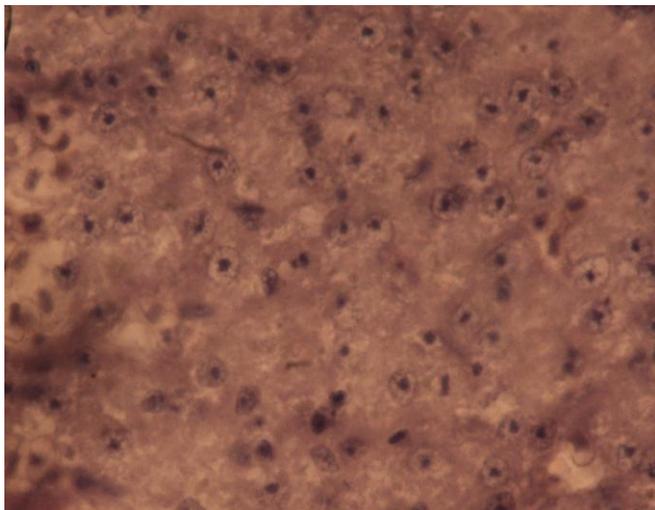


Figure 4. Liver cells at control (H&E, 750X).

egg hatch time and increase of larvae survival. Zn is resistant to deposition and lingering in environment for long time. Acute damages caused by Zn on gill include lamella fusion, hyperplasia, hyperemia, cell necrosis and telangiectasis after 12 h. These disasters in fish are kind of responses to environment conditions, so that fusion of secondary filaments is due to mucosa glycoprotein on cells (Chreck and Moyle, 1990) which lead to increase exposure of lamella with air or oxygen for collapse and adhesiveness, hyperemia and hyperplasia that indicated an immune response to chemical matters such as heavy metals (Rostami et al., 2000). Hypertrophy and hyperplasia caused to disaster in gill epithelium cells because of reduction in water flow between gill filaments that decreased respiratory function (Marioara et al.,

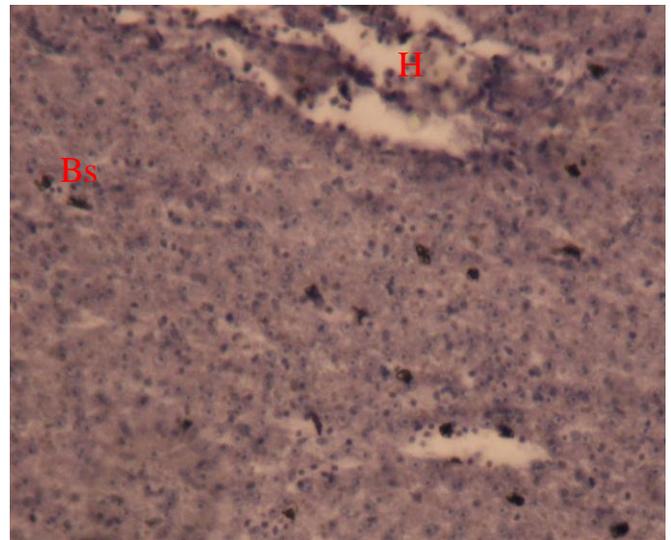


Figure 5. Hyperemia (H), bile stagnation (Bs) (H&E, 750X).

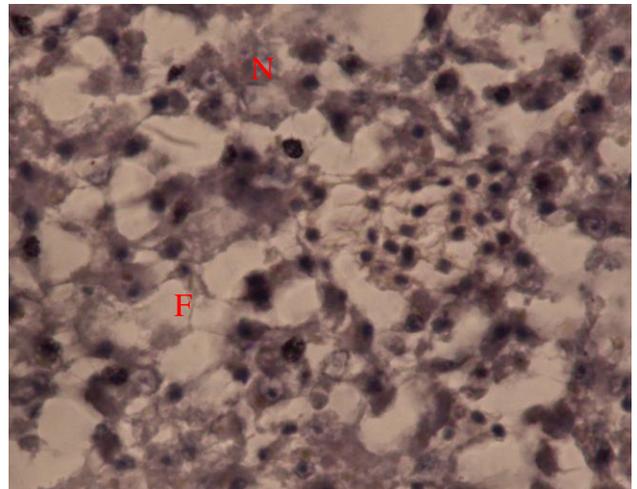


Figure 6. Necrosis of cells (N), fatty degeneration at concentration (F) (H&E, 750X).

2009). Some similar effects have been reported by Marioara et al. (2009) and Athikesavan et al. (2006) about Cadmium effects on Silver Carp; Naji et al. (2007) about Zn effects on Common Carp; Gorouiee et al. (2008), Colins and Brown (1998), and Jeney (1992) about aluminum sulfate effects on *Rutilus frisi kuttum*, *Onchorhynchus mykiss* and *Cyprinus carpio*, respectively. Fernandez et al. (2008) indicated that long term exposure to heavy metals cause respiration, blood circulation and osmoregulation disasters in fishes. Alvarado et al. (2006) observed that high increase in chloride cells of gills led to thickness in epithelial cells, increase in migration of chloride cells into secondary lamella edge, hypertrophy and fusion of secondary

lamella of gills.

Liver play a main role in detoxication. Histological studies of liver showed cell atrophy, cell necrosis, hyperemia and bile stagnation after 12 h exposure to Zinc nitrate. Rostami et al. (2000), and Rostami and Soltani (2009) reported cell necrosis, increase of Hemosydrine in Common Carp exposed to Zn. Kamaraju and Ramasamy (2011) indicated that increase in NiCl₂ concentration lead to decrease in glycogen level. Yilmaz et al., (2011) showed that with increase of CdSO₄ concentration in *Leuciscus cephalus*, some disasters in liver such as fatty degeneration, cell necrosis and kopfer cells were severed according to present study results. Based on obtained results at present study, Zinc nitrate induced some histopathological disasters and lead to fish death. Toxicity rate of Zn in fishes was severe with the increase of concentrations and days.

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