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Asian Journal of Animal and Veterinary Advances

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Asian Journal of Animal and Veterinary Advances 7 (7): 588-596, 2012
ISSN 1683-9919 / DOI: 10.3923/ajava.2012.588.596

Histological Changes Induced by Ammonia and pH on the Gills of Fresh Water Fish *Cyprinus carpio* var. *communis* (Linnaeus)

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ABSTRACT

Ammonia is one of the serious problems in fish culture especially in recirculation systems, aquaria and fish ponds. The effect of ammonia exposure at different pH levels on gills of common carp *Cyprinus carpio* was studied for 96 h. The median lethal concentration (LC_{50}) of ammonia for 96 h was 22 ppm, ammonia with low pH (5.0) was 27 ppm and ammonia with high pH (8.0) was 18 ppm. In the present study ammonia and different pH levels induced several changes in the fish gills like lamellar fusion, edema, hyperplasia, chloride cell proliferation and fusion in secondary lamella as compared with control. The impact of gill epithelium was less in ammonia and ammonia with low pH exposed fish than ammonia with high pH exposed fish. These parameters are indicative of pH dependence of ammonia toxicity and gill histology may be used as early indicators of toxicity in aquatic animals.

Key words: Ammonia, pH, histology, *Cyprinus carpio*, toxicity

INTRODUCTION

The aquatic environment is particularly sensitive to the toxic effects of contaminants since a considerable amount of the chemicals used in industry, urbanization and in agriculture enters marine and other aquatic environments (Osman *et al*., 2009). Ammonia is an organic pollutant which arises from sewage, industrial effluent and agricultural wastes as opined by Bloxham *et al*. (1999). Sahin and Williot (1991) have also stated that ammonia is the end product of protein catabolism that represents about 60-80% of nitrogenous excretion of fish. Ammonia is measured as total ammonia nitrogen (TA-N) which represents the sum of un-ionized ammonia (NH_3) and ionized (NH_4) forms (Wajsbrot *et al*., 1993; Abbas, 2006). The toxicity of NH_3 is ascribed to the fact that in the form of ammonia, it has high lipid solubilizing property and lacks ionic charge, so that it readily diffuses across the gill membranes. On the other hand, the ionized NH_4 form is hydrated and has charged entities which will not allow this through the gill membrane (Scobodova *et al*., 1993). Ammonia is one of the serious problems in fish culture especially in recirculation systems, aquaria and fish ponds. There are numerous studies on different fish species concerning acute and chronic ammonia toxicity (Lemarie *et al*., 2004; Abbas, 2006). In aquatic animals, homeostasis is maintained by one important factor the pH and either increase or decrease of pH causes disturbances in acid-base balance, ion regulation and ammonia excretion (Wood, 2001).

Fish exposed to alkaline waters shows increased plasma ammonia, because a significant proportion of excreted ammonia remains as NH_3 in the water. In alkaline waters the amount of H^+ available to react with NH_3 and produce NH_4^+ is low and in this condition the NH_3 plasma-water gradient is reduced, decreasing NH_3 excretion and consequently accumulating in the plasma and tissues (Wilkie and Wood, 1996). Studies by Tomasso *et al.* (1980), Thurston *et al.* (1984) and Frances *et al.* (2000) have depicted that exposure of fish to ammonia causes histopathological changes in gill and liver. Likewise, elevated ammonia and low pH induced gill damage in juvenile brook trout (*Salvelinus fontinalis*) was also reported by Mueller *et al.* (1991). However, acute ammonia toxicity does not cause serious morphological changes in gills of fish (Tomasso *et al.*, 1980; Thurston *et al.*, 1984; Frances *et al.*, 2000). On contrary, studies on the toxicity of ammonia in carps are relatively scarce as stated by Benli and Koksal (2005). Most of these studies measured the effects of ammonia on survival, growth and some LC_{50} (the concentration that kills 50% of the organisms) estimates have also been published. On the otherhand, ammonia with different pH levels on gills of fish *C. carpio* has not been studied in Indian condition and this study was planned to find out the effects of ammonia and different pH levels on gill histology of fish *Cyprinus carpio*. This fish was chosen for this toxicity test due to its regional ecological and economic importance.

MATERIALS AND METHODS

In the present study the healthy species of *Cyprinus carpio* were procured from the local fish farm from Vadalore, Cuddalore District, Tamil Nadu, India and it was acclimatized to the laboratory conditions for 20 days. Water was changed daily and fish were fed *ad libitum* with rice bran and groundnut oil cake twice a day. Fish ranging from 7-8 cm in length and weighing 8-10 g were selected for experimental purpose. The qualities of the water was determined according to the method of APHA (1976) and were as follows: dissolved oxygen 6.2±0.02 mg L^{-1}; pH 7.2±0.2; water temperature 25.0±2.0°C; salinity 0.2±0.07 ppm; total hardness 13±2.0 mg L^{-1}; calcium 5.0±0.1 mg L^{-1}; magnesium 8.0±2.0 mg L^{-1} and total alkalinity 20.0±06 mg L^{-1}. Preliminary studies were carried out to find out the median lethal concentration (LC_{50}) for 96 h by probit analysis method of Finney (1978).

The concentration, at which 50% survival/mortality occurred, was taken as a median lethal concentration (LC_{50}) for 96 h which was 22 ppm L^{-1} for ammonia, 27 ppm for ammonia with low pH (5.0) and 18 ppm for ammonia with high pH (8.0). The acute toxicity study was conducted for 96 h with four replicates of all the three treatments along with a common control (total of sixteen tanks). The first tank was filled with 20 L of water and 22 ppm L^{-1} of ammonia was added and in the second tank the pH of the water was lowered to pH 5.0 by adding 0.1 N sulphuric acid drop by drop and 27 ppm L^{-1} of ammonia and the third tank was filled with 20 L of water and the pH of the water was increased upto pH 7.5 by adding 0.1 N sodium nitrite drop by drop then 18 ppm L^{-1} ammonia solution was added. Twenty fish were introduced into each tub. A common control (pH 7.2) was also maintained. Toxicant was renewed daily in all the experimental tanks. No mortality was observed throughout the experimental period. At the end of every 24 h, 10 fish from each experimental tank was collected for histopathological studies, gills were subsequently dissected using a sterile scalpel and were rinsed with distilled water in order to remove the adhering body fluid and were fixed in Bouin's fixative and later processed following the methods of Pearse (1968); Roberts (1978) and Humason (1979). Histopathological changes were examined under a light microscope.

RESULTS AND DISCUSSION

The histopathological observations for control, ammonia and ammonia with low and high pH exposed fish gill images are displayed in Fig. 1, 2 and 3. In control fish, primary lamellae appeared normal and mucus free with well defined secondary lamellae branched from them (Fig. 1a). In ammonia treated fish, the gills showed necrosis, disintegration of epithelial cells and degenerative changes (Fig. 1b-e). In fish, exposed to ammonia with low pH concentration, lifting up of the epithelium, swelling, hyperplasia, hypertrophy and proliferation of chloride cells, were noticed (Fig. 2b-e). In ammonia with high pH treated fish, fused lamellar filaments, desquamated epithelium, hemorrhage and complete damage of epithelial cells of lamellae were noticed (Fig. 3b-e).

Histopathology provides information to detect effects of irritants in various organs (Altinok and Capkin, 2007). The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs. Gills are a well known target organs in fish, being the first to react to unfavourable environmental conditions. In the present study, hyperplasia, hypertrophy, deformation of the lamella and lamellar fusion were recorded in the gills of ammonia exposed fish. Bais and Lokhande (2012) noticed minor alterations like chloride cells of the secondary lamellae in zebra fish exposed to cadmium concentration of 3 μg L^{-1}. Osman *et al.* (2009) also noted congestion of gill arch and lamellar blood vessels, together with edema, hemorrhage, leukocytic in filtration, lamellar epithelial hyperplasia was observed at 2 weeks post-intoxication. Several authors have reported similar alterations on the gills of different fish species exposed to ammonia (Mallatt, 1985; El-sherif *et al.*, 2008; Spencer *et al.*, 2008). Larmoyeux and Piper (1973) determined aneurysms

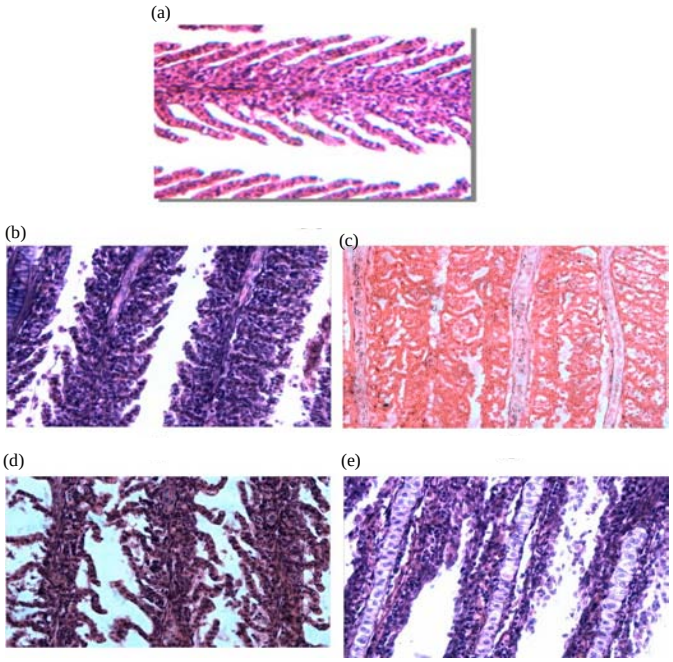

Fig. 1(a-e): Transverse section of gills of fish *Cyprinus carpio* var. *communis* exposed to acute ammonia concentration for 96 h. (Magnification X 400), (a) control, (b) after 24 h as ammonia exposure, (c) after 48 h as ammonia exposure, (d) after 72 h as ammonia exposure and (e) after 96 h as ammonia exposure

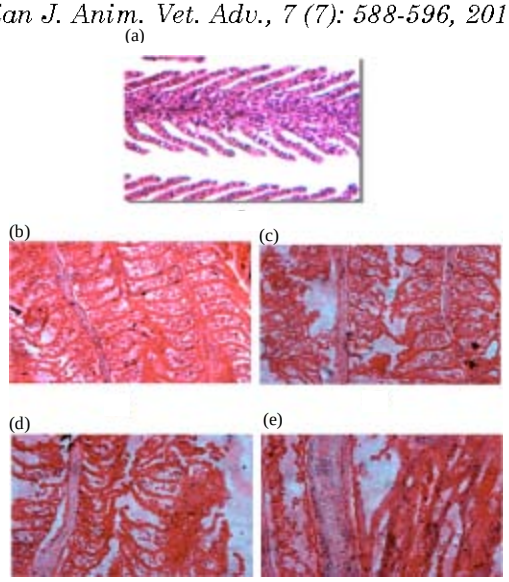

Fig. 2(a-e): Transverse section of gills of fish *Cyprinus carpio* var. *communis* exposed to acute ammonia with low pH concentration for 96 h. (Maganification X 400), (a) control, (b) affer 24 h as ammonia with low pH exposure, (c) affer 48 h as ammonia with low pH, (d) affer 48 h as ammon with low pH exposure and (e) affer 96 h as ammonia with low pH exposure

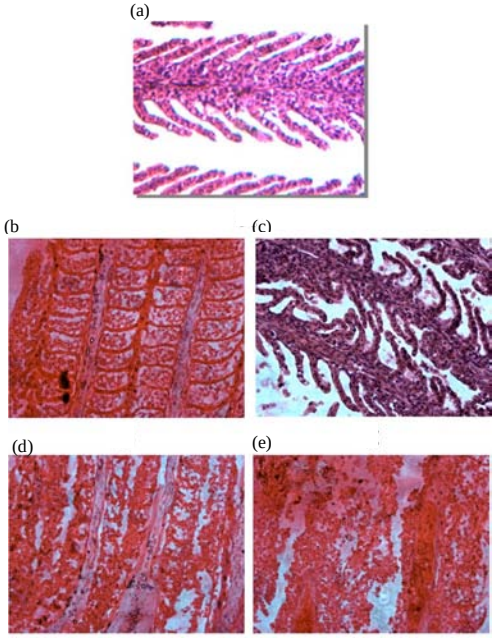

Fig. 3(a-e): Transverse section of gills of fish *Cyprinus carpio* var. *communis* exposed to acute ammonia with high pH concentration for 96 h. (Maganification X 400), (a) control, (b) affer 24 h as ammonia with high pH exposure, (c) affer 48 h as ammonia with high pH, (d) affer 48 h as ammonia with high pH exposure and (e) affer 96 h as ammonia with high pH exposure

and fused lamella of rainbow trout (*Salmo gairdneri*) gill epithelium cells. Furthermore, Kirk and Lewis (1993) reported that the gills of the rainbow trout exposed to 0.1 mg^{-1} ammonia for 2 h exhibited deformation of the lamellae. Salin and Williot (1991) observed that in Siberian sturgeon (*Acipencer baeri*) (270 g) exposed to more than 60 mg^{-1} of ammonia revealed a modification of the epithelium of the secondary lamellae and the base of the filament was slightly turgescent.

Miron *et al.* (2008) observed the major changes in the gills of Silver catfish, *Rhamdia quelen* exposed to ammonia levels of 96 h- LC_{50} (mainly in those exposed to pH 6.0) exhibited edema and fusion of the secondary lamellae. The above authors further stated that these lesions may reduce gill functional surface for gaseous exchange, impairing respiratory function. In the present study also, a similar mechanism might be operating, thus reducing the gill functional surface for gaseous exchange (Abbas and Ali, 2007). Wood *et al.* (1988), Venkataraman *et al.* (2007) and Moharram *et al.* (2011) reported that thickening of the lamellar epithelium increased diffusive distance of the gill. The thickening of the gill epithelium (via. cell hypertrophy) is sometimes considered to be an indicator of cell degeneration and eventually necrosis (Tietge *et al.*, 1988; Peuranen *et al.*, 1993; Jiraungkoorskul *et al.*, 2006; Lamchumchang *et al.*, 2007). The lifting and hypertrophy of cells greatly increases the diffusion distance (water-blood distance) (Ingersoll *et al.*, 1990). A similar reason may be attributed for the fusion of primary and secondary lamella and hypertrophy in the present study too. In the present study the ammonia with low pH treated fish gills showed swelling of primary and secondary lamellae, fusion of adjacent secondary lamellae, increased mucus production, secondary lamellae appeared thickened and shortened with extremely rough surface and considerable mucus. A similar observation was also made by Tandjung (1982), Segner *et al.* (1988) and Lamchumchang *et al.* (2007) in brown trout, *Salmo trutta* and *Oreochromis niloticus*, respectively. Daye and Garside (1976) observed hypertrophy and separation of epithelial cells from the supporting pillar cells in brook trout, *Salvelinus fontinalis* in chronic acid pH (5.5) treatment and this condition greatly increased the diffusion distance (water-blood distance). The gill epithelium represents the primary site for oxygen exchange and ionoregulation (Lease *et al.*, 2003; Jiraungkoorskul *et al.*, 2006; Joseph and Raj, 2011) and disruption of these processes leads to decreased ability to function (Smart, 1976). Lamellar fusion which is the fusing of adjacent lamellae, affects gas exchange and can be a direct result of lifting pavement cells (Frances *et al.*, 2000). In the present study, swelling of primary and secondary lamellae, fusion of adjacent secondary lamellae, increased mucus production, appearance of thickened and shortened secondary lamellae and necrosis of gill epithelium of fish may be due to direct deleterious effect of ammonia with low pH toxicity. Similar observation like gill edematosis, foamy vacuolation especially in the proximal part of the secondary lamellae, swollen, prominent in chloride cells, epithelial lifting and telangiectasis in the secondary lamellae were also found by Mahjoor and Loh (2008) and Mobarak and Sharaf (2011).

The gills of ammonia with higher pH exposed fish showed several histological changes like, separation of secondary lamellae, desquamated primary and secondary and lamellae and necrosis. Similar observations were made by several others (Tomasso *et al.*, 1980; Frances *et al.*, 2000; Lemarie *et al.*, 2004). Meade (1985) in chinook salmon (*Oncorhyncus tshawytscha*) reported an extensive hyperplasia of gill epithelium after exposure to 0.005 mg L^{-1} of NH_3-N for 6 weeks. Kirk and Lewis (1993) reported that the gills of the rainbow trout exposed to 0.1 mg L^{-1} ammonia for 2 h exhibited deformation of the lamellae. The filamental and lamellar epithelium was covered with shallow, circular depressions in which the integrity of microridges was maintained. Salin and

Williot (1991) observed that Siberian sturgeon *(Acipenser baeri)* (270 g) exposed to more than 60 mg L^{-1} of ammonia revealed a modification of the epithelium of the secondary lamellae and the base of the filament was slightly turgescent. Smith and Piper (1975) and Smart (1976) found that the most characteristic feature for chronic exposure of rainbow trout to ammonia was the appearance of swollen, rounded secondary gill lamellae or telangiectatic capillaries in the secondary lamellae. Hemorrhage is a result of blood channel disruption and is indicative of severe physical damage. Smart (1976), Mallatt (1985) and Abdel-Moneim *et al.* (2008), who observed, desquamation, necrosis, the lifting of the lamellar epithelium, oedema, aneurism, hyperplasia of epithelial cells and fusion of the secondary lamellae were seen in the gills of fish Silver Sailfin Molly (*Poecilia latipinna*) after exposure to dyestuff and chemical wastewater. Edema with apparent separation of epithelium from the underlying basement membrane and an increase in the interstitial fluid spaces are common pathological findings in toxicity with heavy metals and many other chemical pollutants. In the present study, gill hyperplasia, degeneration of epithelium was noticed in ammonia with high pH, which might be due to direct deleterious effect of ammonia with high pH toxicity. Considering the above facts, the present study concludes that effect of ammonia toxicity was very high in ammonia with high pH condition.

CONCLUSION

Ammonia is an environmental toxicant that is especially problematic for aquatic organisms. Its concentrations in water systems can increase due to agricultural run-off and decomposition of biological waste. In addition, in intensive fish culture, high stocking densities and feeding rates also increase the probability of exposure of the animals to elevated concentrations of nitrogenous wastes, particularly ammonia. In the present study, exposures to ammonia concentrations at 22 ppm, ammonia with low pH (5.0) at 27 ppm and ammonia with high pH (8.0) at 18 ppm for 96 h resulted in definite histopathological changes in the gills. In the present study, swelling of primary and secondary lamellae, fusion of adjacent secondary lamellae, increased mucus production, appearance of thickened, shortened secondary lamellae and necrosis of gill epithelium of fish may be due to direct deleterious effect of ammonia toxicity. The histological results found in the present study indicate that the ammonia with high pH tends to be more harmful than ammonia and ammonia with low pH. Further intensive studies are needed to maintain the safe level of ammonia for aquaculture/aquarium practices.

ACKNOWLEDGMENT

The authors are grateful to the authorities of CAS in Marine Biology, Annamalai University, Tamil Nadu - India for providing all the necessary facilities to carry out the present work and deeply acknowledge to University grant commission for financial assistance.

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