Pathological Study of Effect of Short and Long-term Copper Sulfate Bath on Gill in Grass Carp, *Ctenopharyngodon idella*

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Abstract

Grass carp were exposed to two therapeutic concentrations of copper sulfate (10 mg·L^{-1} in 1 hour and 1 mg·L^{-1} in 24 hours). Twenty-four hours after the copper sulfate baths, the histopathology of the gills was studied. The observed lesions in gills after short and long term baths were: mucus coagulation and accumulation of cellular debris on the epithelium of lamellae and inter lamellar regions, lamellar edema, hemorrhage, hyperplasia, epithelial cell necrosis, congestion and aneurysm (telangiectasis) in the secondary lamellae. In the 24 hour copper sulfate bath, the percentage of gill lesions that were observed correlated directly with the copper sulfate concentration ($p<0.05$). In 0.2, 0.5, 1, 2 and 5 mg·L^{-1} subgroups, a total of 42.2, 37.5, 49.2, 50 and 62.5% respectively of fishes showed extensive pathological lesions in their gills. Accumulation of cellular debris in the epithelium of lamellae and epithelial cell necrosis (100% in 5 mg·L^{-1} for 24 h) were the most prevalent lesions in these groups. In the 1 hour bath groups the total percentage of fishes with extensive gill lesions in 1, 5, 10, 20 and 30 mg·L^{-1} subgroups were: 26.6, 44, 42.5, 31.7 and 46.6% respectively. There was no significant correlation between copper sulfate concentrations and the percentage of gill lesions in these groups ($p>0.05$). Epithelial necrosis (89% in 10 mg·L^{-1} for 1 h) was the most prevalent lesion in these groups.

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Introduction

Copper sulfate is one of the chemicals that are frequently used for the control of some fungal, parasitic and bacterial diseases of fish. Some of the diseases that are controlled by this chemical include Myxobacteriosis, Saprolegniosis and some ectoparasite infestation such as Oodiniasis and Monogeniasis. It is also used as an algaecide, molluscicide and herbicide in aquaculture, irrigation and municipal water treatment systems (Stoskopf 1993). Although copper sulfate ($CuSO_4$) has been widely used, it can still be highly toxic to fish, so the concentrations required for controlling algae or pathogen agents must be below the toxicity threshold for fish.

Copper is one of the most toxic heavy metals to fish (Mance 1987) and increasing copper concentrations in water can be very dangerous. The 96-hour LC50 values of copper has been reported to range from 0.06-0.34 mg$\cdot$L^{-1} for juveniles of the Japanese eel *Anguilla japonica* (Yang and Chen 1996) to 1.97 mg$\cdot$L^{-1} for juveniles of the pompano *Trachinotus carolinus* (Birdsong and Avault 1971).

Gill is the most important and sensitive organ of fish that can be affected by water born irritant chemicals such as copper sulfate. The effect of copper sulfate on fish has been studied exhaustively and, as expected, some species have been found to be more susceptible to copper than others (Boyd 1982; Wedemeyer 1996). Daoust et al (1984) reported some lesions in the gill of rainbow trout after acute exposure to copper sulfate but there is no report about gill pathology in grass carp after exposure to copper sulfate bath. This study was carried out to determine the extant of gill lesions caused by therapeutic concentrations of copper sulfate baths in grass carp in warm water culture condition and relatively hard water condition.

Materials and Methods

Chemicals

Copper sulfate ($CuSO_4$, 5 H_2O) was supplied by Merk company-Germany.

Animal and maintenance

One hundred and twenty grass carp fingerlings were caught from a fish farm in Khuzestan province (Southwest of Iran) and transferred to an aquarium room. The grass carps were in 10-20 gram weight range and were apparently normal. The fish were held in dechlorinated tap water (temperature, 22-24 °C; hardness, 368 mg·L^{-1} as CaCO3; pH, 7.6) and kept off-feed for 2 days before the experiments.

Experiments and sampling

Sixty fish were randomly distributed in six groups for long-term bath: one control, and test solution containing the following nominal concentration: 0.2, 0.5, 1, 2 and 5 mg·L^{-1} CuSO4. The other 60 fish were exposed to 1 hour copper sulfate bath (short-term bath). The concentrations used in this group were: 1, 5, 10, 20 and 30 mg·L^{-1} (10 fish per exposure concentration). Ten fish were considered as control (control fishes were held in dechlorinated copper free tap water). After bath, the fish were transferred to copper free water. Twenty four hours later, each fish was stunned by a blow on the head and the second left gill arch was cut and immersed in cold buffered formalin. For histopathological study, after routine histopathological preparation using an automatic tissue processor, 5 µm thick sections were prepared and stained by hematoxylin-eosin (H&E) method.

Statistical test

To study the correlation between percentage of gill lesions and copper sulfate concentrations in long- and short-term baths, Spearman rank correlation method was used. The p values less than 0.05 were considered significant.

Results

The results of histopathological examinations in different concentrations are summarized in tables 1 and 2. The observed lesions in the gills of experimental groups (long- and short-term baths) were mucus coagulation and accumulation of cellular debris in the epithelium of lamellae and inter-lamellar regions, hemorrhage, lamellar edema, hyperplasia, epithelial cell necrosis, aneurysm (telangiectasis) and congestion. Normal and some typical gill lesions are presented in figures 1-6.

Table 1. Lesions recorded on the gill of grass carp in long-term bath with copper sulfate and prevalence of lesions in each group (%)

Type of gill damage	Concentrations ($mg \cdot L^{-1}$)				
	0.2	0.5	1	2	5
Lamellae surface changes	89	87.5	66.6	90	100
Hemorrhage	11.1	12.4	33.3	20	-
Edema	77.7	12.5	11.1	50	-
Hyperplasia	11.1	-	89	70	-
Epithelial cell necrosis*	22.2	37.5	77.7	50	100
Aneurism	-	-	22.2	40	16.6
Hyperemia	-	-	44.5	30	33.3

* There is significant correlation between lesion and the concentrations ($p<0.05$, $r=0.9$).

Table 2. Lesions recorded on the gill of grass carp in short-term bath with copper sulfate and prevalence of lesions in each group (%)

Type of gill damage	Concentrations ($mg \cdot L^{-1}$)				
	1	5	10	20	30
Lamellae surface changes	20	80	44.5	33.3	30
Hemorrhage*	-	30	22.2	33.3	50
Edema	20	20	-	11.1	-
Hyperplasia	20	-	55.5	11.1	40
Epithelial cell necrosis	40	50	89	77.7	70
Aneurism	20	-	22.2	44.5	50
Hyperemia	20	40	22.2	11.1	40

* There is significant correlation between lesion and the concentrations ($p<0.05$, $r=0.9$).

In the 0.2, 0.5, 1, 2 and 5 $mg \cdot L^{-1}$ subgroups, a total of 42.2, 37.5, 49.2, 50 and 62.5% respectively of fishes showed extensive pathological lesions in their gills. The lesion was considered extensive when observed in more then half of the gill arch lamellae. Accumulation of cellular debris in the epithelium of lamellae and epithelial cell necrosis (100% in 5 $mg \cdot L^{-1}$ for 24 h) were the most prevalent lesions in these groups.

Fig. 1. Light micrograph of saggital section through normal grass carp gill. Micrograph shows relatively normal secondary lamellae (arrow) (H&E, ×1000)
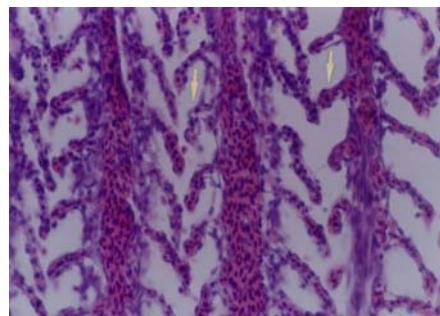

Fig. 2. Necrosis of epithelial cells and destruction of secondary lamellae (arrows) in grass carp after copper sulfate bath (H&E, ×400)
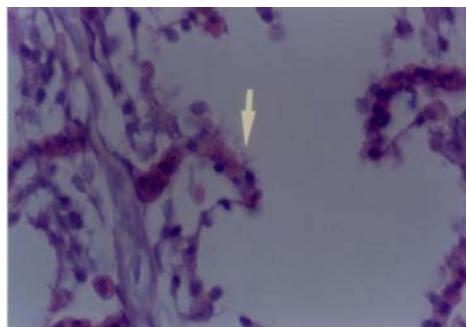

Fig. 3. Grass carp gill with accumulation of cellular debris on epithelial cells of secondary lamellae and coagulation of mucus (arrow) after copper sulfate bath (H&E, ×1000)

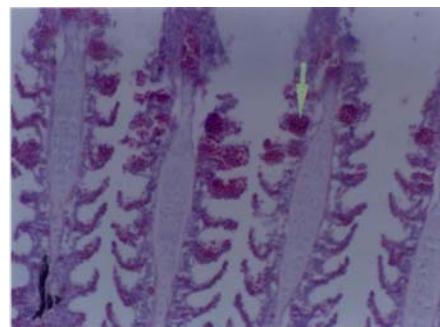
Fig. 4. Blood-filled aneurysms (telangiectasis) of secondary lamella (arrow) can be seen in gill lamellae after copper sulfate bath. (H&E, ×200)
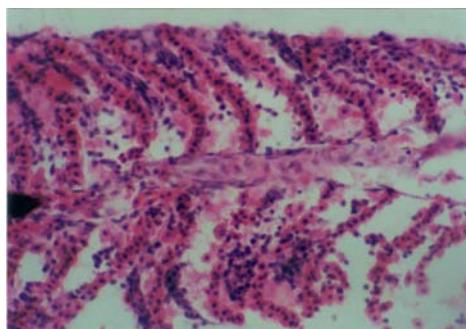

Fig. 5. Gill with hyperplasia secondary lamellae after copper sulfate bath. Inflammatory and hyperplastic cells can be seen in the inter-lamellar space (H&E, ×400)
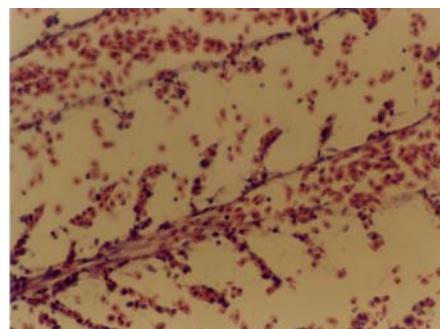

Fig. 6. Grass carp gill with hemorrhage and necrosis of secondary lamellar cells after copper sulfate bath, blood cells have filed the inter-lamellar space (H&E, ×400)

In the long-term bath, a significant correlation was observed only between epithelial cell necrosis and the concentrations ($p<0.05$, $r=0.9$).

In the 1 hour bath groups, the total percentage of fishes that had extensive gill lesions in the 1, 5, 10, 20 and 30 $mg \cdot L^{-1}$ subgroups were: 26.6, 44, 42.5, 31.7 and 46.6% respectively. There was no significant correlation between copper sulfate concentrations and the percentage of gill lesion in these groups ($p>0.05$). Epithelial necrosis (89% in 10 $mg \cdot L^{-1}$ for 1 h) was the most prevalent lesion in these groups. In the short-term bath, the significant correlation was observed only between hemorrhage and the concentrations ($p<0.05$, $r=0.9$).

Discussion

Fish is naturally exposed to a variety of metals including both essential and non-essential elements. Copper is one of the essential metals that is transported by metallothionein to the blood circulation after absorption from gills and intestines, and some of it accumulate in different internal organs especially in the liver and kidney. Our histopathological study showed relatively extensive acute lesions in the gills of the experimental groups. Copper sulfate in high concentration is very toxic to fish. Its toxicity to fish varies with the species and the physical and chemical characteristics of the water. Even at recommended rates of application, this metal may be poisonous to fish, especially in soft or acid waters. However, its toxicity to fish generally decreases as water hardness increases but our study showed that even in relatively hard water it can lead to severe pathological changes in gills. Gills of adult freshwater fish, which typically make up about 50% of the total body surface area, are thinner and more delicate than the skin epidermis. Copper sulfate can be corrosive to the gill lamellae so edema, congestion and hyperemia of gill lamellae may be due to a direct effect of copper sulfate on the gill. Cerqueria and Fernandes (2002) found hyperplasia and thickening in thr gill as well as lamellar telangiectasis in *Prochilodus scrofa* as a result of copper exposure. Heerden et al (2004) also reported some pathological changes of gills after copper sulfate exposure. These changes are reported as thickening of the epithelium as well as telangiectasis. Daoust et al (1984) reported same lesions in the gill of rainbow trout after acute exposure to 0.135 $mg \cdot L^{-1}$ copper sulfate at 48 hours. Tissue changes such as cell hyperplasia, mucus secretion and cell hypertrophy due to exposure to metals are reported in many investigations and are believed to be a compensatory response to

keep metals from entering through the gill cells (Mallat 1985; Dang et al 2000).

Copper treatment has been reported to cause hyperplasia of the gill epithelium (Nelson et al. 1999). In our study, however, the time after bath was not enough to induce severe proliferation. This effect of copper can have an adverse impact on osmo-regulation in freshwater fish.

Accumulation of cellular debris on the gill lamella was one of the histopathologic findings. Contact of fish gill with copper sulfate can cause access mucus secretion and because of substantial net negative charge of gill surface, gill have a high affinity for cationic metals. Therefore the accumulation of superficial debris may be a result of precipitation of copper ions in mucus secretions.

In summary our histopathological study showed relatively extensive acute lesions in the gills of experimental groups especially in long-term bath groups. These lesions can lowered the respiratory efficiency of the gill therefore using extra aerators during the treatment period will be necessary to prevent asphyxiation and death of affected fish.

Acknowledgements

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