Histopathological Response of Milkfish *Chanos chanos* Forsskal Fingerlings to Potassium Permanganate*

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Static 96 h bioassays were conducted on milkfish fingerlings at concentrations ranging from 1.00 to 1.80 mg/l KMnO_4 . Histopathological analyses of gills, liver, and kidney tissues revealed significant changes even in non-lethal concentrations tested. Damage became severe with increasing concentration and longer exposure to the chemical. Partial to complete recovery was observed in gills, liver, and kidney cells of fish exposed to KMnO_4 for 96 h and then maintained in KMnO_4 -free seawater for 240 h.

Potassium permanganate (KMnO_4), a strong oxidizing agent, has long been used in aquatic systems for various purposes. These include improvement of available oxygen in water (LAY, 1971; TUCKER and BOYD, 1977), treatment of infectious diseases (PHELPS *et al.*, 1977; TUCKER and BOYD, 1977; JEE and PLUMB, 1981), detoxification of fish poisons (LAWRENCE, 1956; MARKING and BILLS, 1975), and control of algae (LAY, 1971; TUCKER and BOYD, 1977). The use of KMnO_4 for certain aquatic applications has been approved by the United States Food and Drug Administration (MEYER *et al.*, 1976).

Although KMnO_4 has been used extensively, available literature on tolerance level to fish is limited and none on histopathological effects on fish tissues.

This study describes the histopathological response of milkfish, *Chanos chanos*, fingerlings exposed to different concentrations of KMnO_4 .

Materials and Methods

Milkfish fingerlings (3-5 g) were acclimatized for five days under laboratory conditions. Fish were fed daily with live brine shrimp (*Artemia* sp.) adults but were starved 24 h prior to and

during the experiment.

Static 96 h bioassays were conducted following standard procedures for toxicity tests (APHA, 1971). The completely randomized design (CRD) was used for the experiments. Tests were conducted in 50 l glass aquaria with 10 fish/30 l seawater (temperature, $27 \pm 1^\circ\text{C}$; salinity, 30 ppt; pH, 8.4) with aeration. Test concentrations ranged from 1.0-1.80 mg/l KMnO_4 plus a control. All test concentrations were done in three replicates.

The median lethal concentration (LC50) values at different time intervals are not included in this report, but will be presented in a separate paper.

Moribund or freshly dead fish were sampled after 24 h at the end of the experiment for histological analyses of the gills, liver, and kidney. Tissues were fixed in 10% buffered formalin, washed in running tapwater, dehydrated with ethyl alcohol, cleared in xylene, infiltrated with paraffin, and embedded in paraffin wax. Tissues were sectioned at 6μ and stained with hematoxylin and eosin.

All surviving fish after the 96 h exposure were transferred to a KMnO_4 -free seawater and maintained for another 240 h. Fish were likewise sampled at the end of the 240 h period for histological analyses of the gills, liver, and kidney tissues.

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Fig. 1. Gill filament from control milkfish with normal gill lamellae. H & E. 200 $\times$.

Fig. 3. Gill lamellae exposed to 1.4 mg/l KMnO_4 for 24 h showing epithelial lifting. H & E. 200 $\times$.

Fig. 2. Gill filament from milkfish exposed to 1.35 mg/l KMnO_4 for 24 h showing lamellar hyperplasia. H & E. 200 $\times$.

Fig. 4. High power view of Fig. 3. The epithelial layer lifted away from the pillar cell system. H & E. 1000 $\times$.

Results

Fish Behaviour

Immediately upon exposure to KMnO_4 , all fish were affected even at concentrations that caused no mortality during the test. Fish exhibited rapid opercular movements and swimming activity. In fish exposed to higher concentrations, that behaviour continued for 30 minutes followed by gulping of water at the surface and blackening of the dorsal portion of the body. The snout and fin tips were clearly oxidized as evidenced by the brownish color. Fish affected by the chemical were less sensitive to external noise or movement.

Histopathology

Histopathological changes in the gills, liver, and kidney were observed in all treatments as early as 24 h after exposure. Lesions observed were essentially similar in all treatments and exposure time. However, tissue damage became severe with increasing concentrations and longer exposure to the chemical.

1. Gills

Fig. 1 shows normal structure of milkfish gill filament. Fish exposed to the chemical after 24 h exhibited epithelial hyperplasia (Fig. 2), clubbing of lamellar tips, fusion of adjacent lamellae, lamellar detachment, epithelial lifting (Figs. 3, 4), and extensive disruption of the gill system.

Fig. 5. Gill filaments exposed to 1.45 mg/l KMnO_4 for 96 h with multiple aneurysms. H & E. 100 $\times$.

Fig. 7. Severe hyperplasia of gill epithelium resulted to fusion of lamellae after exposure to 1.80 mg/l KMnO_4 for 96 h. H & E. 200 $\times$.

Fig. 6. Higher-power view of Fig. 5. Aneurysm contains disorganized mass of pillar cells and blood cells. H & E. 1000 $\times$.

Fig. 8. Liver hepatocytes from control milkfish. H & E. 1000 $\times$.

After 96 h, gills had severe pathological changes. In many filaments, several lamellae exhibited bloodfilled aneurysms or lamellar telangiectases (Fig. 5). In each of these swollen and rounded secondary lamellae, the epithelium enclosed a disorganized mass of pillar cells and blood cells (Fig. 6). Severe hyperplasia of epithelium resulted in extensive fusion of lamellae (Fig. 7).

2. Liver

In control fish, the liver parenchyma is composed of polyhedral hepatocytes separated by sinusoids (Fig. 8). Following exposure to KMnO_4 , the liver parenchyma had undergone severe degeneration consisting of vacuolation and necrosis. Vacuolation of the hepatocytes was

observed in all treatments tested. Fish exposed to 1.80 mg/l KMnO_4 exhibited extensive vacuolation of the hepatocytes (Fig. 9). Also, the well-defined liver structural pattern was lost. The extent of liver structure disruption generally corresponded to the degree of cytoplasmic vacuolation of the hepatocytes.

Necrotic lesions walled-off by thick layers of fibrin (Fig. 10) were also often noted. Liver cell nuclei were pyknotic and sinusoids were dilated (Fig. 11). Hemorrhagic areas were frequently seen, especially in blood vessels and sinusoids presumably demonstrating stasis (Fig. 12). In some, liver hepatocytes showed fibrosis (Fig. 13).

3. Kidney

Fig. 9. Extensive vacuolation of hepatocytes after exposure to 1.80 mg/l KMnO_4 for 24 h. Vacuoles were of different sizes and shapes. The well-defined liver structure was also disrupted. H & E. 1000 $\times$.

Fig. 11. Pyknotic nuclei and sinusoidal dilation were evident after exposure to 1.40 mg/l KMnO_4 for 24 h. H & E. 1000 $\times$.

Fig. 10. Necrotic area is walled-off by thick layers of fibrin after exposure to 1.25 mg/l KMnO_4 for 24 h. H & E. 200 $\times$.

Fig. 12. Hemorrhage occurred in blood vessels and sinusoids after exposure to 1.25 mg/l KMnO_4 presumably demonstrating stasis. H & E. 200 $\times$.

The milkfish kidney is composed of the glomerulus, renal tubules and hematopoietic tissue (Fig. 14). A marked dilation of the glomerular capillaries and thickening of the walls occurred 24 h after exposure to the chemical (Fig. 15). In some, there was a consequent hypertrophy of the glomerulus and space of Bowman's capsule was not observed (Fig. 16). Also, some glomeruli were shrunken (Fig. 17).

Cloudy swelling (Fig. 18) and vacuolation (Fig. 19) of epithelial cells in the renal tubule were often observed. The shape of the affected cells were indistinguishable and the lumen of the tubules either decreased in size, or completely

disappeared. Hemorrhage also occurred as erythrocytes consequently filled up the renal tubule. Recovery from KMnO_4 induced lesions.

The gills, liver, and kidney of milkfish exposed to 1.0 to 1.50 mg/l KMnO_4 for 96 h and then transferred to a KMnO_4 -free environment for 240 h apparently recovered. Most had normal gill structures (Fig. 20) although hyperplasia, clubbing, and some lamellar detachment were still evident. There was no fusion of adjacent lamellae, epithelial lifting, nor aneurysms observed. Although the liver exhibited vacuolation of the hepatocytes after 240 h in untreated seawater, vacuolation was less than in their coun-

Fig. 13. Liver hepatocytes showing fibrosis after exposure to 1.0 mg/l KMnO_4 for 24 h. H & E. 1000 $\times$.

Fig. 15. Glomerulus from fish exposed to 1.35 mg/l KMnO_4 for 24 h. Note dilation of the glomerular capillaries. H & E. 1000 $\times$.

Fig. 14. Kidney from control fish showing glomerulus, renal tubules and hematopoietic tissue. H & E. 100 $\times$.

Fig. 16. Glomerulus from fish exposed to 1.0 mg/l KMnO_4 for 96 h. Glomerular capillaries were extremely dilated and space of Bowman's capsule was not observed. H & E. 1000 $\times$.

terparts prior to recovery (Fig. 21). The renal tubules appear to be normal after the 240 h recovery period (Fig. 22).

Discussion

Changes observed in milkfish behaviour during exposure to KMnO_4 clearly indicate that the fish are under stress. Increased opercular rates have been suggested as a sensitive indicator of physiological stress in fish exposed to pollutants and may be caused by decreased efficiency in oxygen uptake or transport (DAVIS, 1973; THOMAS and RICE, 1975).

In fish, the gills, liver, and kidney are usually

the organs of choice for histological studies of chemically induced lesions (COUCH, 1975). These organs are easily damaged by low concentrations of numerous toxic and metabolic disturbances (CAHN, 1975; ROBERTS, 1978). The primary pathological changes we observed were found in fish exposed to 1.25 mg/l KMnO_4 , and higher concentrations.

Epithelial hyperplasia was suggested as an initial response of gills to toxicity following exposure to various pollutants. In this study, varying degrees of hyperplasia were observed. Gill hyperplasia was more uniform and extensive

Fig. 17. Glomerulus shrunk after exposure to 1.25 mg/l KMnO_4 for 24 h. H & E. 1000 $\times$.

Fig. 18. Cloudy swelling of epithelial cells of renal tubules after exposure to 1.30 mg/l KMnO_4 for 96 h. H & E. 1000 $\times$.

Fig. 19. Vacuolation of epithelial cells of renal tubules after exposure to 1.35 mg/l KMnO_4 for 96 h. H & E. 1000 $\times$.

Fig. 20. Gill filaments after exposure to 1.0 mg/l KMnO_4 for 96 h and then maintained in untreated seawater for 240 h. H & E. 200 $\times$.

in fish treated with concentrations higher than 1.40 mg/l KMnO_4 . Severe proliferation of epithelial cells which leads to fusion of adjacent lamellae decreases the surface area of the gills, and consequently, the ability of the animal to absorb oxygen (BURROWS, 1964; GARDNER and YEVICH, 1970).

Another gill damage frequently reported in toxicity related studies is epithelial lifting. In our study, the respiratory epithelia lifted away in a continuous sheet from the pillar cell system. In severe cases, the epithelial layer was completely ruptured thereby exposing the capillaries to water. SKIDMORE and TOVELL (1972) suggested epithelial lifting as an acute inflammatory reaction to pollutants. Disruption of the epithelial layer has

been implicated in asphyxiation, partial or complete loss of secretory or excretory function, or to loss of plasma electrolytes or proteins from open or exposed gill lesions (MITCHELL *et al.*, 1978).

Gill lamellar telangiectasis has been suggested as a diagnostic lesion of fish associated with chemical pollution (ELLER, 1975; WALSH and RIBELIN, 1975; ROBERTS, 1978). In the present study, lamellar telangiectasis was observed in fish treated with 1.45 mg/l KMnO_4 or higher concentration, and only after 96 h of exposure. Telangiectasis resulted to dilation of the capillary and pooling of the blood which thromboses and eventually fuses with the adjacent lamellae. If there

Fig. 21. Liver hepatocytes after exposure to 1.0 mg/l KMnO_4 and then maintained in untreated seawater for 240 h. H & E. 1000 $\times$.

Fig. 22. Renal tubules after exposure to 1.25 mg/l KMnO_4 for 96 h and then maintained in untreated seawater for 240 h. H & E. 200 $\times$.

are several telangiectatic lamellae, respiratory function may be hindered, and if such fish are further traumatized, rupture or fatal hemorrhage may follow (ROBERTS, 1978).

Gill alterations are easily observed in chemical-induced studies designed to measure short-term acute toxicity. In this study, the most consistent gill lesions were lamellar hyperplasia, fusion of adjacent lamellae, epithelial lifting, and lamellar telangiectasis. Such changes may be specific tissue reactions to toxicosis as noted in previous reports mentioned. Gill changes are often directly related to disorders in gill function which consequently may affect the physiology or cause the death of fish.

The liver pathology of fish is less diverse than that of the mammals (ROBERTS, 1978). The most common type of degenerative changes we encountered are vacuolation of the hepatocytes and focal necrosis. Vacuolation of the hepatocyte was common in all concentrations tested. The severity of cell vacuolation was related to concentrations of KMnO_4 . The large, smooth-edged vacuoles of the hepatic cells is a characteristic of advanced pathological response (COUCH, 1975). Liver cell vacuolation is considered as the most common non-specific liver lesion found in toxic conditions and indicates abnormal accumulation of fats (COUCH, 1975; ROBERTS, 1978). ROBERTS (1978) associated degenerative fatty change of hepatocytes to unsuitable dietary fats. The disruption of the well-defined liver structure as a

consequence of cytoplasmic vacuolation is an indication of nutritional deficiency enhanced by toxic conditions (ELLER, 1971).

Necrosis of liver cells, pyknosis of hepatocyte nuclei, sinusoidal congestion, and fibrosis are also commonly observed in toxic conditions (COUCH, 1975; ROBERTS, 1978; PIERCE *et al.*, 1980).

The types of pathological changes in kidneys of milkfish exposed to KMnO_4 were similar in all treatments. In this study, histopathologic lesions in kidney are confined mostly in the glomerulus and renal tubules. In pathological conditions, dilation of the glomerular capillaries and thickening of the walls occur as a result of alterations in the basal lamina (HIBIYA, 1982). This dilation and thickening with a consequent hypertrophy of the glomerulus have been reported in fishes with nutritional deficiencies (WATANABE *et al.*, 1970; YOKOTE, 1970), or fishes treated with alloxan (INUI and YOKOTE, 1975).

Degeneration or cloudy swelling of kidney tubules have been reported in several fish species following exposure to pesticides and heavy metals (KING, 1962; BUHLER *et al.*, 1969; WALSH and RIBELIN, 1975; HIBIYA, 1982).

Results from this study indicate that although milkfish could tolerate as high as 1.40 mg/l KMnO_4 , tissue alterations were evident. Histologically, there was no difference between treatments, but the intensity of tissue damage increases with increasing concentration. However, sublethal pathological changes in fish tissues appear

to be temporary, and once fish return to a KMnO_4 -free environment, they have the ability to recover.

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