Histopathology biomarker responses in Asian sea bass, *Lates calcarifer* (Bloch) exposed to copper

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Abstract  Copper is a trace element necessary for the normal growth and metabolism of living organisms. If exceeds its limit it becomes pollutant and causes pathological effects. Asian sea bass (*Lates calcarifer*) was exposed to sublethal concentrations of copper for 28 days and histopathological alterations were noticed in the gill, liver, muscle and intestine. Epithelial necrosis, hypertrophy, rupture of gill epithelium and haemorrhage at primary lamellae were observed after 7 days of exposure. Lifting of epithelium, oedema and fusion of adjacent secondary lamellae were conspicuous at 28 days of exposure. The experimental liver tissue showed reduction in the size of hepatocytes, vacuolisation, and hypertrophy. The intestine displayed fused microvilli, sloughing of mucus membrane and swollen cells. Muscle bundles with severe oedema and their thickening and separation were more pronounced in sublethal treatment of copper in the muscle. Several histopathological changes observed in various fish organs would serve a useful purpose in evaluating the toxic effects of copper. The present study clearly demonstrated that all the treated body organs exhibited significant damage with response; amongst the body organs the liver is an important target organ for copper toxicity in *L. calcarifer* and this species could be possibly used as a model organism for toxicity studies.

Keywords *Lates calcarifer*; Copper; Histopathology

Background

Copper is a trace element essential for normal growth and metabolism. In vertebrates, it is indispensable for bone formation, development of connective tissue, and cardiac function (Li et al., 1996) and also functions as a cofactor for key enzymes (Puig and Thiele, 2002). However, this metal becomes toxic to cells when its concentration surpasses certain natural levels (Theophanides and Anastassopoulou, 2002). The main sources of copper pollution includes mining, industrial discharge, sewage disposal and fertilisers. Besides this, lixiviation magnifies its levels in aquatic bodies and in turn severely affects the aquatic organisms (Nor, 1987). The effects of water copper contamination on fish are of primal concern. Even sublethal concentrations can induce severe damages, with negative impacts on fish performance and enhanced vulnerability to secondary diseases that potentially cause mortality. Accordingly, copper levels determined during sporadic monitoring of aquatic envi-

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Copper in particular are one of the most detrimental toxicants affecting fish gills by changing their morphology and ultrastructure (Wong and Wong, 2000; Machado and Fanta, 2003). Gills are the first object of waterborne pollutants due to their stable contact with the external environment (Perry and Laurent, 1993). Heavy metal ions impede with respiration and osmoregulation causing cellular damage to gill cells (De Boeck et al., 2001; Pandey et al., 2008). The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, location and blood supply (Van der Oost et al., 2003) it is also one of the organs most affected by contaminants in the water (Rodrigues et al., 1998). The monitorisation of histological changes in the fish liver is a highly perceptive and precise way to assess the effects of xenobiotic compounds in field and experimental studies.

Amongst various organs, very little is known about the effects of Cu on the fish intestine which is believed to be the first organ that comes into contact with food borne contaminants. Also, the intestine is one of the most important sites where food enters and is assimilated. Thus, toxic substances, that enter the intestine, directly affect the vigour of the organism. Therefore intestine can serve as a potent indicator for water borne Cu. Since gills and gastrointestinal tract in fishes are considered the main passage for the entry of pollutants into the internal body organs like the liver and kidney through the blood (Takashima and Hibiya, 1995) the evaluation of these organs for toxicity study is of primary importance. Histological changes associated with heavy metals in fish muscle have been studied by many authors (Thophon et al., 2003; Athikesavan et al., 2006; Giari et al., 2007; Jiraungkoorskul et al., 2007). Previous histopathological studies of fish bare to pollutants exposed that fish organs are efficient indicators of water quality (Cardoso et al., 1996; Barlas, 1999; Cengiz et al., 2001).

Several studies have shown that animals exposed to both acute and sublethal levels of waterborne copper can recover in the long term from harmful metal injury, even in the ambient water. In the shore crab, Carcinus maenus, the recovery processes involved the cytological damage of the gill epithelium (Stentiford and Feist, 2005). A variety of acclamatory effects have also been documented in fish and lobster chronically exposed to sublethal levels of copper (Grosell et al., 1998; Maharajan et al., 2011, 2012a,b, 2013; Paruruckumani et al., 2015a,b).

Lates calcarifer is a significant fish in Asia and it is cultured due to its economic value. In the culture ponds copper sulphate is used as antibiotic and when this copper in lethal concentration reaches the fish organs it causes serious effects. This bioaccumulation of copper makes this fish unfit for human consumption. In the present study, the histological alterations of the gill, muscle, intestine and liver of Asian sea bass, exposed to copper were carried out to analyse the damage occurred.

Methods

Experimental animal collection and maintenance

L. calcarifer specimens, employed in this study, were provided by the Rajiv Gandhi Centre for Aquaculture (Tamil Nadu, India) and acclimated to laboratory conditions for 10 days. During this period, fish were maintained in 150-L capacity aquaria with water and equipped with filter and oxygenation systems. During the acclimatisation period, salinity (15%), density (1.027–1.028 g/cm³), temperature (25–27 °C) and nitrite and nitrate concentrations were measured and kept constant (dissolved oxygen 8–9 mg/L; hardness 100 mg CaCO₃/L and the absence of heavy metals). For the entire duration of the experiment, the animals were maintained under a natural light/dark cycle and fed every second day with commercial fish food. They were starved for 24 h before and during the experiment.

Chemicals used

For the preparation of stock solution, 3.9 g of copper II sulphate pentahydrate (CuSO₄·5H₂O) (Merck) was dissolved in 11 of double-distilled water and used as stock solution. It was stored in a clean standard flask at room temperature in the laboratory.

Experimental procedure

Test concentration

Fish were exposed to nominal 6.83 and 13.66 ppm as copper. Doses were theoretically sublethal, 10% and 20%, respectively, of the maximum acceptable toxicant concentration (MATC), which was 68.3 ppm. The MATC was represented as no observed effect concentration (NOEC) < MAT-C < LOEC (lowest observed effect concentration). The test concentration was estimated using the application factor (AF) concept, by dividing the limits (NOEC and LOEC) of the MATC by the 96-h LC50 (AF = MATC/LC50 = (NOEC–LOEC)/LC50).

System design

A recirculation closed system was set up according to Muthuwan (1998). The experiment was carried out in 360 L glass aquarium (120 × 60 × 50 cm), in which one compartment (50 × 50 × 40 cm) was partitioned by a plastic gauze (mesh size 1.5 mm) to contain a biofilter. Each aquarium was filled with 300 L of natural sea water (salinity of 27 ± 2 ppt), which was pumped continuously over a biofilter column at a rate of 41/min. The water was continuously aerated throughout the experiment.

Test procedure

After 2 weeks of acclimatisation in a holding tank, ten healthy fish (8.06 ± 0.19 cm in length and 11.18 ± 0.67 g in weight) were transferred to each aquarium at a loading density of 0.69 g/L. Three replicates were performed for test concentration and control. Fishes were fed twice daily with chopped fresh fish at 10:00 and 14:00 h. Uneaten food was quickly removed from the system. Fishes were starved for 24 h before sampling. The experimental water (50%) was changed every 2 weeks to keep the water quality within acceptable limits according to APHA (1995); water quality (dissolved oxygen, temperature, pH and salinity) was measured everyday and water chemistry (ammonia nitrogen, nitrite nitrogen, nitrate nitrogen) was measured twice weekly. All chemical parameters were determined following the techniques of APHA (1995).
using analytical grade reagents. The actual concentration of copper was measured weekly before and after its addition to maintain concentrations at the designed level. Water characteristics and the actual copper concentrations are shown in Table 1. Mortality and behaviour were observed everyday in each concentration. Two fishes from each aquarium were sampled at 0, 7 and 28 days post-exposure.

**Histological analysis**

Gill, Intestine, liver and muscle were fixed in 10% buffered formalin for 24 h, dehydrated through a graded ethanol series and embedded in paraffin. Tissue sections (5 mm thick) were stained with haematoxylin-eosin. The thin sections of the tissues were stained by haematoxylin and eosin for observation by the Nikon bright field transmission microscope with Köhler illumination and automatic exposure unit was used.

**Results**

**Histology of gills**

Histological study of the gills shows a typical structural organisation of the respiratory lamellae in the untreated fish. There are four gill arches and each arch is composed of numerous gill filaments with two rows of semi circular secondary lamellae that are aligned along both sides of the primary gill lamellae. The primary gill lamellae (PL) consist of centrally placed rod like central axis (CA) with chloride cells (CC) and with blood vessels on either side. The lamellae are lined by squamous epithelium and many capillaries split by pillar cells (PC) run parallel along the surface. Between the lamellae, the filament is lined by a thick stratified epithelium constituted by several cellular types, such as chloride, mucous and pavement cells. (Plate 2, Fig. A).

**Histopathology of gills**

Copper exposure has (Plate 1, Fig. B–F) induced noticeable pathological changes in fish gill architecture. The changes include curling of secondary lamellae (CSL), a few telangiectasis (lamellar capillary aneurism) at the tip of the secondary lamellae (TSL) and desquamated epithelium at 7 days and 6.83 ppm concentration of copper (Plate 1, Fig. B). Other observations during the experiment includes rupture and breakdown of the pillar cell system (CDPCS) at 28 days and 6.83 ppm concentration of copper (Plate 1, Fig. C and D). Shortened and clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were well marked. Besides these changes pyknotic nuclei, lamellar clubbing, rupture of secondary lamellar tips (RSLT), oedema and rupture of epithelia cells (EREC) were also observed at 7 days and 13.66 ppm concentration of copper (Plate 1, Fig. E). The gill cells show extensive aneurism with rupture in secondary lamellae at 28 days and 13.66 ppm concentration of copper (Plate 1, Fig. F).

**Histology of intestine**

Histological findings showed that the fundamental organisation of intestinal wall is similar to that in other vertebrates and is formed by tunica mucosa with a loose connective tissue lamina propria (LP) tunica submucosa, tunica muscularis (inner circular and outer longitudinal smooth muscles) and tunica serosa layers. The outermost serosa is followed by a well developed longitudinal and circular muscle embedded in loose connective tissue (CE) ornately supplied with blood capillaries. It merges with tunica propria of the underlying mucosal coat. Intestinal mucous-secreting cells or goblet cells (GC) are interspersed amongst the columnar cells (Plate 3, Fig. A).

**Histopathology of intestine**

Lower concentration (6.83 ppm) of copper at 7 days of exposure induced significant changes in the intestine of the experimental fish. In the exposed group a degenerative effect is evident in the mucosal lining and villi of the intestine. The villi tend to become fused (FV) due to excessive hypertrophies and there is sloughing off of the mucosal lining, finally leading to the large lumen (LL) (Plate 2, Fig. B). Hypertrophy of epithelial cells, swelling or oedema of lamina propria (SLP) and flattening of villi (FLV) ultimately leading to rupture of villi at tip, are also evident at 28 days and 6.83 ppm concentration of copper (Plate 2, Fig. C and D). Large areas of intestinal mucosal folds are injured and wreckage of the fragmented secondary mucosal folds observed in the cavities. The nuclei of columnar epithelial cells exhibited pyknosis with fusion of boundaries of columnar epithelial cells. Degeneration of the submucosal layer, i.e., lamina propria and the absorptive columnar epithelial cells resulted in vacuolation in the submucosal layer cytoplasmic boundaries. Longitudinal muscle fibres are loosely arranged and become swollen (SLML). In later stages flattening of microvilli and a cracked clay appearance of the tissue (CCA) are very apparent at 7 & 28 days and 13.66 ppm concentration of copper (Plate 2, Fig. E and F).

**Histology of muscle**

Muscles are poised of elongated muscle fibres, held together by connective tissues. The body musculature is fairly simple in the fish. The fins are usually provided with individual small mus-

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**Table 1** Water quality characteristics and actual copper concentration during sublethal exposure to Asian sea bass, *L. calcarifer*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range</th>
<th>Mean ± S.D.</th>
</tr>
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<tbody>
<tr>
<td>Dissolved oxygen (mg/l)</td>
<td>6.6–7.1</td>
<td>6.87 ± 0.25</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25.6–27.9</td>
<td>26.8 ± 1.15</td>
</tr>
<tr>
<td>Salinity (%)</td>
<td>27.4–30.2</td>
<td>29.0 ± 1.44</td>
</tr>
<tr>
<td>pH</td>
<td>6.81–8.24</td>
<td>7.60 ± 0.71</td>
</tr>
<tr>
<td>Ammonia nitrogen (mg/l)</td>
<td>0.02–0.94</td>
<td>0.57 ± 0.48</td>
</tr>
<tr>
<td>Nitrite nitrogen (mg/l)</td>
<td>0.03–0.97</td>
<td>0.58 ± 0.49</td>
</tr>
<tr>
<td>Nitrate nitrogen (mg/l)</td>
<td>0.69–0.91</td>
<td>0.81 ± 0.11</td>
</tr>
<tr>
<td>Actual copper concentration (mg/l)</td>
<td>0.013–0.026</td>
<td>0.019 ± 0.01</td>
</tr>
</tbody>
</table>
cles, but the most complex organisation is in the head region. Many individual muscles show an arrangement of fibres into bundles separated from each other by connective tissue portions. The nerves generally penetrate a muscle on its side and branch out as they penetrate the connective tissue. Segmentation or metamericism of vertebrate musculature is seen clearly in the lateral muscles of the fishes. They are divided into myotomes or muscle segments, each of which is bent into a single V with the angle directed anteriorly. Each myofibril is composed of two types of short myofilaments which are precisely arranged giving the appearance of transverse banding, the striations (Plate 4, Fig. A).

Plate 1  Histological changes of gills in *L. calcarifer*. Light micrographs of a paraffin section stained with Hematoxylin and Eosin (40×). (A) Control; (B) after 7 days of exposure to 6.83 ppm concentration of copper; (C) and (D) after 28 days of exposure to 6.83 ppm concentration of copper; (E) after 7 days of exposure to 13.66 ppm concentration of copper; (F) after 28 days of exposure to 13.66 ppm concentration of copper. Abbreviations used: PL – primary lamellae; SL – secondary lamellae; PC – pillar cells; CC – chloride cells; EC – epithelial cells; NE – nucleated erythrocytes; TSL – telangiectasia at the tip of secondary lamellae; CSL – curling of secondary lamellae; BPC – breakdown of pillar cells; HEC – hyperplasia of epithelial cells; LSGE – lifting of secondary gill lamella epithelium; CDPCS – completely damaged pillar cell system; RSLT – rupture of secondary lamella tip; EEC – oedema of epithelial cells; RBPC – rupture and breakdown of pillar cell system; EREC – oedema and rupture of epithelial cells.
Histopathology of muscle

In copper treated muscle, oedema and mild lymphocyte infiltration, vacuolar degeneration in muscle bundles and atrophy of muscle bundles are observed. Oedema between muscle bundles and splitting of muscle fibres were seen at 7 days and 6.83 ppm concentration of copper (Plate 3, Fig. B). After 28 days of exposure in the lowest concentration of copper the muscle tissue exhibited dystrophic changes with marked thickening and separation of muscle bundles. The vacuolar degeneration in muscle bundles with aggregations of inflammatory cells between them and inter myofibrillar space (IMFS) get widened ends with disintegration of myofibrils observed (Plate 3, Fig. C and D). The muscle seems to have lost the myoseptum that separates each myotome. Disintegrated epidermis is also seen in the section of muscle. Intramuscular oedema (EMF) is a common feature at the highest concentration (13.66 ppm) at 7 & 28 days of treated fish. Significant changes noted are broken myofibrils and gap formation between muscle bundles (GFMF) which finally lead to degen-

Plate 2  Histological changes of intestine in L. calcarifer. Light micrographs of a paraffin section stained with Hematoxylin and Eosin (40×). (A) Control; (B) after 7 days of exposure to 6.83 ppm concentration of copper; (C) and (D) after 28 days of exposure to 6.83 ppm concentration of copper; (E) after 7 days of exposure to 13.66 ppm concentration of copper; (F) after 28 days of exposure to 13.66 ppm concentration of copper. Abbreviations used: GC – goblet cells; LP – laminar propria; N – nucleus; L – lumen; CE – columnar epithelium; FV – fusion of villi; LL – large lumen; FLV – flattened villi; DCML – damaged circular muscle layer; DL – distended lumen; DLML – damaged longitudinal muscle layer; VF – vacuole formation; SLP – swelling of lamina propria; CCA – cracked clay appearance of the tissues; SLML – swelling of longitudinal muscle layer; DGC – damaged goblet cells; DMM – disarrangement of muscularis mucosa.
operation in muscle bundles (MD) accompanied with focal areas of necrosis as well as atrophy (Plate 3, Fig. E and F).

**Histology of liver**

The surface of the liver is covered with serous membrane and some connective tissue extends inwards into parenchyma. It is composed of parenchymal cells (hepatocytes) (HC) and lattice fibres. Hepatic cells are roundish polygonal, containing clear spherical nucleus (N). They are located amongst sinusoids forming cord like structures known as hepatic cell cords. Blood sinusoids (BS), are irregularly distributed between the polygonal hepatocytes. Fairly large quantities of lipid glycogen granules were also observed in the cytoplasm (Plate 4, Fig. A).

**Histopathology of liver**

The changes observed in the liver tissue on exposure to 6.83 ppm concentration of copper for 7 days included swelling and rounding off of hepatocytes, detachment of cells from
each other. Pancreatic acini appeared to have lost its architecture. Cytoplasm of hepatocytes became more basophilic. These changes include degenerated hepatocytes presenting a homogenous cytoplasm and a large central or sub central spherical nucleus (Plate 4, Fig. B). The important histopathological changes observed in the copper treated groups were pyknotic nuclei and clear cell foci. The liver tissue after 28 days of copper exposure at lowest concentrations revealed vacuolation of hepatocytes, condensation of nuclear chromatin and swelling of hepatocytes (HSHC) (Plate 4, Fig. C). At a high concentration of copper after 7 days of exposure there is extensive vacuolation of hepatic cells (Plate 4, Fig. D) with several foci of coagulative necrosis, blood congestion (BC) and accumulation of dark granules (ADG). Appearance of Blood streaks amongst hepatocytes are a marked change in treated liver. Formation of cytoplasmic vacuoles (CV), along with atrophy, necrosis and disappearance of hepatocytic cell wall and disposition of hepatic cords are also observed. The degenerative changes are intensified in lethal exposures. Hepatic nuclei were either swollen or pyknotic. (NP) (Plate 4, Fig. E and F).
Discussion

The present study showed that the copper accumulated in different organs of the fish *L. calcarifer* and caused many histopathological defects. Extensive architectural loss was observed in the gills of copper treated group. In the present study, after 7 days of exposure to high concentrations of copper, epithelial necrosis, hypertrophy of the epithelial cells, rupture of gill epithelium, haemorrhage at primary lamellae and sloughing of respiratory epithelium were noted (Plate 1, Fig. B and C). The lifting of the epithelium, oedema, epithelial necrosis, fusion of adjacent secondary lamellae and haemorrhage at primary lamellae were observed in the gills of the fish examined after 28 days of exposure to copper (Plate 1, Fig. D).

Another important histopathological change observed in the copper treated group was hyperplasia. Morphologically, hyperplasia refers to augmentation in the number of normal cells that constitute a given tissue. Gill alterations such as hyperplasia of the epithelial cells can be considered adaptive, since they increase the distance between the external environment and blood, serving as a barricade to the entrance of contaminants. Gill hyperplasia might serve as a protective mechanism leading to a decrease in the respiratory surface and an increase in the toxicant-blood diffusion distance. Increased mucus production and fusion of lamellae were obvious on exposure to copper. Extensive epithelial desquamation was also observed in the copper treated group. It is well known that changes in fish gill are amongst the most commonly recognised responses to environmental pollutants (Mallatt, 1985; Laurent and Perry, 1991; Au, 2004). After acute exposure to hexavalent chromium, *Channa punctatus* exhibited marked degenerative changes in the histology of gills, kidney and liver tissues (Mishra and Mohanty, 2008). The gills of copper treated sea bass exhibited lamellar telangiectasis (localised dilation of blood vessel). This appearance of the secondary lamellae results from the collapse of the pillar cell system and breakdown of vascular integrity with a release of large quantities of blood that push the lamellar epithelium outwards (Alazemi et al., 1996). Complete lamellar fusion may have reduced the total surface area for gas exchange. Otherwise, they increase the distance of the water–blood barrier, which together with epithelial lifting and the increase in mucus secretion may drastically reduce the oxygen uptake. Epithelial necrosis and rupture of gill epithelium are direct deleterious effects of the irritants. The histopathological changes of gills can result in hypoxia and respiratory failure problems with ionic and acid-base balance (Alazemi et al., 1996). The histopathological changes observed in the gills of *L. calcarifer* in the present study are in good agreement with the results of (Rao et al., 2003; Jauch, 1979).

While many studies have documented the histomorphological alterations to the gills, kidneys and livers of fish (Fracacio et al., 2003; Au, 2004) only a few have investigated the effect they exert on the intestine (Banerjee and Bhattacharya, 1995; Kamunde et al., 2001). The gastrointestinal tract represents a major route of entry for a wide variety of toxicants present in the diet or in the water that the fish inhabit (Bano and Hasan, 1990; Lemaire et al., 1992) on the other hand there is little information related to the protective mechanisms adopted by the intestine epithelial surfaces of the fish against mercury uptake (Oliveira Ribeiro et al., 2002).

The intestinal villi in copper treated *L. calcarifer* were found to be severely injured (Plate 2, Fig. B). The lamina propria was shrunken and the epithelial layer orientation was found completely collapsed. The tips of the villi were also found to be ruptured (Plate 2, Fig. C). Virk et al. (1987) have also discussed endrin and carbaryl induced damages in the intestine of *Mystus tengara*. Inbarani and Seenivasan (1988) have observed similar pathological lesions in the intestine of phosphamidon treated *Sarotherodon mossambicus*.

In the present study, the result of the effect of copper on the gastrointestinal system of *L. calcarifer* clearly showed that copper exerts toxic effects on the different layers of intestine. Mandel and Kulshrestha (1980) found lesion formation in the villi of *Clarias batrachus* after exposure to sumithion. Necrosis and infiltration of lymphocytes and eosinophils were reported in the intestine of *Gambusia affinis* exposed to deltamethrin (Cengiz and Unlu, 2006). The alterations in the intestine of the sea bass were more severe in higher doses. Toxic lesions most common in the intestine of fishes exposed to copper chloride include hyperaemia, degenerative changes in the tips of villi, loss of structural integrity of mucosal folds, degenerative mucosal epithelium (hypertrophy, vacuolation, hyper-chromasia) necrosis, desquamation of mucosal epithelium, cellular debris, excessive mucus in gut of lumen, necrosis of submucosa and inflammatory infiltration (Plate 2, Fig. E and F).

Like gills, muscle tissue also comes in close contact with pollutants dissolved in water. Hence, reactions in the histopathology of the muscles were spontaneous. In the present study the histopathology of muscle show progressive damage in the structure of muscles with increasing concentrations of copper. Similar observations have been made by Nagarajan and Suresh (2005) in the muscle tissue of the fish *Cirrhinus mrigala* with increasing concentrations of sago effluent. Sakr and Gabr (1991), Abo Nour and Amer (1995), and Das and Mukherjee (2000) have studied the effect of different pollutants on fish muscles.

The copper treated fish showed significant thickening and separation of muscle bundles with severe intracellular oedema (Plate 3, Fig. B and C). A similar observation has been made by (Das and Mukherjee, 2000). Fatma (2009) observed the degeneration of muscle bundles with aggregation of inflammatory cells between them and focal areas of necrosis and also, vacuolar degeneration in muscle bundles and atrophy of muscle bundles in fish exposed to different pollutants. The present investigation closely agreed with a similar report by (Fatma, 2009). The histological findings included degeneration in muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis. Also, vacuolar degeneration in muscle bundles and atrophy of muscle bundles were observed. Alterations in the muscles of several species of the fishes exposed to heavy metals have been described by Oliveira-Ribeiro et al. (2002); Thophon et al. (2003), Gupta and Srivastava (2006), Kaoud and El-Dahshan (2010) which are in sequence with the present investigation. Separation of muscle bundles was an interesting observation. Initial stimulus of copper can induce hyperactivity and excitability in animals, leading to the release of lactic acid and subsequent muscular fatigue (Das and Mukherjee, 2000).

The liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by the elevated concentrations of these compounds, and could subsequently
result in structural damage (Brusle and Anadon, 1996). Fish liver histology could therefore serve as a model for studying the interactions between environmental factors and hepatic structures and functions. Some of these environmental factors include biotoxins, parasites, infectious germs, physiochemical parameters and pollutants, for example pesticides, hydrocarbons, PCB’s (polychlorinated biphenyls) and heavy metals (Brusle and Anadon, 1996).

The parenchymatous hepatic tissue in teleosts, has many important physiological functions and also detoxification of endogenous waste products as well as externally derived toxins, important physiological functions and also detoxification of the hepatocytes in places with areas of diffuse necrosis (Plate 4, sublethal concentrations of copper with marked swelling of vessels, vacuolisation, hypertrophy; pyknotic nuclei, necrosis, and accumulation of blood vessels (Plate 4, Fig. C and D).

Significant changes were observed in the liver tissue at lethal and sublethal concentrations of copper with marked swelling of the hepatocytes in places with areas of diffuse necrosis (Plate 4, Fig. F). Radhiah and Jayantha Rao (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture of blood vessels and appearance of blood vessels amongst hepatocytes and pyknotic nuclei in the liver of Tilapia mossambica exposed to fenvalerate. Sakr et al. (2005) observed histopathological changes induced in the liver after exposing the fish Clarias gariepinus to fenvalerate which are mainly represented by cytoplasmic vacuolisation of the hepatocytes, blood vessel congestion, inflammatory leucocytic infiltration necrosis and fatty infiltrations. In the world, sea bass hatcheries are running successfully even though, in the larval rearing period problems are faced with bacterial and protozoan diseases. To control these diseases, copper sulphate treatment is applied in the hatchery system. Several histopathological symptoms that appeared in fish organs would serve as biomarker responses in copper toxicity of Asian sea bass. These biomarkers reduce the usage of copper sulphate as antibiotic and attempt the implementation of alternative natural remedies. The present study clearly demonstrated that amongst various organs, the liver is an important target organ for copper toxicity in the treated L. calcarifer.

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