Ultrastructure changes in hepatocytes of catfish
Clarias gariepinus from Lake Mariut, Egypt

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Abstract: In the present study, specimens of catfish (Clariidae) were collected from a polluted location (Main Basin) and a relatively clean area (East Basin) in Lake Mariut, one of the Nile Delta Lakes in Egypt. Fifteen fish were taken from each site. Liver preparations of fish from the two sources were comparatively examined for cellular changes using transmission electron microscopy. Fish hepatocytes from the polluted area showed accumulation of the heterochromatin, enlarged nucleoli, and an extremely folded nuclear envelope. Perichromatin granules were increased and progressively formed small clusters closely associated with patches of heterochromatin. In the cytoplasm, fractionation, dilation, and vesiculation of rough endoplasmic reticulum (RER), and elevated amounts of smooth endoplasmic reticulum (SER) tubules were noted. The most frequent pathological modifications were the swelling of mitochondria, cristae regression and changes in the electron-transparency of the matrix. Lysosomes showing myelin-like stacks of membranous material (phagolysosomes), glycogenosomes (i.e., glycogen rosettes enclosed by membranes) and cytoplasmic myelinated bodies were strongly developed. Furthermore, increasing numbers of secondary lysosomes with degraded cell organelles were found. With reference to the storage vesicles, there appeared to be an increase in the lipid droplets (lipidosis) within many hepatocytes. This study reinforces the need to select representative sentinel species from different habitats for biomonitoring purposes and it provides further support for the use of biomarkers in assessing the health of aquatic ecosystems.

Key words: Clarias gariepinus, Liver, Ultrastructure, Pollution, Lake Mariut

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Introduction

Inland water, including seas, rivers and lakes receive massive flux loaded with industrial and anthropogenic wastes that exert huge impact on aquatic life (Barton and Iwama 1991; Adham, 2002; Padmanabha and Belagali, 2008; Toroglu and Toroglu, 2009; Srivastava et al., 2009). In Egypt, lakes of the Nile Delta that used to be of special economic and social importance are now under potential risk due to uninterrupted runoff of many types of polluted discharges (Adham, 2002). Lake Mariut is the smallest of these lakes and the most polluted one. It has suffered over the years from the untreated sewage, agricultural and industrial wastes dumped into it (Hamza, 1999; Amr et al., 2005). Fish quality and quantity were dramatically affected and eventually becoming unfit for human consumption due to the poor water quality of the lake (Adham et al., 1997; Amr et al., 2005). Many fish species like Mugil cephalus, Mugil capito, Anguilla vulgaris, Cyprinus carpio and Morone labrax have disappeared from the lake due to the increased pollution problem (Saleh et al., 1983a,b; Saleh and Hamza, 1986). Only few fish species survived including the Nile sharpooth catfish or Clarias gariepinus. A synonym of Clarias gariepinus is Clarias lazera (Teugels, 1986).

Clarias gariepinus is the most important species for aquaculture among 32 valid species of the genus Clarias recognized by Teugels (1984). It is probably the most widely distributed fish in Africa (Adham, 2002). This fish species grows up to 60-170 cm total length and is of considerable importance as food fish (Admek and Sukop, 1995). It is omnivorous feeder and a general scavenger with a marked tendency to feed on benthic organisms and detritus (Bok and Jongbloed, 1984). As other clariids, this species is highly resistant to muddy waters and can survive extremes of aquatic hypoxia and even desiccation (Bok and Jongbloed, 1984).

Polluted water habitats exert extensive stress impacts upon aquatic animals (Barton and Iwama, 1991). Histopathological biomarkers of xenobiotic exposure and histopathology of fish liver have been increasingly recognized as valuable tools for the detecting adverse chronic effects of contaminant exposure and uptake on aquatic organisms (Myers et al., 1998; Pietrapiana et al., 2002). Hepatic lesions, including neoplasms, focal lesions, degenerative or necrotic lesions are commonly detected in fish from contaminated environments (Myers et al., 1998). Such lesions are similar to those experimentally induced by toxicant or a carcinogen exposure in fish under chronic exposure to contaminated sediments and diets. Therefore, they have been related to environmental contaminant exposure in several field studies (Moore and Myers, 1994).

The use of fish as bio-indicators of pollution in Lake Mariut has received the attention of many workers. Much has been documented about the accumulation of heavy metals and pesticide residues in fish collected from polluted sites in the lagoon (Saad et al., 1982; El-Rayis and Saad, 1990; Amr et al., 2005). In addition, the connections between changes in physiological and molecular parameters such as acetylcholinesterase, alkaline phosphatase, glutathione S-transferase, DNA damage and free amino acid
composition, in fish blood or target organs, and pollution stress in the
lake have been studied (Adham et al., 1997,1999,2001; El-
Demerdash and Elagamy, 1999; Matta et al., 2007). Furthermore,
sperm deformations in fish have been used as a model for predicting
environmental hazards in Lake Mariut (Abdelmeguid et al., 2007).
However, the effects of pollutants on liver ultrastructure of benthic fish
(e.g., catfish) collected from the lake have not been examined so far.
Accordingly, the aim of the present study was to mark the possible
cytological changes in hepatocytes of Clarias gariepinus as affected
by chemical pollution in Lake Mariut.

Materials and Methods

Study area: Lake Mariut is an important fishing lake at the southern
area of Alexandria City, Egypt (Fig. 1). It has no direct connection
with the Mediterranean Sea. Lake Mariut is mainly divided into five
principal basins; these are (main, MB; east, E; northwest, NW;
southeast, SE; southwest, SW) (Adham et al., 2001). The East Basin
(also known as Fish Farm) extends 6 km beside the desert road with an average depth of 130 cm. It receives most of its water
from the New Mariut Hydraulic Pumps and Umoum Drain at the
southern margin. The Fish Farm connects with Qualaa Drain by a
movable gate at its northern extremity. However, this gate is usually
closed (Smaan and Abdel-Moneim, 1986). The Main Basin or Lake
Proper is of about 600 acres, however, its area has reduced greatly
due the recent establishment of many investment projects on the
lake. It is boarded by highways from three sides and by the Nubarriya
Canal and Umoum Drain at the west (Smaan and Abdel-Moneim,
1986). It receives flow from three main sources of pollution; the first is
Qualaa Drain which carries agricultural wastewater, sewage
discharge and industrial effluents to the basin. The second source is
the West Treatment Plant (WTP), while the third one is Umoum Drain
(El-Rayis, 2005). According to El-Rayis and El-Sabrouti (1998), the
surplus water from the lake proper is allowed to flow into the lower
reach of Umoum Drain before pumping the mixed waters (6.8 hm³
d⁻¹) to the Mediterranean Sea at El-Max (Fig. 1). This pumping
process maintains surface water level in the lake at about 2.5 m
below sea level, to work properly as a recipient of different effluents.

Fish sampling: During the summer months (June - August) of
2007, about 15 male specimens of Clarias gariepinus were collected
alive from various fishermen from each of the two sampling sites of
Lake Mariut and were then brought to the laboratory in plastic
containers. Total lengths of specimens ranged between 27.3 and 40.7 cm and their weights fluctuated between 125 and 515 g.
Maturation stages of the collected specimens were visually determined
from the general morphology of gonads, according to Sehriban and
Yilmaz (2007). All examined specimens were mature ones. As soon
as the fish samples reached the laboratory, they were dissected and
liver tissues were taken for transmission electron microscopy (TEM).

Ultrastructure study: Liver samples were fixed by immersion in
2.5% glutaraldehyde in phosphate buffer solution (pH 7.2) at 4°C.
The specimens were post-fixed in 2% osmium tetroxide for 2h at
4°C. They were rinsed again in buffer, dehydrated in a graded
ethanol series, and embedded in epon-araldite mixture. Ultrathin
sections were cut and stained with uranyl acetate and lead citrate
according to Reynolds (1963). The specimens were viewed in Jeol
100 CX TEM. To quantify hepatocellular modifications as a
consequence of pollution impacts, a semi-quantitative evaluation was
carried out by classifying the extent of the changes into six categories:
- = absent; + = very weak; ++ = weak; +++ = strong; ++++ = very
strong.

Results and Discussion

Catfish hepatocytes from the East Basin (relatively clean
area) were distinctly subdivided into an organelle-rich zone around
the centrally located nucleus and a peripheral area with glycogen
rosettes interspersed with lipid deposits (Fig. 2a). Nuclei showed
heterochromatin randomly distributed in the nucleoplasm with small
concentrations underneath the nuclear envelope. The organelle-
rich portion of the cytoplasm was found to be comprised of smooth
endoplasmic reticulum (SER) and rough endoplasmic reticulum
(RER) stacks with non-fenestrated cisternae arranged in parallel
array. In addition, golgi fields consisting of cisternae and occasional

Fig. 1: Map of lake Mariut showing its position in relation to the Mediterranean
sea as well as the supporting canal system (Adham et al., 2001). MB, main
basin (polluted area); E, east basin (relatively clean area); NW, northwest
basin (cleaned area); SE, southeast basin; SW, southwest basin; IN, inner
harbor; WH, west harbor; MC, Mahmoudiya Canal; NC, Nubarriya Canal;
QD, Qualaa Drain; UD, Umoum Drain; WTP, west treatment plant.
Ultrastructure changes in hepatocytes of catfish

lipid droplets were also observed. Thus, the ultrastructure of reference hepatocytes closely resembled that described for control fish hepatocytes in other experiments (e.g., Braunbeck et al., 1989, 1990).

Cytological changes in fish hepatocytes from the polluted area (Main Basin) are summarized and quantified in Table 1. Part of the hepatocytes showed an increasing tendency of the heterochromatin to condense in the nuclear periphery and around the nucleus (Fig. 2b). Perichromatin granules (Fig. 2c) were increased and progressively formed small clusters closely associated with patches of heterochromatin. Significantly enlarged nucleoli indicating enhanced hepatocellular activity were clearly seen. An extremely folded nuclear envelope could also be observed (Fig. 2d). Moreover, cytoplasmic effects comprised a less regular compartmentalization of different components. RER underwent a progressive loss of structural integrity. Although part of the RER cisternae still displayed typical parallel arrays, increasing numbers of cisternae appeared fractionated, dilated and vesiculated. Furthermore, RER cisternae were occasionally transformed into circular arrays (Fig. 2e). In contrast to the RER, elevated amounts of SER tubules were noted. The most frequent pathologica modifications were the swelling of mitochondria, cristae regression and changes in the electron-transparency of the matrix (Fig. 2b). On the other hand, increasing heterogeneity in the lysosomal matrix was commonly observed. Lysosomes showing myelin-like stacks of membranous material (phospholipidosis), glycogenosomes (i.e. glycogen rosettes enclosed by membranes) and cytoplasmic myelinated bodies were strongly developed (Fig. 2b). Furthermore, increasing numbers of secondary lysosomes with degraded cell organelles were found (Fig. 2f). In peroxisomes, no structural changes were evident. However, small clusters of peroxisomes were observed. With reference to the storage vesicles, there appeared to be an increase in the lipid droplets (lipidosis) within many hepatocytes (Fig. 2f).

Table 1: Semiquantitative analysis of ultrastructural alterations in the liver of catfish (Clarias gariepinus) collected from two locations in lake Mariut

<table>
<thead>
<tr>
<th></th>
<th>Relatively clean area (East Basin)</th>
<th>Polluted area (Main Basin)</th>
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<tbody>
<tr>
<td><strong>Nucleus</strong></td>
<td></td>
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<tr>
<td>Irregular outline</td>
<td></td>
<td>+</td>
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<tr>
<td>Dilation of nuclear membrane</td>
<td></td>
<td>+ +</td>
</tr>
<tr>
<td>Amount of heterochromatin</td>
<td></td>
<td>+ + +</td>
</tr>
<tr>
<td>Perichromatin granules</td>
<td></td>
<td>+</td>
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<tr>
<td><strong>Mitochondria</strong></td>
<td></td>
<td></td>
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<tr>
<td>Cristae regression and electron lucent matrix</td>
<td>-</td>
<td>+ + +</td>
</tr>
<tr>
<td>Swelling</td>
<td></td>
<td>+ +</td>
</tr>
<tr>
<td><strong>Peroxisomes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structural integrity</td>
<td></td>
<td>+ + +</td>
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<tr>
<td>Formation of clusters</td>
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<tr>
<td><strong>Lysosomes</strong></td>
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<td>Heterogeneity of lysosomal matrix</td>
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<td>+</td>
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<tr>
<td>Secondary lysosomes</td>
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<td>+ +</td>
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<tr>
<td>Phospholipidosis (myelin formation)</td>
<td>-</td>
<td>+ +</td>
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<tr>
<td>Glycogenosomes</td>
<td></td>
<td>-</td>
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<tr>
<td>Myelinated bodies in cytoplasm</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Vacuoles</td>
<td></td>
<td>+</td>
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<tr>
<td><strong>RER</strong></td>
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<tr>
<td>Parallel stacks of RER cisternae</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>Fragmentation of cisternae</td>
<td>-</td>
<td>+ +</td>
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<tr>
<td>Dilation of membranes</td>
<td></td>
<td>+</td>
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<tr>
<td>Vesculation of membranes</td>
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<tr>
<td>Transition into concentric arrays</td>
<td>-</td>
<td>+</td>
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<tr>
<td><strong>SER</strong></td>
<td></td>
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<tr>
<td>Quantity</td>
<td></td>
<td>+</td>
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<tr>
<td><strong>Golgi fields</strong></td>
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<tr>
<td>Activity</td>
<td></td>
<td>+ +</td>
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<tr>
<td>VLDL-particles</td>
<td></td>
<td>+ +</td>
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<tr>
<td><strong>Storage materials</strong></td>
<td></td>
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<tr>
<td>Amount of lipid</td>
<td></td>
<td>+</td>
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<td>Amount of glycogen</td>
<td></td>
<td>+ + +</td>
</tr>
</tbody>
</table>

Data are given as means from 15 fish
Abbreviations: - = Not present, + = Little developed, + + = Moderately developed, + + + = Strongly developed, RER = Rough Endoplasmic Reticulum, SER = Smooth Endoplasmic Reticulum, VLDL = Very low density lipoprotein
Fig. 2: Ultrastructure of hepatocytes of Clarias gariepinus collected from the East Basin (relatively clean area) (a) and the Main Basin (polluted area) (b-f). (a) The nucleus (N) is large and centrally located. There are low numbers of secondary lysosomes (arrows). The cytoplasm contains mitochondria (M), and RER cisternae. The remainder of the cytoplasm represents glycogen storage, X 7500. (b) Accumulation of heterochromatin (arrows) is evident. Note fractionation of the RER cisternae, degenerated mitochondria (M) and the presence of numerous and large myelinoid bodies (arrowheads) within hepatocyte. Peroxisomes (asterisk) are mostly concentrated in fields of dilated RER cisternae, X 7500. (c) Note large intranuclear inclusion body (arrow), perichromatin granules can also be noted, X10000. (d) The nuclei exhibit severe irregularities in shape, along with the development of SER, X 7500. (e) Note dilation, fragmentation and vesiculation of RER cisternae (arrows) are strongly developed. Degranulation and transition of RER cisternae into circular arrays (arrowheads) enclosing glycogen or, mitochondria (M) are also observed, X 10000. (f) Augmentation of lipid droplets (arrows) with decrease of cellular organelles and proliferation of lysosomal elements (arrowheads) can be seen, X 5000.
Because of the deleterious impact of pollutants in aquatic ecosystems (mainly in continental and coastal areas), the histocytochemical responses of fishes to various classes of xenobiotic compounds need to be determined and characterized (Hinton et al., 1978; Couch, 1988; Braunbeck et al., 1989, 1990). Fish liver structure and ultrastructure proved to be valuable as sensitive indicators of toxicant-induced injury (Hinton et al., 1988; Braunbeck and Volki, 1991). In the present study, liver cells of catfish from polluted area in Lake Mariut showed toxicant-related alterations in ultrastructure. Most ultrastructural changes such as progressive dilation of RER cisternae, irregular nuclear outline, and induction of glycogenosomes and phospholipidosis are of unspécific nature, since they were also reported in fish after exposure to several toxicants such as lindane (Syvlie et al., 1996), polychlorinated biphenyls (Hugla and Thome 1999), and mercury (Giarda et al., 2008). On the other hand, myelin-like figures in the cytoplasm of cells may be used as an index of autophagy of certain damaged membranous components as well as a high degree of turnover of cell organelles (Ghadially, 1997). Myelin-like figures in hepatocytes of catfish collected from the polluted Main Basin were noticed several times.

One of the major alterations in liver cells of catfish was the increase in size and number of lipid droplets. Increase in lipid droplets in hepatic cells of fish exposed to toxicants was reported by Biagianti-Risbourg and Bastide (1995), Biagianti-Risbourg et al. (1996), Braunbeck (1998), Strmac and Braunbeck (2002), and Thophon et al. (2004). A large increase in the number of lipid droplets in the cell cytoplasm reflected the decline of protein synthesis that accompanies hepatocyte injury which blocks the utilization of lipid-protein conjugation (Cheville, 1994). On the other hand, glycogen inclusions declined in hepatocytes of catfish caught from the Main Basin. Similar observation was noted in other fish species such as Channa punctata, Oncorhynchos mykiss, Danio rerio and Liza ramada, following exposure to several toxicants (Murthy and Devi, 1982; Biagianti-Risbourg and Bastide, 1995; Braunbeck, 1998) as well as in fish from natural polluted water (Teh et al., 1997). This could be due to either increased glycolytic activity to meet the energy demands imposed by enhanced metabolic activity, hormone-mediated stress phenomena (Hanke et al., 1983; Gluth and Hanke, 1985) or reduced intestinal absorption of carbohydrates (Braunbeck and Appelbaum, 1999).

The mitochondrial degeneration may account for the impaired oxidative capability of hepatocytes in fish from the polluted location. Indeed, marked ultrastructural changes including the presence of swollen mitochondria with loss of functional cristae have already been reported in the liver tissue of freshwater catfish exposed to methyl parathion (Tripathi and Shukla, 1990). Furthermore, SER proliferation is generally regarded to be indicative of the induction of biotransformation processes (Hinton et al., 1978; Braunbeck et al., 1989, 1990). In addition to SER proliferation, RER proliferation and fenestration (Braunbeck et al., 1989, 1990; Lester et al., 1993) as well as circular ER arrays were all suggested to be indicative of mixed-function oxidases (MFO) induction (Hawkes, 1980). It is worth mentioning that RER changes in catfish from contaminated area may well be interpreted as the morphological counterpart of ethoxyresorufin-O-deethylase (ECOD) and ethoxyresorufin-O-deethylase (EROD) induction. Similar findings were reported in rainbow trout after exposure to endosulfan and disulfoton (Arnold et al., 1995), and in the demersal fish following intraperitoneal injection of benz(α)pyrene (Au et al., 1999). Most likely, the increase in perichromatin granules (observed in the present study) also represents aberrations in protein synthesis (Ghadially, 1997). In agreement, Adham et al. (2001) reported improper growth, protein inadequacy, and functional impairment in Oreochromis niloticus inhabiting polluted sites of Lake Mariut, in particular the Main Basin. These were reflected by data of specific formulae as RNA/DNA and the relative RNA content in cells of the liver and gill arches as well as by the relative mobilization of serum protein fractions.

In conclusion, our results suggest that water pollution in Lake Mariut is capable of inducing morphological alterations in liver, which may cause physiometabolic dysfunction in clariid species. This study reinforces the need to select representative sentinel species from different habitats for biomonitoring purposes and it provides further support for the use of biomarkers in assessing the health of aquatic ecosystems.

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