

Environmental effects on liver tissue of Tilapia fish *Oreochromis sp* from Wadi Hanifah Stream, Riyadh, Saudi Arabia.

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Abstract: Histological and ultrastructural studies were carried on the liver fish *Oreochromis sp* collected from highly polluted and less polluted areas (control area) from Wadi Hanifah stream. The major histopathological changes in liver fish collected from polluted area were disruption of the normal tissue arrangement with congestion of blood vessels and leucocytes infiltration. Most hepatocytes appeared vacuolated with peripheral necrotic nuclei. Electron microscopic observations revealed irregular and pyknotic nuclei with marginated nucleoli and heterochromatin. Moreover, dilatation and fragmentation of the rough endoplasmic reticulum cisternae, degeneration and swelling of mitochondria were noted. Myelin-like figures, numerous secondary lysosomes, as well as multivesicular electron-dense bodies were occurred in the cytoplasm of most hepatocytes. There was also, an increase in the amount of lipid droplets while the Golgi bodies were hypertrophied. The histopathological and ultrastructural alterations demonstrated in the present study are useful biomarkers for field evaluation and tilapia fish was shown to be appropriate for environmental monitoring.

[Jehan M.Sorour and Dalal Al Harbey. **Environmental effects on liver tissue of Tilapia fish *Oreochromis sp* from Wadi Hanifah Stream, Riyadh, Saudi Arabia.** *Life Sci J* 2013; 10(3): 1592-1599] (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 239

Keywords: Histopathology, ultrastructure, liver, *Oreochromis sp*, Wadi Hanifah

1.Introduction

Fishes can be easily exposed to toxic substances in a similar way of the other vertebrates and could be used to assess the presence of substances that cause teratogenic and carcinogenic in human (Biagini *et al.*, 2009). Evidence arising from histo-cytopathological examinations is a valuable tool to evaluate the impact of pollutants on fish (Au,2004; Giari *et al.*,2008; Grund *et al.*,2010). It also allows examining specific target organs, including liver, gills and kidney, that are responsible for vital functions (Gernhofer *et al.*, 2001; Camargo and Martinez, 2007; Sorour and Al Harbey, 2012). The liver is the major organ of accumulation, biotransformation and excretion of contaminants in fish. Hence, the evaluation of histological changes in fish liver is important for monitoring environmental exposure to toxicity in the aquatic ecosystem (Koehler *et al.*,2004; Matos *et al.*, 2007; Liu *et al.*,2010).Tilapia fish is considered one of the most commercial and common fresh water fish in Saudi Arabia. Thus, it is was used in the present study as a bioindicator of the pollution in different sites from Wadi Hanifah stream in Riyadh, through examination of histopathological features and ultrastructural changes in the liver tissue.

2. Material and Methods:

About 47 mature specimens of Tilapia fish (*Oreochromis sp.*) weighting between

250- 650 g were collected alive from two sampling sites of Wadi Hanifah stream in Riyadh. Control (less polluted) site and highly polluted site have been chosen according to Siddiqui and Al-Harbi (1995).

For histopathological study, liver specimens from the dissected fish were fixed in 10% neutral buffered formalin or in Bouin's fluid, dehydrated, embedded in paraffin, sectioned and stained with haematoxylin and eosin.

For ultrastructural study, the liver specimens were cut into small pieces, immediately fixed in 3% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated and embedded in Epon 812.

Semithin and ultrathin sections were cut on LKB ultramicrotome.

The semithin sections were stained with toluidine blue and examined with light microscope while ultrathin sections were double stained with uranyl acetate and lead citrate and examined with Jeol 100S electron microscope at King Saud University.

3.Results:

At the light microscopic level, the liver of fish collected from control area revealed the typical appearance of hepatocytes which were polygonal in shape with central spherical nuclei. The hepatocytes were arranged in cord-like strands around small sinusoids and separated by the hepatopancreas (Fig.1).

Endothelial cells and Kupffer cells lined the luminal surface of the sinusoids (Fig.2).

Histopathological changes were identified in liver fish collected from polluted area. The major changes were disruption of the normal arrangement of liver tissue with dilatation and congestion of blood vessels (Fig.3). Leucocytes infiltration was observed along the hepatocytes and around the blood vessels (Fig.4). Moreover, cytoplasmic and nuclear degeneration could be recognized in the hepatocytes. Some hepatocytes appeared swelling with cloudy and granular cytoplasm while most of them appeared vacuolated with unstained areas (Fig.5). This vacuolization in the hepatocytes may be associated with lipid accumulation in the cytoplasm. The large vacuoles forced the nuclei to the periphery of the hepatocytes which appeared necrotic with irregular nuclear profiles (Fig.6).

Electron microscopic observations of liver fish collected from less polluted area (control area) revealed that the centrally spherical nucleus of hepatocytes exhibited little heterochromatin (Fig.7). The cytoplasm was mainly filled with large amounts of glycogen particles, some lipid droplets and small amounts of smooth endoplasmic reticulum. Long parallel cisternae of rough endoplasmic reticulum were located around the nucleus and along the plasma membrane. Circular and elongated mitochondria of varying lengths and well developed cristae were located prominently near the nucleus in close association with rough endoplasmic reticulum (Fig.8).

On the other hand, ultrastructural alterations were observed in most hepatocytes of liver fish collected from polluted area. Some nuclei exhibited abnormal features and appeared indented and irregular in form with marginated nucleoli and heterochromatin (Fig.9). Other nuclei appeared pyknotic and almost damaged (Fig.10). The cytoplasm appeared nearly diffused and vacuolated in most examined hepatic cells (Fig.11). Meanwhile, the cell junctions between the cells showed dilatation of intercellular spaces (Fig.12). Most cytoplasmic organelle were clearly affected. The most common structural alterations were dilatation and fragmentation of the rough endoplasmic reticulum (rER) cisternae (Fig.11). The overall amount of rER was slightly reduced with dissociation of ribosome particles from their cisternae (Fig.13).

The mitochondria of most hepatocytes were found at varying stages of degeneration (Fig.14). They appeared swollen with incipient disintegration of crests and partial rupture of inner and outer membranes with appearance of laminated membranes and myelin-like figures (Fig.15).

Numerous secondary lysosomes, as well as multivesicular electron – dens – bodies occurred in the cytoplasm of many hepatic cells (Fig.16). There was also an increase in the amount of lipid while Golgi bodies were hypertrophied and appeared with dilated cisternae and large vacuoles (Fig.17).

Besides these cytoplasmic and nuclear alterations, different types of leucocytes could be noticed in many hepatocytes (Figs.: 18 &19).

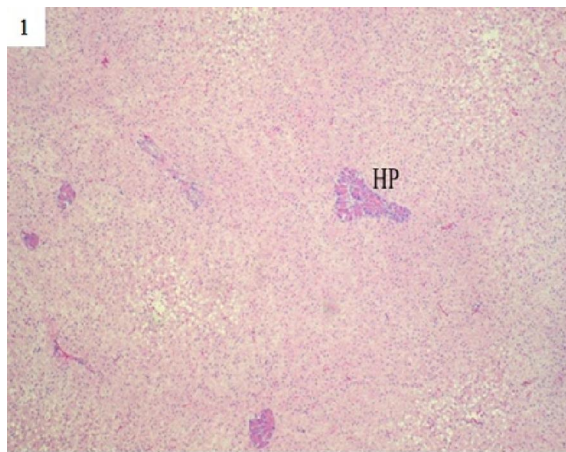


Fig.1: Light micrograph of liver section from fish collected from less polluted area (control area) revealed the typical appearance of liver lobules separated by the hepatopancreas (HP). H&E. X 100

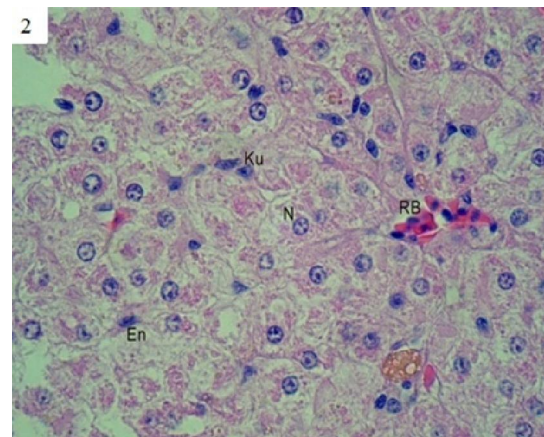


Fig. 2: Light micrograph of liver section from fish collected from less polluted area (control area) showing the hepatocytes with central spherical nucleus (N), endothelial cells (En) and Kupffer cells (Ku) line the luminal surface of the sinusoids which contain red blood cells (RB). H&E. X 1000

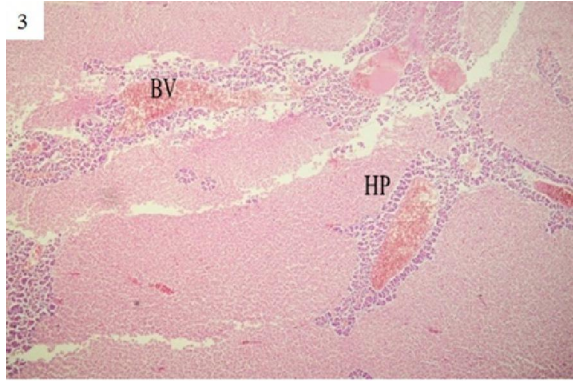


Fig. 3: Light micrograph of liver section from fish collected from polluted area. Note disruption of the normal arrangement of liver tissue and dilatation of blood vessels (BV). HP, hepatopancreas. H&E. X 100

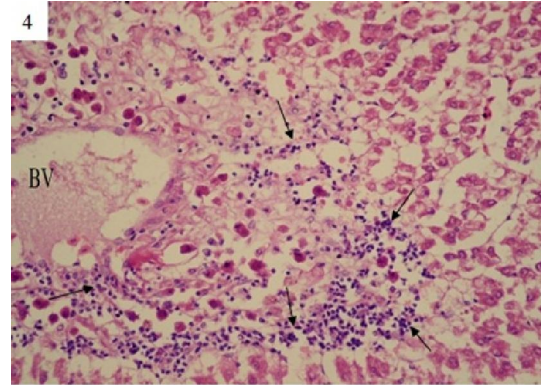


Fig. 4: Light micrograph of liver section from fish collected from polluted area. Leucocytes infiltration (arrows) is apparent along the hepatocytes and around the blood vessels (BV). H&E. X400

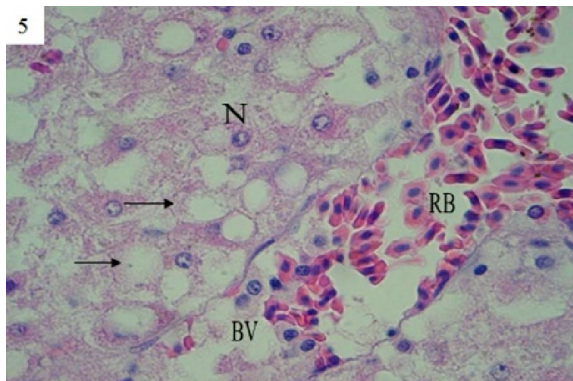


Fig. 5: Light micrograph of liver section from fish collected from polluted area. Vacuolization of most hepatocytes associated with lipid accumulation in the cytoplasm (arrows) and nuclear atrophy (N). Congestion of blood vessel (BV) which filled with red blood cells (RB). H&E. X 1000

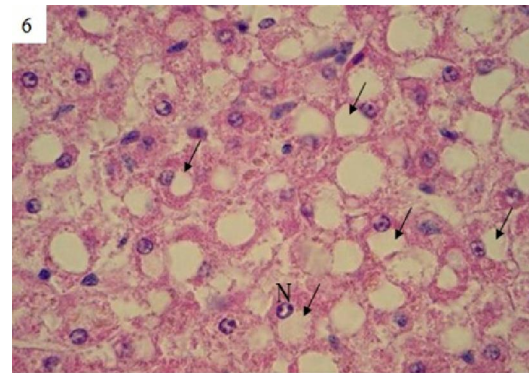


Fig. 6: Light micrograph of liver section from fish collected from polluted area showing clearly visible as white unstained areas within the hepatic cells (arrows) caused by lipid accumulation. Note the peripheral necrotic nuclei (N). H&E. X1000

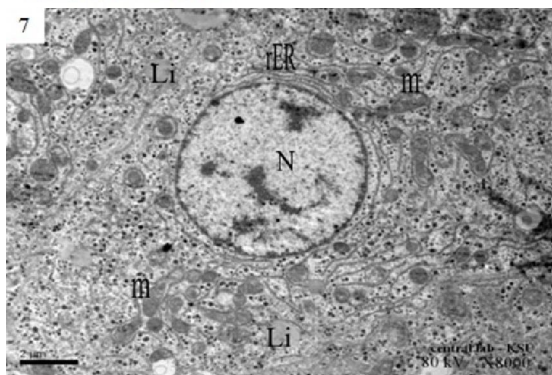


Fig. 7: Electron micrograph of liver fish collected from control area. The hepatocytes appeared with clear spherical nucleus (N) with little heterochromatin. Long parallel cisternae of rough endoplasmic reticulum (rER) and numerous mitochondria (m) surround the nucleus. Li, lipid droplets. X8000

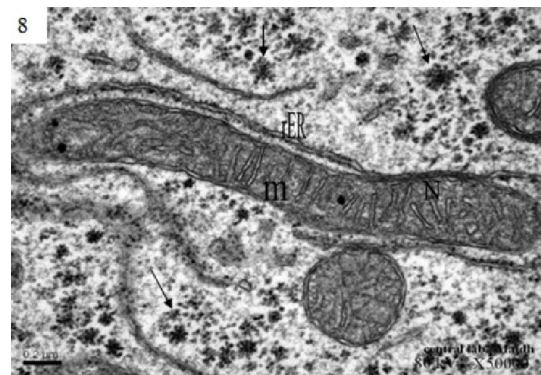


Fig. 8: Higher magnification of hepatocyte cytoplasm showing circular and elongated mitochondria (m), glycogen particles (arrows) and rough endoplasmic reticulum (rER). X50000

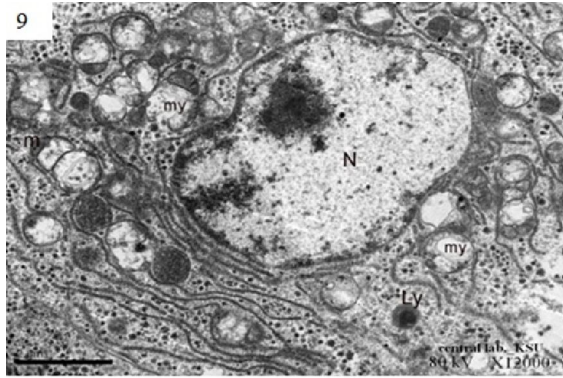


Fig.9: Electron micrograph of liver fish collected from polluted area. The hepatocyte nuclei (N) appeared irregular in form with margined nucleolus and heterochromatin. Degenerated mitochondria (m) and appearance of myelin-like figures (my) in the cytoplasm. Ly, lysosomes. X12000

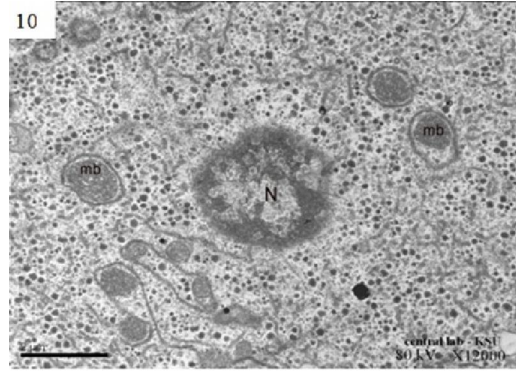


Fig.10: Electron micrograph of liver fish collected from polluted area. The nucleus (N) appeared almost damaged, with absence of most organelle around it. mb, multivesicular electron- dense bodies. X12000

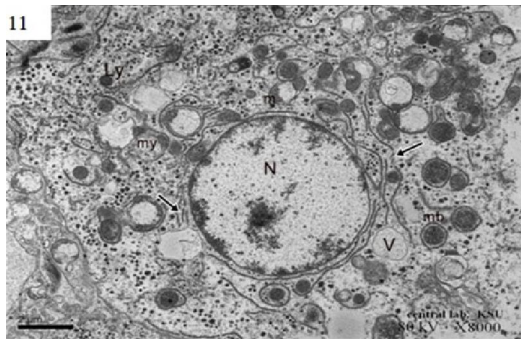


Fig.11: Electron micrograph of liver fish collected from polluted area showing diffusion and vacuolization of the cytoplasm (v). Note swollen of most mitochondria (m) and fragmentation of rough endoplasmic reticulum cisternae (arrows). X8000

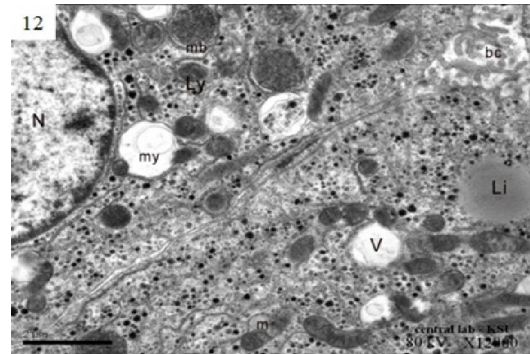


Fig.12: Electron micrograph of liver fish collected from polluted area. The cell junctions between the hepatocytes showed dilation of intercellular spaces. Bile canalicule (bc) appeared with numerous microvilli, multivesicular electron- dense bodies (mb) occurred in the cytoplasm. Li, lipid droplets; my, myelin like - figures; V, vacuoles. X12000

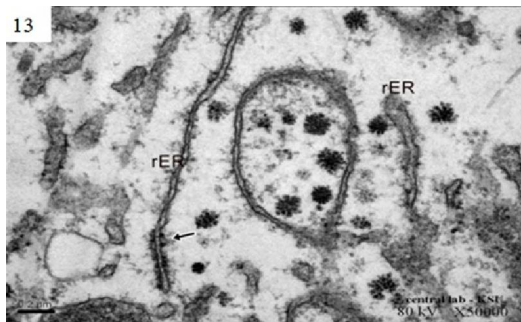


Fig.13: Electron micrograph of liver fish collected from polluted area. The rough endoplasmic reticula (rER) appeared broken into fragments with dissociation of ribosome particles from their cisternae (arrow). X50000

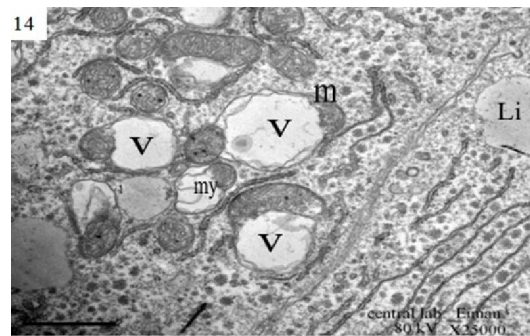


Fig.14: Electron micrograph of liver fish collected from polluted area showing variable degenerative stages of mitochondria (m). Li, lipid droplets; my, myelin like -figures; V, vacuoles. X25000

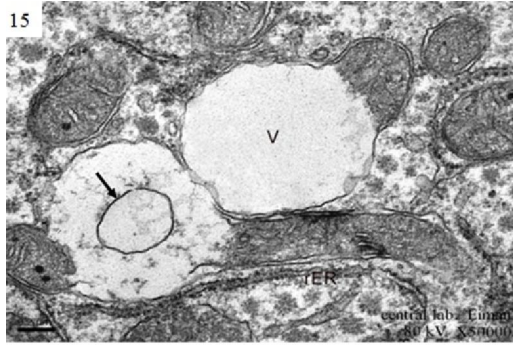


Fig.15: Higher magnification showing disintegration of mitochondrial cristae and appearance of laminated membranes (arrow).rER, rough endoplasmic reticulum; V, vacuoles. X50000

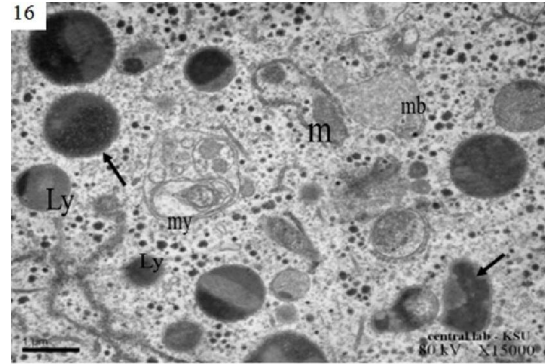


Fig.16: Electron micrograph of liver fish collected from polluted area. Numerous lysosomes (Ly) appeared throughout the cytoplasm. Arrows indicate secondary lysosomes. m, mitochondria; mb, multivesicular bodies; my, myelin like – figures. X15000

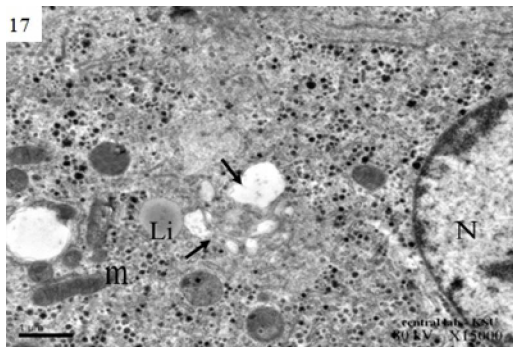


Fig. 17: Electron micrograph of liver fish collected from polluted area showing hypertrophied Golgi bodies with dilated cisternae and large vacuoles (arrows). Li, lipid droplets; m, mitochondria; N, nucleus. X10000

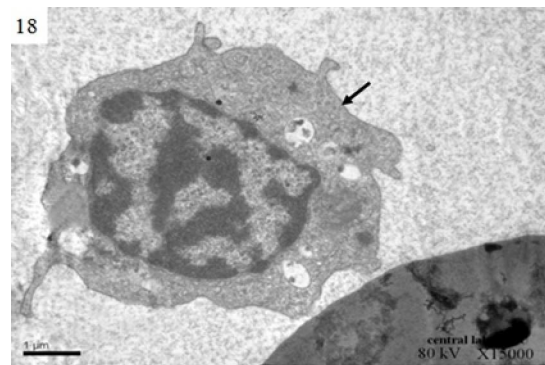


Fig. 18: Electron micrograph of liver fish collected from polluted area showing the presence of lymphocyte (arrow). X15000

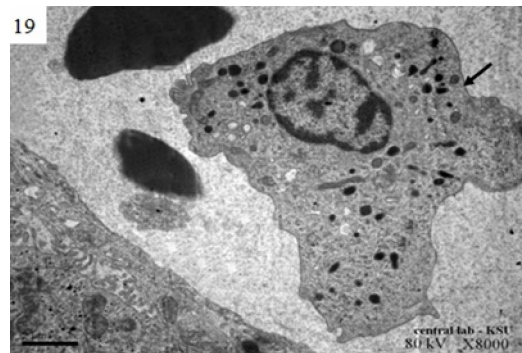


Fig.19: Electron micrograph of liver fish collected from polluted area showing the presence of monocyte. X 8000

4.Discussion:

Light microscopic observations revealed histopathological changes in liver fish collected from polluted area. These changes were swelling of hepatocytes with cloudy, granular cytoplasm and/ or vacuolization of hepatocytes associated with lipid accumulation. Congestion of blood vessels, leucocytes

infiltration and nuclear degeneration were also noticed. These degenerative changes may have been the result of various biochemical lesions. They are correspondent to several previous reports and they are not considered metal specific but generally associated with the response of hepatocytes to toxicants and heavy metals (Hinton and Lauren, 1990; Fanta *et al.*,

2003; Matos *et al.*, 2007; Van Dyk, 2007). Fahprathanchai *et al.* (2007) reported that vacuolization of hepatic cells are associated with the inhibition of protein synthesis, energy depletion, desegregation of microtubules, or shifts in substrate utilization.

Concerning the lipid accumulation observed in the hepatocytes, they were similarly noticed by Braunbeck *et al.* (1990) and Mela *et al.* (2007) in the cytoplasm of hepatocytes after exposure to various toxicants and pesticides. They speculated that these lipid vesicles might be the morphological expression of a blockage in the metabolism of hepatic triglycerides due to a defective synthesis of lipoproteins which are involved in the transport and mobilization of hepatic triglycerides to extra hepatic tissue.

Moreover, blood congestion was previously reported in sinusoids of liver fish exposed to heavy metals (Thophon *et al.*, 2003; Wangsongsak *et al.*, 2007). The increase in number of leucocytes could be suggested as an attempt of fish defense mechanism to overcome the negative impact of pollutants (Matsuyama and Lida, 1999; Coimbra *et al.*, 2007).

Stressor-associated alterations of *Oreochromis sp.* hepatocytes were found in the nucleus as well as in the cytoplasm. Necrotic and irregular nuclear profiles have been reported after fish exposed to several toxicants like pesticides (Jiraungkoorskul *et al.*, 2002; Ortiz *et al.*, 2003).

Examination of electron micrographs of liver sections of fishes collected from polluted area revealed various ultrastructural alterations. The most frequently alterations were nuclear deformation, fragmentation and dilatation of rough endoplasmic reticulum cisternae, degeneration and swelling of mitochondria, increase in number of lysosomes, multivesicular bodies and myelin like- figures.

Heterochromatine and nucleolus margination were recorded by many others in liver fish treated with different toxicants as Yang & Chen (2003) and Mela *et al.* (2007).

Dilatation and fragmentation of the rough endoplasmic reticulum observed during this study, have been previously observed in fish liver after chemical intoxication (Kohler, 1990; Holm *et al.*, 1993). Such modifications clearly reflect disturbance in protein synthesis (Rez, 1986) and initiation of hepatic detoxication mechanisms (Lemaire *et al.*, 1992; Hugla and Throne, 1999). Meanwhile, the dissociation of ribosomes from the surface of the rough endoplasmic cisternae could be the result of toxic disorganization of rough endoplasmic reticulum (Khangarot, 1992; Biagiante- Risbourg *et al.*, 1996).

Observations made on the mitochondria in the hepatocytes of fishes collected from polluted area

indicated that these organelle exhibited extreme vacuolization, lysis of cristae followed by fragmentation of the membranes and formation of myelin like- figures. Similar alterations have been described as reversible changes affecting liver ultrastructure in contaminated fish (Strmac and Braunbeck, 1999; Rangsayatorn *et al.*, 2004). Stevens *et al.* (2002) stated that the first ultrastructural evidence of sub-lethal cell damage is mulling of membrane- bound organelles, particularly endoplasmic reticulum and mitochondria. Mitochondria behave as osmometers, and the, mulling that develop after injury reflects a deregulation of mitochondrial membrane transport and entry of solutes and water into the mitochondrial matrix (Cheville, 1994).

An increase in lysosomal activity in hepatocytes of fishes collected from polluted area was marked in our investigations. These changes might be associated with the obligation of damaged cytoplasmic debris, which is a frequent phenomenon of liver injury (Yang and chem., 2003). Biagiante- Risbourg *et al.*, (1996) indicated that moderate increase in the number of autophagosomes might be viewed as a defense mechanism that allows the segregation and elimination of altered parts of the cytoplasm. Moreover, the accumulation of large number of myelin like- bodies is a common non-specific reaction to various chemical hepatotoxicants (Biagiante- Risbourg *et al.*, 1998). The appearance of multivesicular bodies in the electron micrographs could be related to the accumulation of heavy metals inside the cell (Mc Gee *et al.*, 1992; Sorour, 2001).

There was an increase in Golgi bodies activity, as shown by the dilated cisternae in some examined electron micrographs. This hypertrophy of the Golgi bodies demonstrated a general increase in the synthetic capacities of the hepatocytes (Braunbeck and Volki, 1993) and may be an adaptive response by the cell.

From the previous results it could be noticed that the liver tissue was slightly to moderately damaged and recuperation is still possible, if the water quality improved. Further Chemical analysis of Wadi Hanifah water is recommended to confirm a causal relationship between toxicants and tissue damage.

It is also indicated that histopathology and ultrastructural alterations are useful biomarkers for field evaluation, in particular in areas that are subject to a multiplicity of environmental variations. They discriminated between reference area and polluted area in Wadi Hanifah stream and did not suffer any interference from seasonal variations.

Moreover, the liver showed to be one of the organs affected by stressors to which the fish were subjected and the boliti fish *Oreochromis sp.* was

shown to be appropriate for in situ tests and environmental monitoring.

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