CATRINA (2014), 9 (1): 25 -31

Histopathogical Alterations in Fish Organs as Potential and Direct Biomarkers of Pollution

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ABSTRACT

In the present study, the histological structures of the liver, intestine, stomach and ovary of *Lates niloticus* from Lake Nasser (Kalabsha station) were studied during spring season, 2013. Several histopathological changes were observed in liver, intestine, stomach and ovary of the studied fish. In the liver, vacuolar degeneration, focal areas of necrosis, destruction of hepatoportal blood vessels and haemorrhage between the hepatocytes were observed. Besides, intravascular haemolysis and dilation and congestion were noticed in sinusoids. The histological changes in stomach and intestine included fused villi, the outer membrane of villi were broken, hemorrhage in the sub mucosa region and cells swelling. In the ovary, degenerative and atretic oocytes, proliferative changes in the granulosa of the oocytes, haemorrhage between the oocytes and intravascular haemolysis in some ovarian blood vessels were seen.
Keywords: Lake Nasser, *Latesniloticus*, histopathology, liver, intestine, stomach, ovary.

INTRODUCTION

Pollution of aquatic ecosystem by chemical used in industry and agriculture is increasing day by day, heavy metals, pesticides, antifouling agents, fertilizers and agricultural drainage from water bodies adversely affects on growth and survival of aquatic animals. Today there is no fresh or marine environment that is entirely free from pollution, due to the consistent rise in use of toxic chemicals Generally, metals enter the High Dam Lakes (Nasser and Nubia) from a variety of sources including: rocks and soils directly exposed to waters dead and decomposing vegetation and animal matter, wet and dry fallout of atmospheric particulate matter and human activities, including the discharge of various treated and untreated wastes to the water body(Abo El Ella *et al*., 2005).

Any change in the natural conditions of aquatic medium causes several adjustments in fish and metals are the main culprit for these undesirable changes in water quality **(**Garg *et al.*, 2009). Due to their toxicity, long persistence, bioaccumulative and no biodegradable properties in the food chain, metals constitute a core group of aquatic pollutants. In spite of their natural occurrence in the aquatic ecosystem, metals represent a major environmental problem of increasing concern, and their monitoring has received significant attention in both the field **(**Pandey *et al.*, 2003; Barnhoorn and van Vuren, 2004) and under laboratory conditions (Long *et al*., 2003; Osman *et al*., 2007).

Histopathology deals with the study of pathological changes induced in the microscopically structure of body tissue. Any alteration in normal structure of tissue indicates presence of disease or the effect of toxic substances like heavy metal and pesticides. Sprague (1973) described histopathology as important tool for evaluating the action of any toxicant at tissue level. Histopathology provides data concerning tissue damage. Histopathological alterations can be used as indicators for the effect of various anthropogenic pollutants on organisms and are a reflection of overall health of the entire population in the ecosystem.

These histopathological biomarkers are closely related to the other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism (Muhammad *et al.*, 2009). More than one tissue may be studied for assessment of the biological effects of a toxicant on localized portions of certain organs and also for assessment of subsequent derangements (degradations) in tissues or cells in other locations and this allows for diagnoses of the observed changes (Adeyemo, 2008). Mohamed (2008) investigated the concentration of some heavy metals (Fe, Zn, Cu, Pb, Cd and Co) in water and liver, gills, intestine, testis, heart and muscle of Nile tilapia (*Oreochromis niloticus)* and Nile perch (*Lates niloticus)* obtained from four khors (El-Ramla, Kalabsha, Korosko and Toushka) of Lake Nasser.

The intestine and liver are the most important organs in digestion and absorption of nutrients from food, and therefore monitoring of these organs is considered necessary (Takashima and Hibiya, 1982; Roberts, 1989). Histological alterations have been reported in the intestine of fish as a result of exposure to different toxicants (Cengiz *et al.*, 2001; Cengiz and Unlu, 2006; Giari *et al*., 2007, 2008). Stomach assumes different shapes according to the availability of space in the body cavities of different fishes. Stomach of teleosts presents a variety of different shapes. An acidic gastric juice is secreted by the mucosa of the stomach in carnivore's fish.

So that stomach is important organ in the digestive system of teleosts fishes. So that histopathological study of stomach helpful in assessment of health status as well as metabolism of fish (Bais and Lokhande, 2012).

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Histopathological changes were seen in the ovary of *Puntius conchonius* exposed to zinc (Kumar and Pant, 1984), in the ovary of *Oreochromis mossambicus* exposed to malathion (Shukla*et al*, 1934), in the gonads of winter flounder and cod infected with the hemoflagellate *Trypanosoma murmanensis* and then exposed to Venezuelan crude oil (Khan, 1987), in the ovary of *Oreochromis niloticus* infected by Streptococcus sp. (Chang and Plumb, 1996) and in the gonads of *Tilapia zillii* exposed to phenol (Mohamed, 2001a). In the present work we describe a case of a free living Nile perch *Lates niloticus* from Kalabsha station in Lake Nasser. Histopathological studies on fish are a noteworthy and promising field to understand the structural organization that occurs in the organs due to pollutants in the environment. Water quality characteristics influence histopathological manifestations of toxic effects (Galat *et al*., 1985).

MATERIALS AND METHODS

Study area

Lake Nasser khors are more productive than the main channel of the lake due to the fact that the mean value of chlorophyll concentration in khors is 30 - 40% higher than that outside of the khors. These provide the most important habitat for fish breeding and feeding.

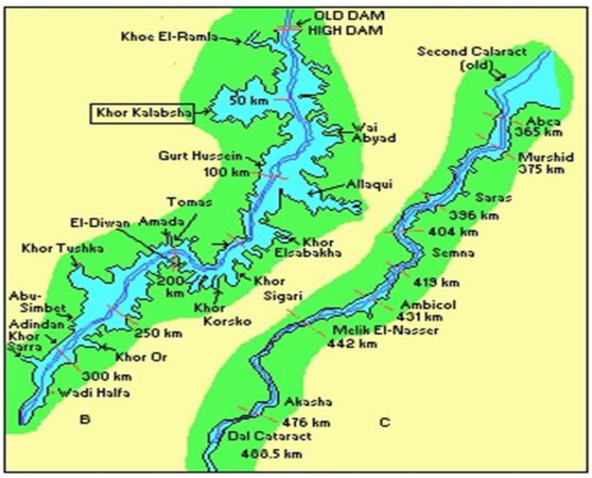


Figure (1): Map of Lake Nasser showing study area at Kalabsha station.

The selected location Kalabsha (Fig. 1) is considered as one of the most important fishery stations in Lake Nasser. The pelagic zone in Kalabsha, has a highest water transparency, conductivity and bicarbonate, which preferred by the fish *Tritrodontus fahaka* and dominated by the fish *Oreochromis niloticus*, *Sarotherodon galilaeus*, Lates niloticus and *Angola dentex*. *Oreochromis niloticus* dominated fish communities in the littoral zone of all locations. In contrast, large size Nile perch (*L. niloticus*) of high total weight are found close to locations forming open-structure vegetation dominated by Potamogetons. shweinfurthii, such as Kalabsha and Garf Hussin at the northern sector of Lake Nasser. This structure of submerged vegetation is preffered by *L. niloticus* and increases their chances of capturing smaller fish of medium weight (*O. niloticus*) which dominate these locations. Dense vegetation slow down fish movement and their foraging efficiency (Killgore *et al*., 1991).

Histopathological studies

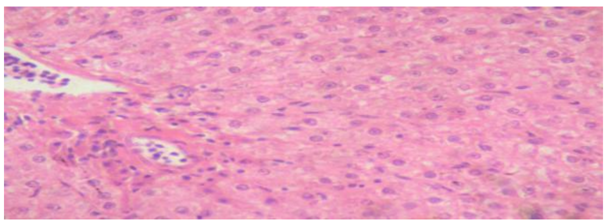
Twenty Samples of fish *Lates niloticus* were collected from khor Kalabsha. The fish measured about 22.7 to 30.2m in total length and 205.9 - 379.1g in total weight was collected during spring season 2013.

Fish were dissected and pieces of liver, intestine, stomach and ovary were immediately isolated and fixed in Bouin´s fluid for 24-48 hrs. After fixation, the tissues were washed in 70% ethyl alcohol to get rid of excess fixative and then dehydrated through ascending grades of ethyl alcohol (70% 1 hr, 80% 1 hr, 90% 1 hr and 100% changed twice during 1 hr). The specimens were cleared in xylene for 15-20 min and infiltrated with and embedded in paraffin wax. The paraffin wax block was sectioned at the thickness 4-6 *xm.* Sections were mounted on clear glass slides and were stained with Harris' haematoxylin and eosin

RESULTS

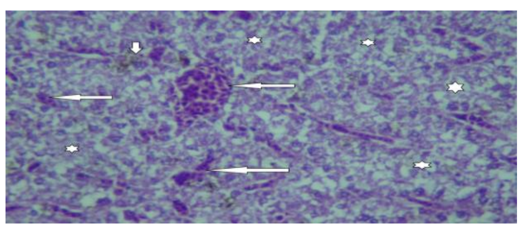
Liver

Figure 2 shows the normal histological structures of the liver.

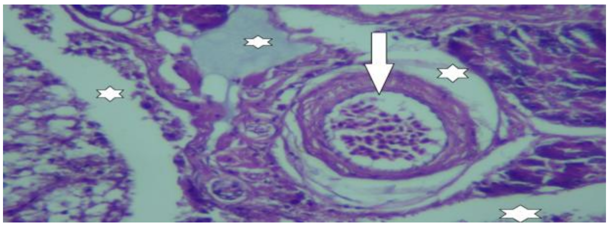


Figure(2): Liver of fish showing the normal structure. H&E(X100).

Liver showed vacuolar degeneration of the hepatocytes in addition to congestion of the central vein and sinusoid, hemorrhage between heptocyte sand edema (Figs.3 and 4).

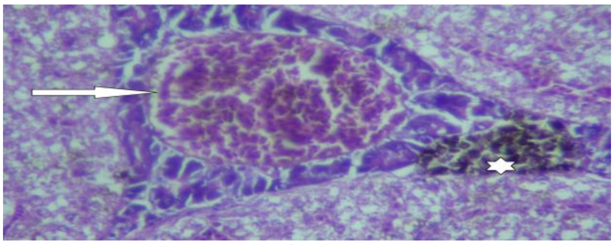


Figure(3): Section in liver, of *L. niloticus* showing, congestion between hepatocytes (long arrows), focal area of necrosis (stars) Heamosidrine (short arrows). H&E (X 100).



Figure(4): Section in liver, of *L. niloticus* Showing congested vein (arrow) and edema (stars). H&E (X 400).

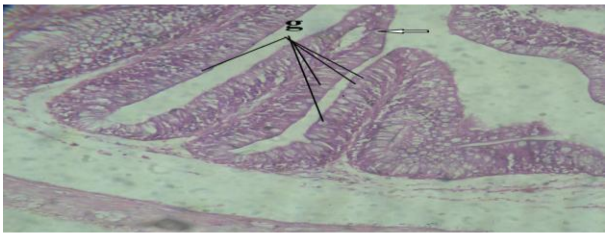
Liver exhibited severe diffuse vacuolar degeneration of hepatocytes and necrosis of some hepatocytes, there was thrombosis in hepatoportal blood vessels and melano macrophage centre "MMC" closed to it (Fig. 5).



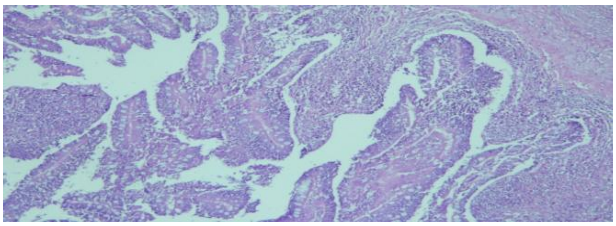
Figure(5): Section in liver, of *L. niloticus* showing thrombosis in hepatoportal blood vessels (arrow) and melano macrophage centre(MMC) closed to it (star). H&E(X400).

Intestine

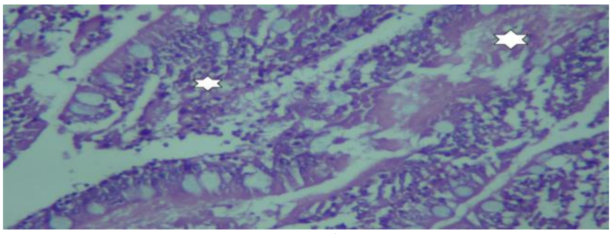
Figure 6 shows the normal histological structures of the intestine. The pathological findings in the intestine of *L. niloticus* included atrophy in the muscularis, severe degenerative and necrotic changes in the intestinal mucosa and submucosa with necrotized cells aggregated in the intestinal lumen (Fig. 7), haemorrhage in the submucosa and aggregations of inflammatory cells in the mucosa and submucosa with muscle fibers were seen. oedema between them and atrophy in the submucosa. Dilation was observed in the blood vessels of serosa. Moreover hypertrophide goblet cells in the intestine of fish and oedema was observed (Fig.8).



Figure(6): Intestine of fish showing normal structure, villi (arrow) and goblet cells (g). (X100).



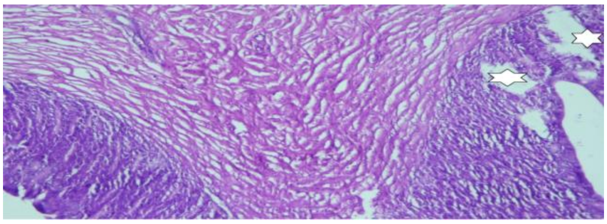
Figure(7): Section in intestine of *L. niloticus* showing atrophy of goblet cells (X100).



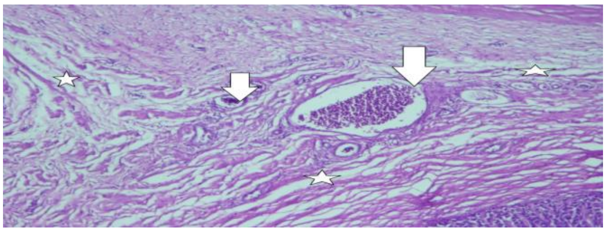
Figure(8): Section in intestine of *L. niloticus* showing sever necrotic changes in intestinal mucosa degeneration of epithelial cells (stars).

Stomach

Fusion of stomach villi, focal areas of necrosis in mucosa layer (Fig. 9), and oedema in submucosa, villi region and haemorrhage in the submucosa and aggregations of inflammatory cells in the mucosa and submucosa with muscle fibers were seen (Fig. 10).



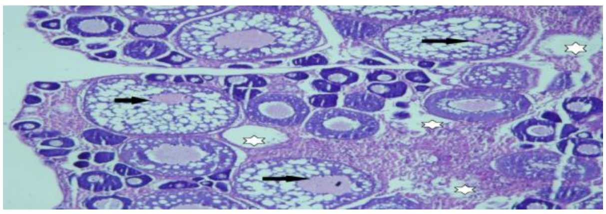
Figure(9): Section in stomach of *L. Niloticus* showing oedema and focal area of necrosis in mucosa (stars). (X 100**).**



Figure(10): Section in stomach of *L. niloticus* showing congested veins in submucosa (arrow) and oedema (stars**).** (X 200**).**

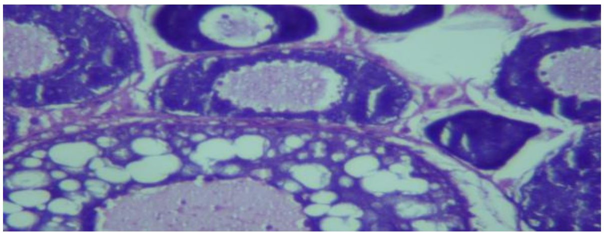
Ovary

The ovary of *L. niloticus* showed degenerative changes (Artesia) in some oocytes (Fig. 11) and proliferative changes in the granulosa of some oocytes, resulting sometimes in adhesion of the cellular coat of the oocytes (Fig. 12). Besides, some oocytes collapsed and became abnormally irregular in shape (Figs. 13 and 14). Moreover, haemorrhage was seen between the oocytes (Fig. 14).

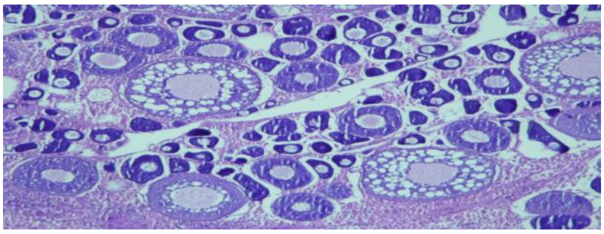


Figure(11): Section in the ovary showing disintegrated nuclei(arrows), large fat vacuoles filled large oocytes and spent oocytes (stars). (X40).

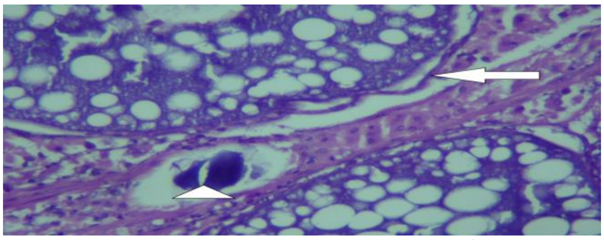
In addition, separation of the follicular layers from the oocytes was observed (Fig. 15). The predominant cell type being previtellogenic oocytes contain large fat vacuoles which fill all oocytes and the nucleus was disintegrated. Most of the oocytes exhibited signs of disrupted yolk accumulation and increased atretic oocytes.



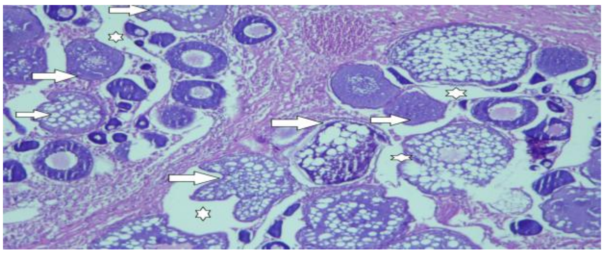
Figure(12): Section in the ovary showing large fat vacuoles in oocyt and spent oocytes. (X100).



Figure(13): Section in the ovary showing atretic young oocytes, large fat vacuoles filled large oocytes and abundant vitellogenic fluid. (X40).



Figure(14): Section in the ovary showing large fat vacuoles in oocyt and degenerate zona radiate (arrow), absorbed young oocyte (head arrow) (X100).



Figure(15): The ovary showing atretic oocytes (arrows) and edema (stars). (X40).

DISCUSSION

Result of the present study clearly revealed that fish *Lets niloticus* manifest histopatholoigcal changes in liver. It is possible that these alterations in liver could be a direct result of the heavy metals, pesticides, fertilizers and salts, which are entered to the river with drainage water (Reddy and Baghel, 2012). The histopatholoigcal alterations studied in *L. niloticus* are in agreement with those observed by many investigators who have studied the effects of different pollutants (Depledge *et al*, 1993; Decaprio, 1997; Adams; 2002). These changes may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals (NRC, 1997). The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in parenchymal cells and the rate of their release into the circulatory system.

The cellular degeneration observed in the liver of the studied fish may be due to the vascular dilation and intravascular haemolysis observed in the blood vessels with subsequent stasis of blood (Mohamed, 2001b). The necrosis observed in the liver of the fish may be attributed to the destruction of the hepatoportal blood vessels which causes invasive infiltration of leucocytes and detrimental focal necrosis resulting in the complete dissolution of the hepatocytes (Ram and Singh, 1988).The cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular haemolysis observed in the blood vessels with subsequent stasis of blood (Mohamed, 2001). Many authors have reported similar histopathological alterations in the liver of fish exposed to metals (Athikesavan *et al.*, 2006; Triebskorn *et al.*, 2007; Van Dyk *et al.*, 2007).

Uptake of metals occurs mainly through gills but may also occur *via* intestinal epithelium. Histopathological gastrointestinal alterations including damage to the mucosal lining, loss of villi and desquamated epithelial cells of gastric mucosa have also been observed by earlier workers. Hence causes irritation and destruction of the mucous membrane of the intestine, thereby hampering absorption (Anderson *et al*, 1992). It was suggested that lead increases the formation of gastric ulcers by interfering with the oxidative metabolism in the stomach that increased the incidence of gastric ulcer (Olaleye *et al.*, 2007).

Histological examination revealed great variability in the intestinal lesions severity existed among most fish caught including focal deformation with necrosis of mucosal epithelial layer of villi, enlargement of the intestinal villi due to vacuolar degeneration or hyaline degeneration, lymphocytic infiltration, dissociation and reduction of muscular bundles. In some instance, the columnar epithelial layer in between the intestine villi carry hair like extensions and lymphatic sinuses and heavily cellular infiltration were detected in the intestinal tissue underlying. This may be representing important link in the intestinal immune system which catch antigen and pass it into macrophage and lymphocyte underlying it to activate immune responses against antigen (Ali *et al.*, 2008). According to Bhatnagar *et al.*, (2007) the observed irritation and destruction of the mucosa membrane of the intestine, hampering absorption. The histopathological alterations were observed by many investigators on the effects of different toxicants on fish intestine. Epithelial degeneration, inflammatory cells infiltration in the submucosa as well as submucosal oedema were seen in the intestine of tilapia fish exposed to carbofuran. The implication of this is that lead causes an increase in the formation of free radicals, which, if not mopped up by free radical scavengers, will expose the stomach to inflammation and gastric mucosal damage. These adverse effects of lead as well as its inhibition ofen zyme activities might be the main inducer of the obtained intestinal histopathological damage of the exposed mollies fish (Dai *et al.*, 2009; Abdallah *et al*., 2010). The Intestine is the first organ which is come into contact with food borne contaminants (Braunbeck *et al.*, 1999). Mandal and Kulshrestha (1980) describe the lesion formation in villi of *Clarias batrachus* after exposure to sumi-thion. Histological analysis of Intestine tissue of *Channa striatus* and *Heteropneustes fossilis* inhabiting the polluted water showed degenerative changes in the serosa, mucosa and sub mucosal layers, necrosis, proliferation and desquamation of the superficial parts of the villi (Kumari and Kumar, 1997). Braunbeck *et al*., (1999) have also found that in the intestine, exposure of Endosulfan is associated with changes in the epithelial lining, which indicates disturbance of intestinal absorption. Cengiz *et al*., (2001) reported oedema, degeneration, accumulation of lymphocytes and eosinophils in the intestine of *Gambusia. affinis* exposed to delta-methtin (Cengiz and Unlu, 2006).

The present results are in agreement with those observed by many investigators about the effects of metals on fish intestine (Giari *et al*., 2007; Hanna *et al*., 2005). There was a destruction of epithelial layer and evidence of degeneration of oocyte and disorganization of nucleus of oocytes. This damage observed in the ovary might be due to the direct effects. The ovary of teleost fish has been regarded as the destination of vitellogenin, with the liver believed to be the primary place for synthesis of vitellogenin (Wahli, 1988). As a consequence, most efforts to date have focused on the deposition and accumulation of vitellogenin in oocytes. Similarly, the histopathological alterations observed in the gonads of the studied fish are in agreement with those observed in *Salmo gairdneri* exposed to cyanide (Sylvia *et al*., 1979), in *Puntius conchonius* exposed to zinc (Kumar and Pant, 1984), in *Oreochromis mossambicus* exposed to malathion (Shukla *et al*., 1984) and in *Barytelphusa guerini* exposed to Zn sulphate (Sarojini *et al*., 1990). Pre-ovulatory atretic follicles have been identified as a key histological feature that shows EDC (endocrine disturbance condition) effects on ovarian development and spawning (Leino *et al*., 2005). Estrogenic exposure leads to relative increase in atretic follicles and a decrease of vitellogenic oocytes, intensity depending on the level of exposure (Van den Belt *et al*., 2002). The vitellogenic fluid which was observed, described as a proteinaceous (vitellogen rich) fluid (Van der Ven *et al*., 2003) is likely to be caused by degeneration of mature vitellogenic oocytes due to chemical exposure.

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