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Full Length Research Paper

Effect of zinc toxicity on liver histology of Nile tilapia, *Oreochromis niloticus*

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In the present study the toxic effects of zinc (Zn) on the liver structure of Nile tilapia, *Oreochromis niloticus* were investigated. Additionally, we estimated whether Zn concentration and exposure period influence the degree and nature of histological changes in the liver of exposed fish. Two hundred and forty fish (the average weight was 24.30± 2.85 g) were distributed randomly in twenty four glass aquaria (80 L) with stocking density of 10 fish for each. The aquaria were divided into two groups: the first group was exposed to 2, 4 and 6 mg/L of $ZnCl_2$ which represent 0.25, 0.50 and 0.75 of Zn LC50 respectively for one week (short exposure period); while the second group was exposed to the same concentrations of $ZnCl_2$ with the same replicates for 4 weeks (long exposure period). In addition, control group were not exposed fish to $ZnCl_2$ (0.00 LC50) were stocked with three replicates for each period. Fish mortality was significantly increased in a dose-dependent manner, without any significant effect of the exposure duration. Histological changes in the liver included hepatocyte degeneration, nuclear pycnosis, cellular swelling, and congestion of blood vessels. There was a marked difference in these changes among the different treatments of Zn concentrations in addition to the intensity of histological changes were however influenced by the extent of exposure period.

Key words: Nile tilapia, Oreochromis niloticus, liver, histological changes, LC50 of Zinc.

INTRODUCTION

Heavy metals occur naturally in the environment and are found in varying levels in the ground and surface water. Anthropogenic activities do, however, cause an increased discharge of these metals into natural aquatic ecosystems. Sometimes, aquatic organisms are exposed to unnaturally high levels of these metals. Fish are relatively sensitive to changes in their surroundings environment. Fish health may therefore reflect and give a good indication of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance. Histological study appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the liver. Exposure to heavy metals may cause histological changes in the liver. Fish liver histology could therefore serve as a model for studying the interactions between environmental factors and hepatic structures and functions (Hinton and Lauren, 1993; Gernhofer et al., 2001). It has been noted that heavy metals had a negative impact on all relevant parameters and caused histo-pathological changes in fish. Zinc (Zn) is one of the most important trace elements in the body, and participates in the biological function of several proteins and enzymes (Maity et al., 2008). Despite being an essential trace element, Zn is toxic to most organisms above certain concentrations (Ho, 2004). Since the range-finding acute test is conducted to pinpoint exposure concentrations; the definitive acute test is firstly conducted to estimate LC50 of the chemical to which organisms are exposed (Rand, 2008). Therefore, heavy metals have been recognized as strong biological poisons because of their persistent nature, toxicity ,tendency to accumulate in organisms and undergo food chain amplification (Kamble and Muley, 2000; Dinodia et

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al., 2002). Among heavy metals, zinc is used in various forms which eventually find its way into the river or sea. Excessive zinc enters the environment as a result of human activities such as mining, purification of zinc, lead and cadmium ores, burning of coal and burning of waste. Although, small quantities of zinc are required for the normal development and metabolism (Srivastava and Sharma, 1996; Srivastava and Kaushik, 2001; Shukla et al., 2002), but if its level exceeds the physiological requirements, it can act as a toxicant. This results in general enfeeblement, retardation of growth and may bring about metabolic and pathological changes in various organs in fish (Ambrose et al.,1994; Sharma and Sharma,1994; Singh and Gaur, 1997). Accumulation of zinc in various organs of fish has been described by Gupta and Sharma (1994), Pandey et al. (1995) and Singh and Gaur (1997). The effect of two heavy metals, cadmium (Cd) and zinc (Zn), on the histology of the liver on the fresh water fish species Oreochromis mossambicus exposed to 5 and 10% concentrations of LC50 of Cd and Zn over both short and long term exposure was studied by Van Dyk et al. (2007).

In the present study, the effect of zinc, on the histological structure of liver in Nile tilapia, *Oreochromis niloticus* was investigated. The objective of the study was to determine the toxic effect of Zn on liver of this fish species, after exposing to 0.25, 0.50 and 0.75 of Zn LC50 (LC50 was previously determined as 8 mg/L for 96 h exposure period) over both short and long-term exposure periods, so as to assess the damage and get an insight in its functional consequences. Furthermore, short- term exposure periods may provide an indication of how long after the onset of exposure cellular damage will start to occur. Long-term exposure periods will then indicate whether the damage identified after the short-term exposure period, increased in intensity, decreased in intensity or remained the same throughout the exposure period.

MATERIALS AND METHODS

Experimental fish

Fingerlings of Nile tilapia O. niloticus were collected from the fish seed hatchery of King Abdulaziz City for Sciences and Technology (KACST) Deerab, Riyadh, Saudi Arabia. Fish were acclimated to laboratory conditions for two weeks before the experiments.

Estimation of LC50

To determine the 96 h LC50 of exposure, seventy fish were randomly distributed in seven small aquaria (40 L). The fish were exposed to various concentrations (2, 4, 6, 8, 10, 12 and 14 mg/L) of zinc chloride ($ZnCl_2$) which previously dissolved in distilled water and added to the aquaria. Fish were exposure to the above concentrations for 96 h. Mortality in each aquarium was recorded daily and removed. To find out the survival time in each concentration of $ZnCl_2$ observations were recorded, and then the LC50 value was calculated from the regression line drawn according to Finney (1964). The 96 h LC50 was 8 mg /L.

Experimental design

Two hundred and forty acclimated fish weighted 24.30± 2.85 g were divided into two groups; the first group was exposed to 2, 4 and 6 mg/L of $ZnCl_2$ which represent 0.25, 0.50 and 0.75 of Zn LC50 respectively for one week (short exposure period) in addition to 0.00 LC50 as a control. 80 L glass aquaria (100 × 50 × 40 cm) were used with three replicates for each concentration. The second group was exposed to the same concentrations of $ZnCl_2$ with the same replicates for 4 weeks (long exposure period). Fish were fed twice daily at a rate of 2% of body weight with 32% crude protein diet. The water quality parameters were monitored biweekly and were kept within the optimal ranges as described earlier by Ali et al. (2008).

Histological examination

Liver samples of the control and treated fish were fixed in 10% neutral- buffered formalin, and then the samples were processed for routine wax histological evaluation (dehydrated and embedded in paraffin). Sections of 5µm were done and stained with hematoxylin and eosin stains as described by Luna (1968) and Bernet et al. (1999).

RESULTS

Control fishes

The liver tissue generally exhibited a normal mural architecture with polygonal shaped hepatocytes, having a large spherical nucleolus and variable amount of dispersed and peripheral heterochromatin (Figures 1 and 6). Hepatocytes were located among blood capillaries called sinusoids forming cord-like structure known as hepatic cell cords. The lumen of sinusoids contained mainly erythrocytes. Kupffer cells were found to rest on the luminal surface of the sinusoids endothelium (Figures 1 and 6).

Short exposure period (acute exposure)

Light microscopic study of the fish liver exposed to Zn for one week showed several changes. The liver cells were degenerated; the normal architecture of liver was markedly disorganized. Hypertrophy of hepatocytes which had pycnotic nuclei was quite evident in liver of fish exposed to all metal concentrations from 2 to 6 mg/L of LC50 zinc chloride (Figures 2 to 5). In addition, dilated sinusoids with congestion were noticed ,the intra hepatic blood vessels were dilated and congested with blood cells in liver of fish exposed to metal concentrations 4 and 6 mg/L of LC50 zinc chloride (Figures 3 and 4). Moreover, a marked increase in numbers of Kupffer cells was observed in liver of fish exposed to metal concentration of 6 mg/L zinc chloride (Figures 4 and 5).

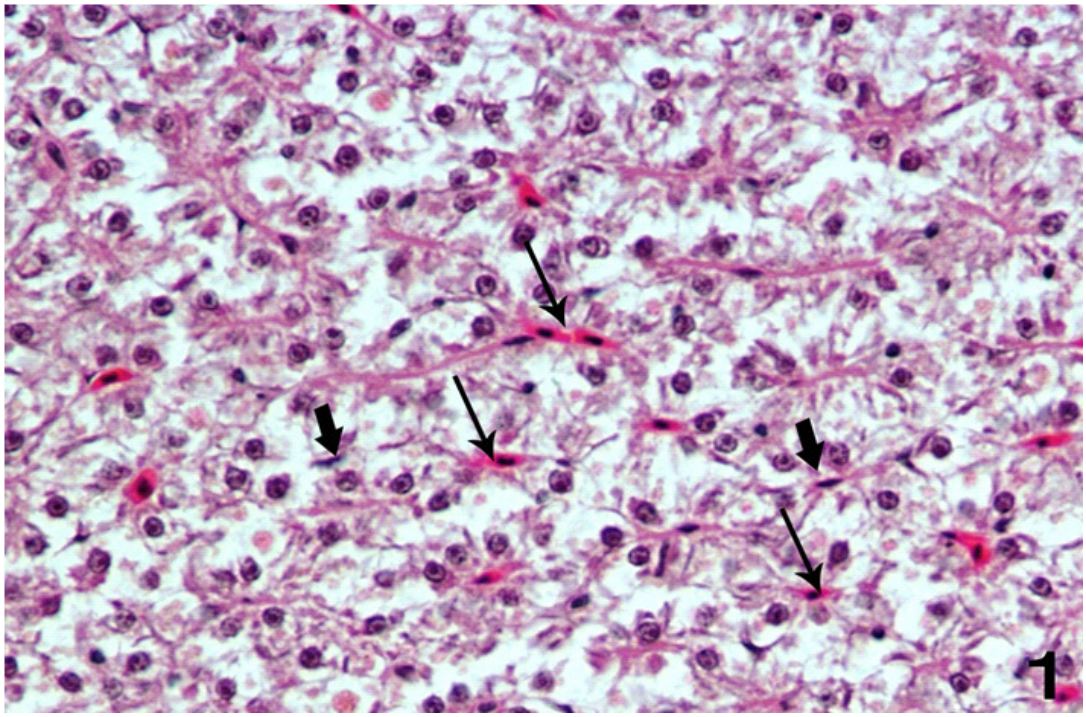

Figure1. Liver section micrography of *O. niloticus* in the control of first group, showing normal shaped hepatic cells, sinusoids (arrows), Kupffer cells (short arrows) hematoxylin and eosin × 400.

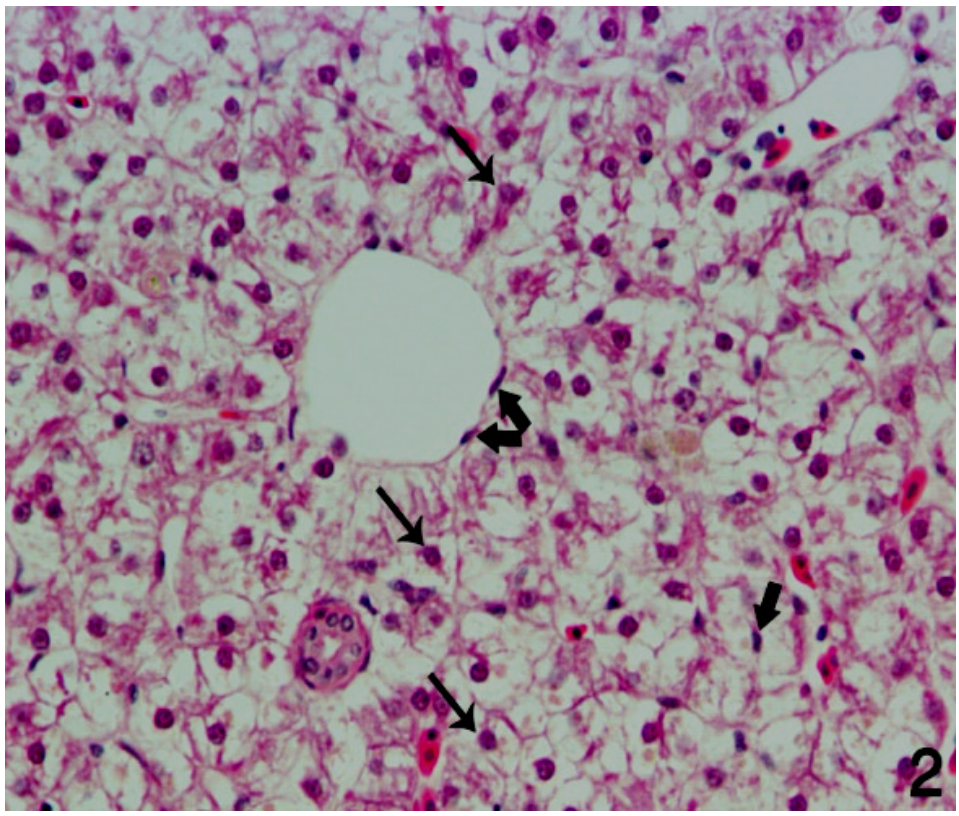

Figure 2. Liver of fish exposed to 2 mg/L zinc chloride showing pycnotic nuclei (arrows), increased Kupffer cells (short arrows) hematoxylin and

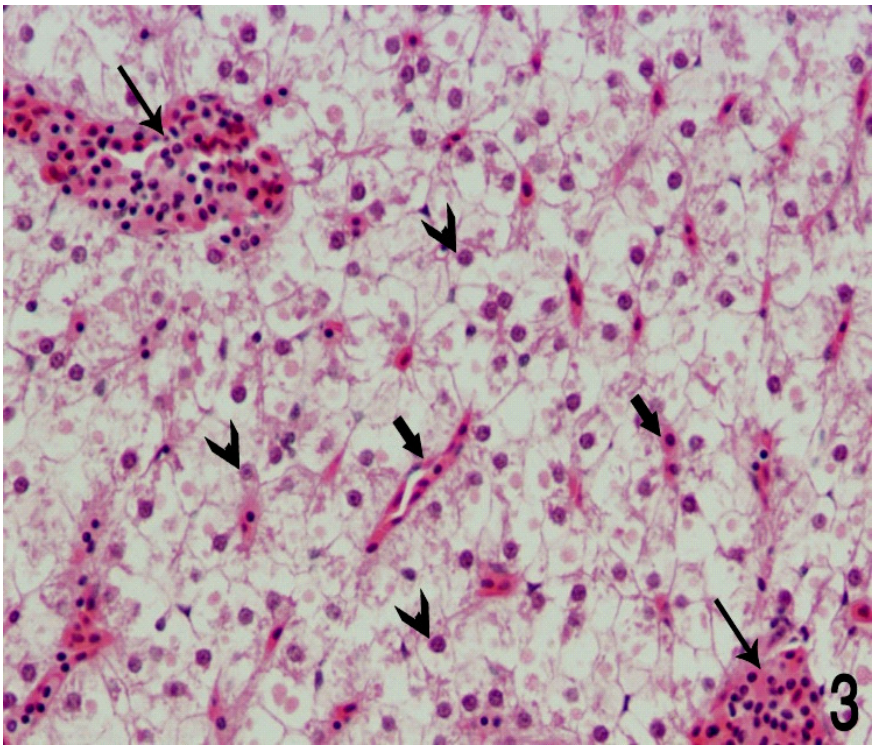

Figure 3. Liver of fish exposed to 4 mg/l zinc chloride showing pycnotic nuclei (arrow head), dilated sinusoids (short arrows), and congested blood vessel (arrows) hematoxylin and eosin × 400.

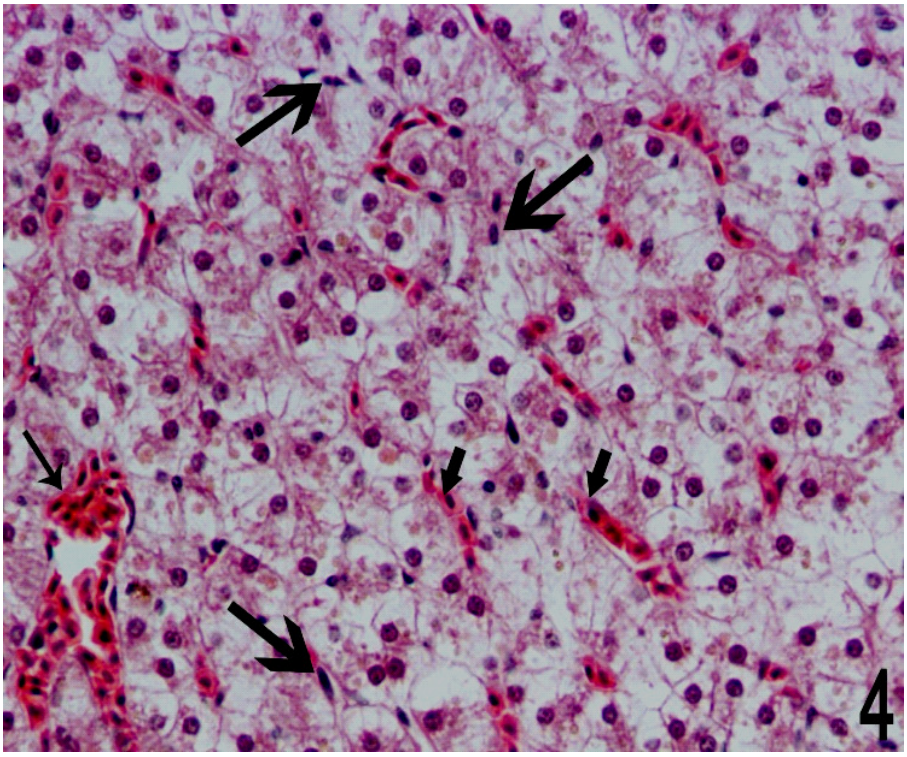

Figure 4. Liver of fish exposed to 6 mg/L zinc chloride showing dilated sinusoids (short arrows), increased Kupffer cells (big arrows) hematoxylin and eosin × 400.

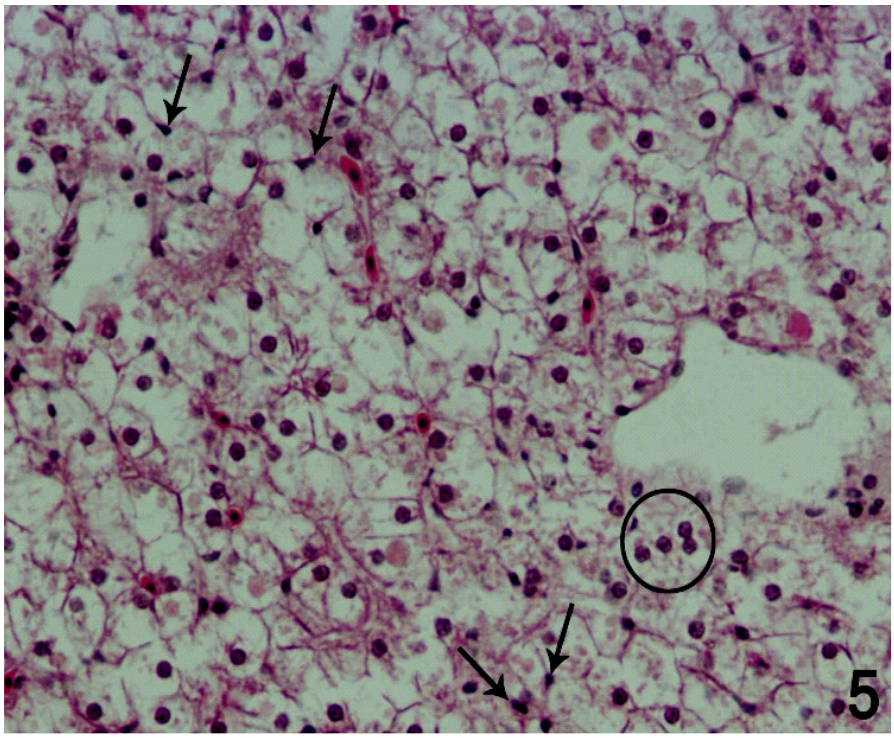

Figure 5. Liver of fish exposed to 6 mg/L zinc chloride showing increased pycnotic nuclei (round) increased Kupffer cells (arrows) hematoxylin and eosin × 400.

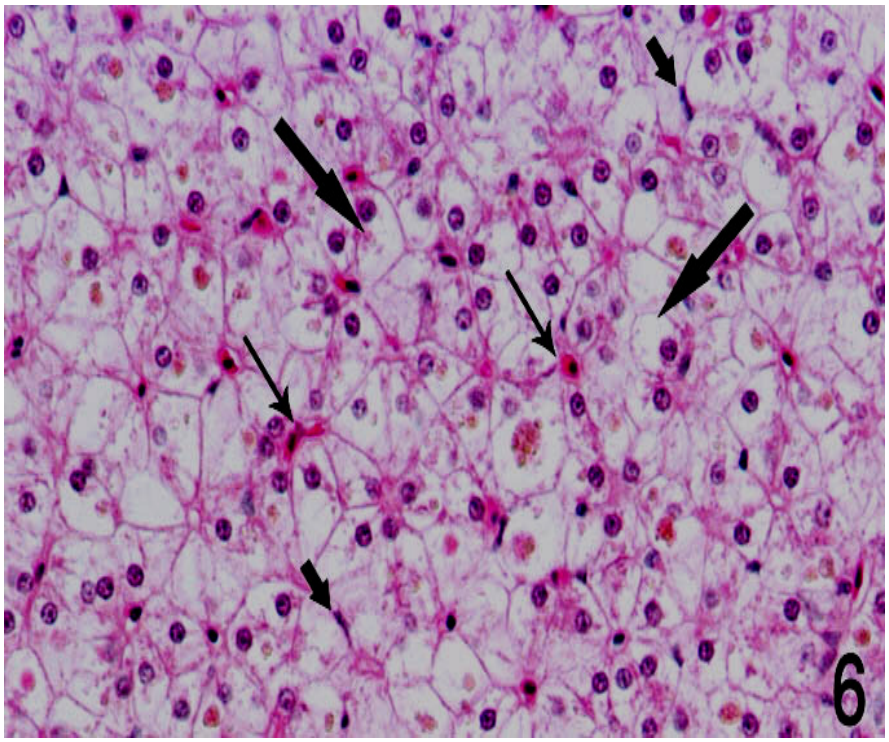

Figure 6. Liver section micrography of *O. niloticus* in the control of second group showing normal shaped hepatic cells (big bold arrows), sinusoids (arrows) shaped hepatic cells (big bold arrows), sinusoids (arrows) Kupffer cells (short bold arrows) hematoxylin and eosin ×400.

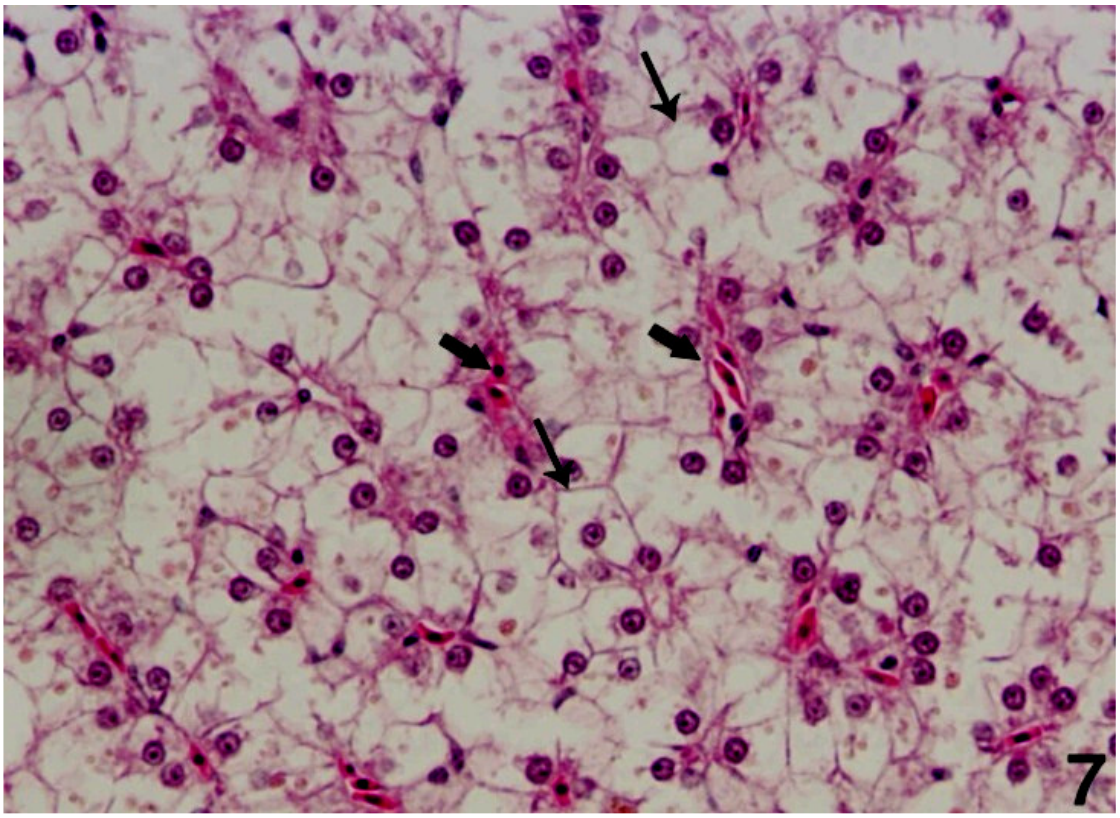

Figure 7. Liver of fish exposed to 2 mg/L zinc chloride showing hypertrophied hepatocytes (arrows), dilation of sinusoids (short arrows) hematoxylin and eosin × 400.

Long exposure period (chronic exposure)

Liver of fish exposed to effluent levels for 4 weeks revealed varying degrees of histopathological alteration due to damaging of cell structure with increase of hypertrophied hepatocytes in liver of fish exposed to all Zn concentrations (Figures 7 to 11). The nuclei displayed pleomorphism with peripheral nucleoli in liver of fish exposed to metal concentration of 4 mg/L zinc chloride (Figure 8). Furthermore, the hepatocytes exhibited focal necrosis resulting in complete disintegration of cellular components as evidenced by the presence of darkly stained eosinophilic debris in liver of fish exposed to metal concentration of 6 mg/l zinc chloride (Figure 11). In all metal concentrations (2 to 6 mg/L of LC50 zinc) there were extensive dilation of sinusoids with blood congestion (Figures 7 to 11). In some places hypertrophy and hyperplasia of bill duct cells and sign of blood vessels fibrosis were noted at concentration of 6 mg/L zinc (Figures 9 and10).

DISCUSSION

Zinc is generally regarded one of the less hazardous metals (Robinson, 1996) but frequently occurs in nature together with other metals of which cadmium is one of the most common (Dallas and Day, 1993). Previous studies have shown that, exposure to either cadmium or zinc caused histopathology of the kidney and epidermis (Somasundaram et al., 1985), the gills (Grobler et al., 1989), and the liver (Morsey and Protasowicki, 1990; Van Dyk et al., 2007). Also, Abdel-Tawwab et al. (2007) reported that environmental copper toxicity caused histopathology on liver, kidney and gills on Nile tilapia.

In this study, the accumulative effect of low and high concentrations (2 and 6 mg/L) of zinc on the histology of the liver was investigated. The results showed a histological response in exposed specimens with the most prevalent histological characteristics identified being congestion of blood vessels, and cellular swelling. However, these histological changes were not considered metal specific but changes generally associated with the response of hepatocytes to toxicants (Hinton and Laurén, 1990). Histological responses that have previously been reported in the liver of various fish species exposed to cadmium included the followings: atrophy andnecrosis of hepatic cells, decrease in the size of the nuclei and nucleoli, and in distinguishable cell membranes (Cyprinus carpio) (Morsey and Protasowicki, 1990); formation of macrophage granulomas (Carassius auratus) (Tafanelli and Summerfeldt, 1975); and increase in connective

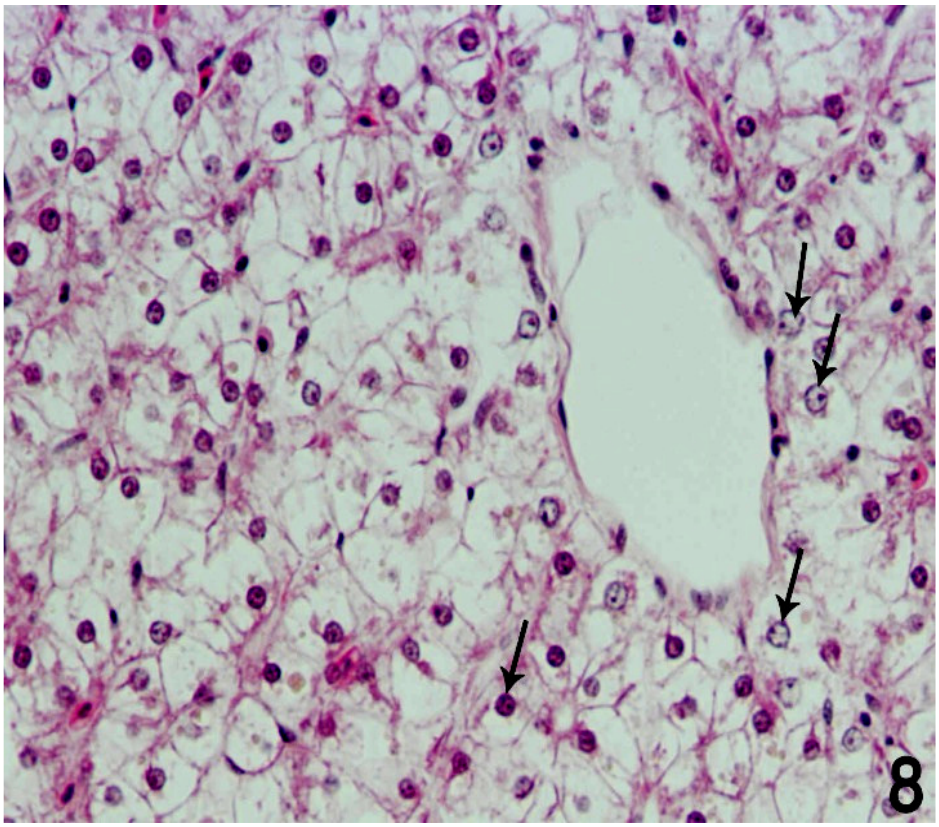

Figure 8. Liver of fish exposed to 4 mg/L zinc chloride showing pleomorphitic nuclei (arrows) hematoxylin and eosin × 400.

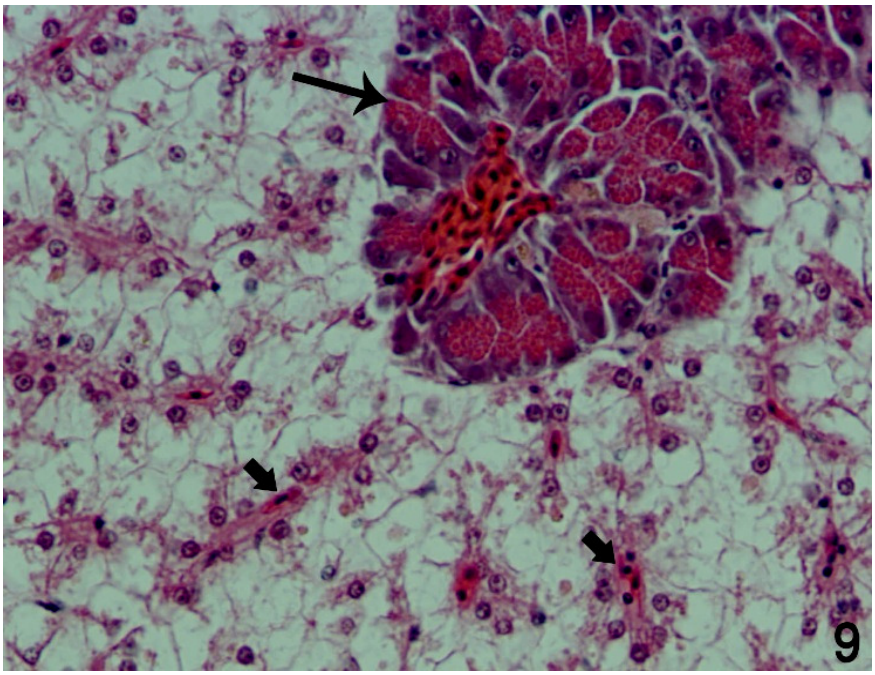

Figure 9. Liver of fish exposed to 6 mg/l zinc chloride showing hypertrophy and hyperplasia of bill duct cells (arrows), dilation of sinusoids (short arrows) hematoxylin and eosin × 400.

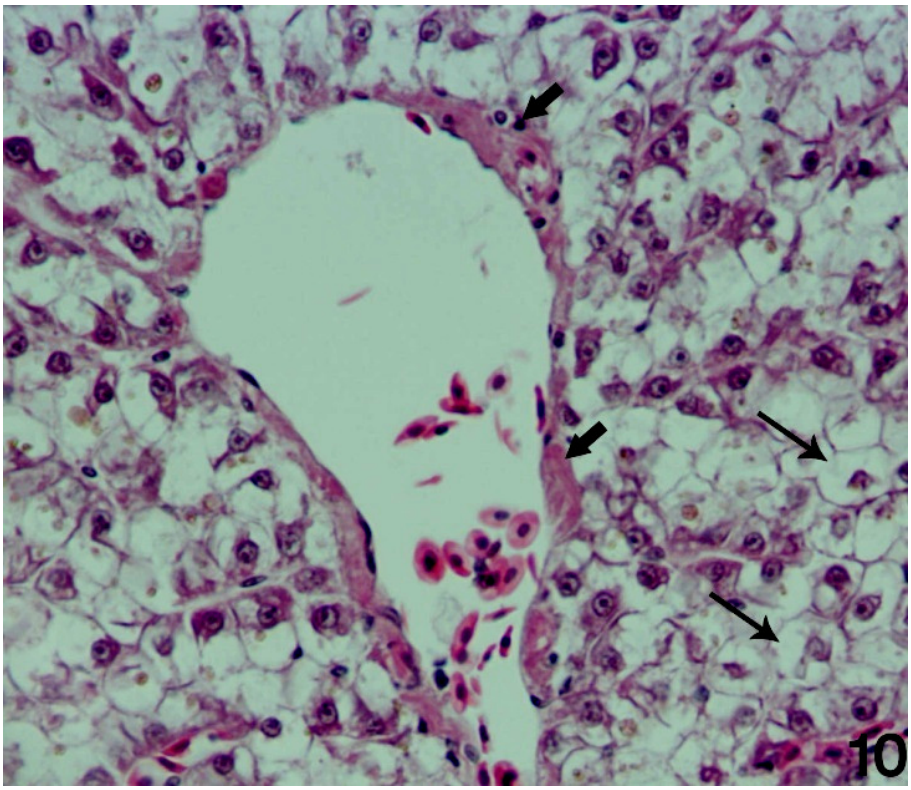

Figure 10. Liver of fish exposed to 6 mg/l zinc chloride showing hypertrophied hepatic cells (arrows), fibrosis of blood vessel (short arrows) hematoxylin and eosin × 400.

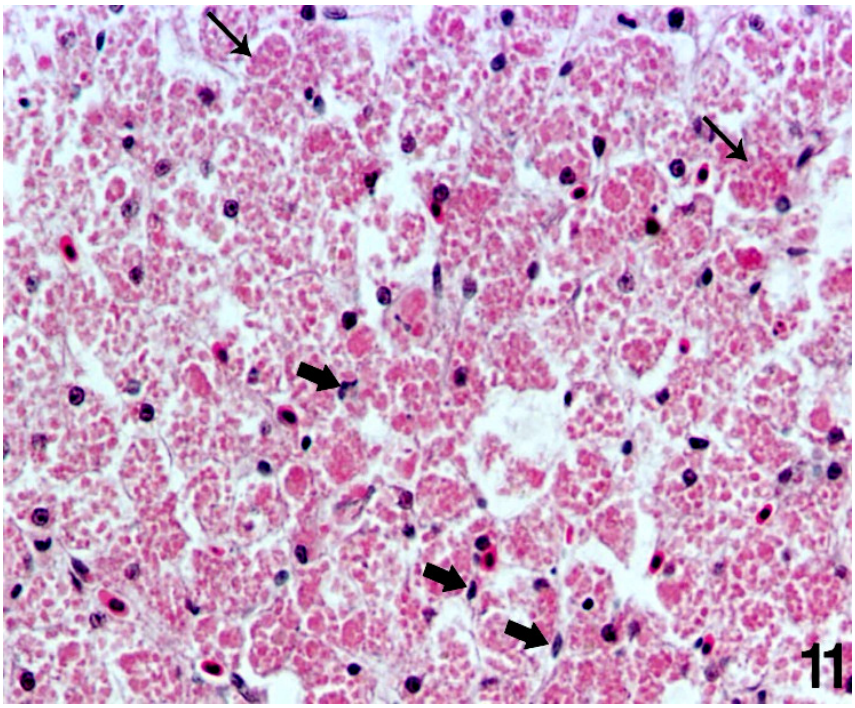

Figure 11. Liver of fish exposed to 6 mg/L zinc chloride showing eosinophilic debris (arrows), increased Kupffer cells (short arrows) hematoxylin and eosin × 400.

tissue and hepatocyte nuclei (*Halobatrachus didactylus*) (Gutierrez et al., 1978).

Histological changes in specimens exposed for one and four weeks to 2,4 and 6 mg/L, indicating a toxic response, including cellular swelling and congestion of blood vessels, suggesting that exposure of fish to different zinc concentrations poses great stress on the fish and elicits severe changes in their histology. The histological changes identified within the hepatocytes in this study may have been the result of various biochemical lesions. According to Cheville (1994), protein inclusion bodies are common in metal toxicities. Cellular swelling occurs either directly by denaturation of volume-regulating ATPases or indirectly by disruption of the cellular energy transfer processes required for ionic regulation (Hinton and Laurén, 1990).

Also, these results were in accordance with those obtained by VanDyk et al. (2007) who reported that congestion of blood vessels occurred in *O. mossambicus* after exposure to cadmium and zinc, especially with the portal veins. Also, a definitely higher level of congestion was observed in the 10% of LC50 exposure group compared to the 5% exposure group.

Senthil et al. (2008) noted that the liver appears to be one of the most important sites for Zn accumulation in Channel punctatus as it was also evident from some of the earlier findings of Seymore et al. (1994). The high levels of Zn in liver can be ascribed to the bindings of Zn to metallothionein (MT) which was at highest concentration in liver (Kendrick et al., 1992).

Severe necrosis, haemorrhage nuclear pyknosis and degeneration of hepatocytes were witnessed in the liver tissue *of Labeo rohita* exposed to zinc (Loganathan et al., 2006), and *Heteropneustes fossilis* subjected to thiodan toxicity (Narayan and Singh, 1991). Therefore, Van Dyk et al. (2007) noted that cellular swelling or hydropic degeneration was observed in a few livers of *O. mossambicus* exposed over 96 h in the 5% of LC50.

However, cells were swollen and the cytoplasm appeared cloudy and granular.

Fish mortality significantly increased in a dose-dependent manner, without any significant effect of the exposure duration. This was in agreement with Abdel-tawwab et al. (2011) who found that large increases in fish mortality are associated with the increases in exposure concentrations of Zn on Nile tilapia fingerlings.

Also, Shetty Akhila et al. (2007) reported that the determination of acute toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. Likewise, De Schamphelaere and Janssen (2004) reported that fish mortality might be a more sensitive endpoint for assessing effect of Zn exposure.

According to the results obtained, it was evident that the degree and nature of histological changes in the liver of exposed fish was affected by the exposure period. It can therefore be concluded that concentrations of zinc caused histological alterations in the livers of exposed fish, allows the liver of *O. niloticus* to be used as a biomarker of prior exposure to zinc. Histological changes were mainly observed in fish exposed over the short-term exposure periods while regenerative responses were noted in fish exposed over the long-term period.

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