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HISTOPATHOLOGICAL EFFECT OF ENDOSULFAN ON THE LIVER OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) (SILURIFORMES: CLARIIDAE)

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Abstract

Pesticides are transported into aquatic ecosystems, where they enter organisms via food webs and water contact. Endosulfan is a neurotoxic organochlorine insecticide that is used to keep insects at bay. This insecticide is particularly hazardous to fish, and its use disrupts the aquatic food. As the major organ of metabolism and detoxification, the fish liver comes into direct contact with hazardous compounds acquired by the body from polluted water. This causes histopathological changes in the liver, which serves as a useful tool for detecting the pollutants effects. In the present study, exposure of *Clarias gariepinus* to Endosulfan in various sublethal concentrations resulted in structural alterations like binucleated condition of liver, hemorrhage and rupture of blood vessel, nonnucleated condition of liver cells, multinucleated hepatocytes, presence of polynucleus hepatocytes, hepatocellular cytoplasmic vacuolization, cytoplasmic degeneration, nuclear vacuolization, irregular shaped hepatocytes, lesions, hydropic degeneration, leukocyte infiltration, nuclear pleomorphism, clumping of hepatocytes leading to swelling, destruction of hepatic cords, cytoplasm degradation, vascular congestion, cell cytoplasm destruction, infiltration of sinusoids, necrosis and fibrosis.

Keywords: *Clarias gariepinus*, Endosulfan, Histopathological, Insecticide, Liver, Neurotoxic, Organochlorides.

Introduction

The usage of synthetic organic pesticides started around 1940 and for the next few decades intensive use of these pesticides has resulted in maximum production of quality crops. But now, The increased usage of pesticides has become a matter of concern because of the damage caused by these pesticides in environment and health of many non-target organisms. Their incorrect application and improper handling is a matter of great risk. In India, 51% of food commodities are contaminated with pesticide residues (Gupta, 2004). Pesticides of the Organochloride group are the most prevalent toxicant in the aquatic environment. These exhibit a large variety of structures with much diverse chemical properties. Due to high atomic weight of chlorine, these compounds are found to be denser than water. Mammals have high chances of increased Organochlorine concentration as most of them occupy high trophic levels in food chains and food web, thus accumulating more of the toxic compounds in their body (Singh *et al.* 2016).

The pesticide Endosulfan belongs to the Organochloride group and can be absorbed in the body either through ingestion, inhalation and through skin. This pesticide is extremely toxic to fish and its use results in the disruption of the aquatic food chain. It is particularly toxic to juveniles (Dutta & Arends, 2003). Exposure of Organochlorine pesticides in water may damage liver, kidney, central nervous system and other organs. In humans, short term exposure causes headache, dizziness, nausea, vomiting, muscle weakness, slurred speech, confusion salivation and sweating. Direct or indirect entry of pesticides in the water can lead to mortality of fish, reduced fish productivity, and poison edible fish tissue. The toxic effects of the chemicals may be physiological, biochemical and pathological in nature. Pesticide toxicity in fish has been studied by several workers who have shown that at chronic levels, it causes diverse effects including oxidative damage, inhibition of AchE activity, histopathological changes as well as developmental changes, mutagenesis and carcinogenicity (Murthy *et al.* 2013).

Histopathological assessment of fish tissue allows for early warning signs of disease and detection of long-term injury in cells, tissues or organs (Reddy & Ravat, 2013). Monitoring histopathological changes can help to evaluate pathological side effects of water born pollutions. Such studies have provided information to bio monitoring plan designed for various aspects of environmental risk assessments (Kazempoor *et al.* 2015). Liver plays a key role in biochemical transformations of pollutants under detoxification process. The liver carries out essential body functions including regulation of metabolism, synthesis of plasma proteins, energy storage, storage of certain vitamins and trace metals and transformation and excretion of steroids and detoxification of pollutants (Salamat & Zarie, 2012). Fish liver comes in close contact with toxic chemicals absorbed by the body from polluted water as it is the primary organ of metabolism and detoxification. This results in histopathological changes of liver which serves as valuable tool for the detection of effect of these contaminants. The present study records the histopathological changes in the liver of the catfish *Clarias gariepinus* (Burchell, 1822).

Material and Methods

Young *Clarias gariepinus* fishes (12-13 gm and 10-11 cm long) were purchased from the market and acclimatized under laboratory conditions for 15 days and later treated with Endosulfan 35% EC (Endocel).

Chronic Toxicity measures long-term effects of exposure (typically 21-28 days). Sub lethal or safe level concentrations were derived from 96h LC 50 (APHA, 1992). In the present study the 96 h LC_{50} value of Endosulfan in *Clarias gariepinus*, was found to be 4.355µg/l with a 95% confidence limit ranging from 3.428µg/l (lower confidence limit) to 5.651µg/l (upper confidence limit). LC_{50} values of 24, 48 and 72 h of Endosulfan in *Clarias gariepinus* are 5.912µg/l, 5.459µg/l, 4.927µg/l respectively. Chi-square test showed that the calculated values

were less than the table values and is significant ($p<0.05$). Liver tissue from each group of fishes was dissected post- treatment, fixed in Bouin's and stained with Delafield's Haematoxylin – Eosin (Humason, 1962).

Results and Discussions

Exposure of *Clarias gariepinus* to sublethal concentrations of Endosulfan resulted in histopathological changes in liver tissue. Exposure of 0.215µg/l Endosulfan for 5 days showed cytoplasmic degeneration, lesions, focal area of mild necrosis, destruction of hepatic cords, nuclear pleomorphism, accumulation of pyknotic nuclei and cytoplasm vacuolation. Tissue exposed to 0.43µg/l Endosulfan for 5 days exhibited irregular shaped hepatocytes, leukocyte infiltration, cell hypertrophy and vacuolation, ruptured hepatocyte, accumulation of pyknotic nuclei and tissue damaged. Exposure of liver tissue for 5 days at 0.86µg/l concentration resulted in loss of primary structure of hepatocyte, presence of polynucleus hepatocyte, increased vacuolation in hepatocyte, sinusoid congestion and extreme hepatocyte vacuolation

Treatment for 10 days to 0.215µg/l concentration of Endosulfan exhibited binucleated condition of liver cell, hemorrhage and rupture of blood vessel, hydropic degeneration, nuclear pleomorphism, deformed central canal and vacuolar degeneration. Concentration of 0.43µg/l Endosulfan resulted in non-nucleated condition of liver cells and multinucleated hepatocyte, vacuolar degeneration, cytoplasm vacuolation, pyknotic nuclei and ruptured hepatocyte. 10 days exposure at 0.86µg/l concentration of Endosulfan showed changes like nuclear vacuolization, cell lesions, rupture of blood cells, rupture due to necrosis of hepatocyte, cell atrophy and visible oedema of the tissue.

15 days treatment of Endosulfan at 0.215µg/l concentration resulted in nuclear vacuolization, leukocyte infiltration, necrosis, cytoplasm degradation, ruptured hepatocyte and accumulation of pyknotic nuclei and binucleated hepatocyte. At 0.43µg/l concentration of Endosulfan, necrosis, cell atrophy, cytoplasmic degeneration, extreme vacuolation, destruction of hepatic cords, vascular congestion, cell cytoplasm destruction, destruction of hepatic cords, infiltration of sinusoids were observed. 15 days exposure at 0.86 µg/l concentrationof Endosulfan resulted in atrophy, necrosis and hepatocyte vacuolization, oedema of hepatocyte, damaged hepatocyte due to necrosis, focal area of necrosis showing extreme damage and destruction of hepatocyte. The results of these studies clearly indicate that sublethal concentration of Endosulfan has diverse effects on fish liver (Figs. 1-12).

The liver plays a key role in the metabolism and biochemical transformations of pollutants from the environment, which inevitably reflects on its integrity by creating lesions and other histopathological alterations of the liver parenchyma (Doherty *et al.* 2013). This organ is associated with the detoxification and paradoxically, due to its function, position and blood

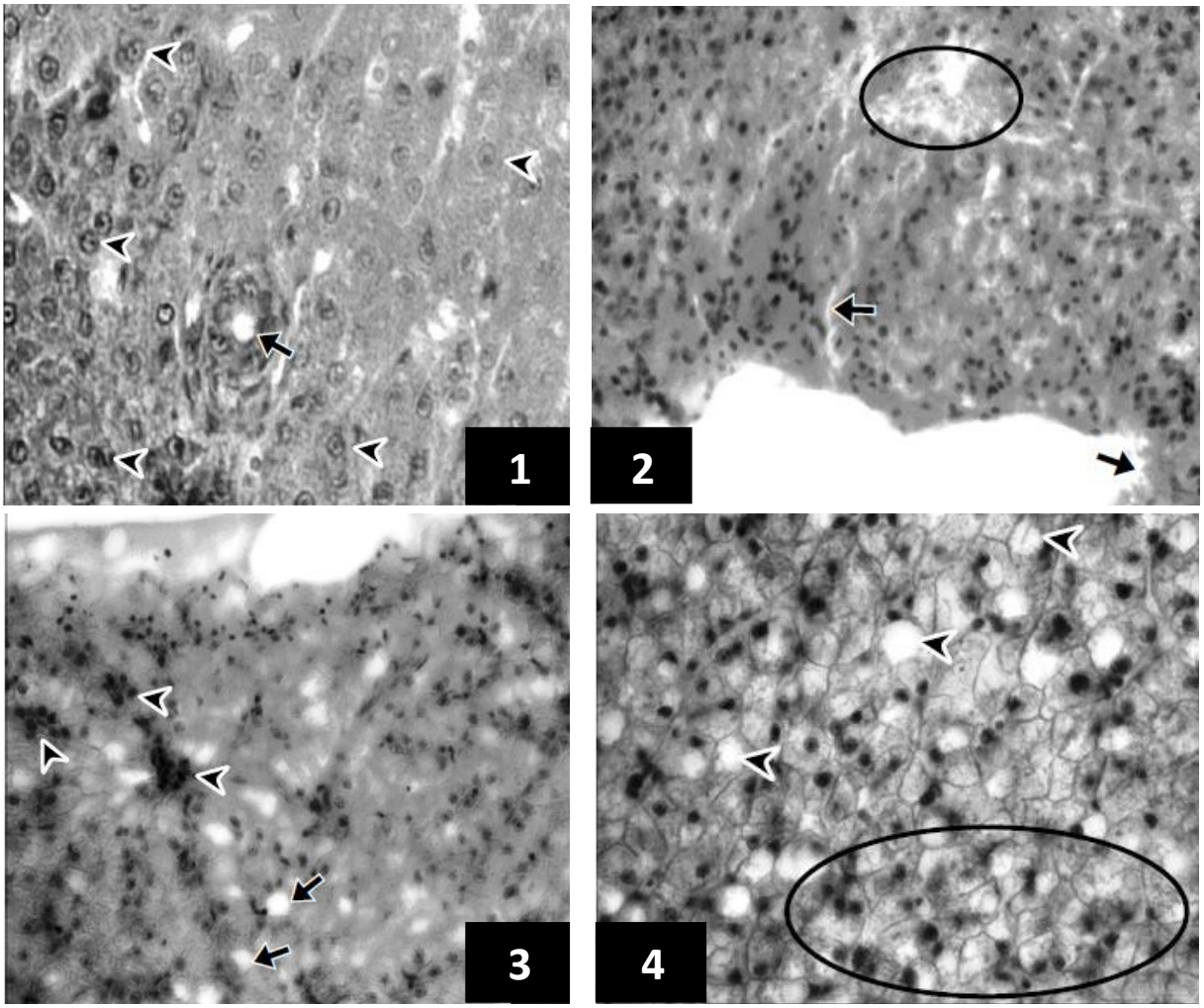
supply, also one of the foremost organs in fishes most affected by contaminants in water (Camargo & Martinez, 2007).

In the present study, exposure of *Clarias gariepinus* to Endosulfan in various sublethal concentrations resulted in structural alterations like binucleated condition of liver, hemorrhage and rupture of blood vessel, nonnucleated condition of liver cells, multinucleated hepatocytes, presence of polynucleus hepatocytes, hepatocellular cytoplasmic vacuolization, cytoplasmic degeneration, nuclear vacuolization, irregular shaped hepatocytes, lesions, hydropic degeneration, leukocyte infiltration, nuclear pleomorphism, clumping of hepatocytes leading to swelling, destruction of hepatic cords, cytoplasm degradation, vascular congestion, cell cytoplasm destruction, infiltration of sinusoids, necrosis and fibrosis. These pathological alterations are in agreement with the observations of many other reports. Sakr & Lail (2005) reported histopathological changes like cytoplasmic vacuolization, inflammatory responses and necrosis in *Clarias gariepinus* exposed to Fenvalerate.

Liu *et al*. (2006) described increased vacuolization of the hepatocytes as a signal of degenerative process that suggests metabolic damage, related to exposure to contaminated water. Degenerative changes like necrosis and hypertrophy were reported by Altinok & Capkin (2007) in *Onycorhynchus mykiss* exposed to Methiocarb. Velisek *et al*. (2009) noticed degeneration and vacuole formation in liver cells of *Oncorhynchus mykiss* exposed to Bifenthrin. Mohammed (2009) also reported vacuolar degeneration and necrosis in *Tilapia zillii* and *Solea vulgaris.* Butchiram *et al*. (2009) reported structural changes like atrophy, formation of vacuole, rupture of blood vessels and necrosis in liver tissue of *Channa punctatus* exposed to Alachlor. In *Labeo rohita*, hepatocellular necrosis and cytoplasmic vacuolization was reported with exposure of Endosulfan by Indirabai *et al.* (2010). Parikh *et al.* (2010) observed vacuolar degeneration and swelling of lymphocytes in *Oreocromis mossambicus* exposed to Dimethoate. Salim & Majeed (2014) also reported vacuolisation and enlargement of hepatocytes by the effect of different pesticides in *Cyprinus carpio.* According to Reethamma (2014) who studied the effect of Fluben Diamide on the juveniles of fish *Etroplus maculates,* the liver of the fish exposed to both low as well as high dose showed vacuolar degeneration, swelling in the hepatocytes with indistinguishable cellular outline. These changes are attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all type of toxins and chemicals and there is a temporal sequence of the events that starts with vacuolization, swelling and necrosis and similar situation is also found in the present study.

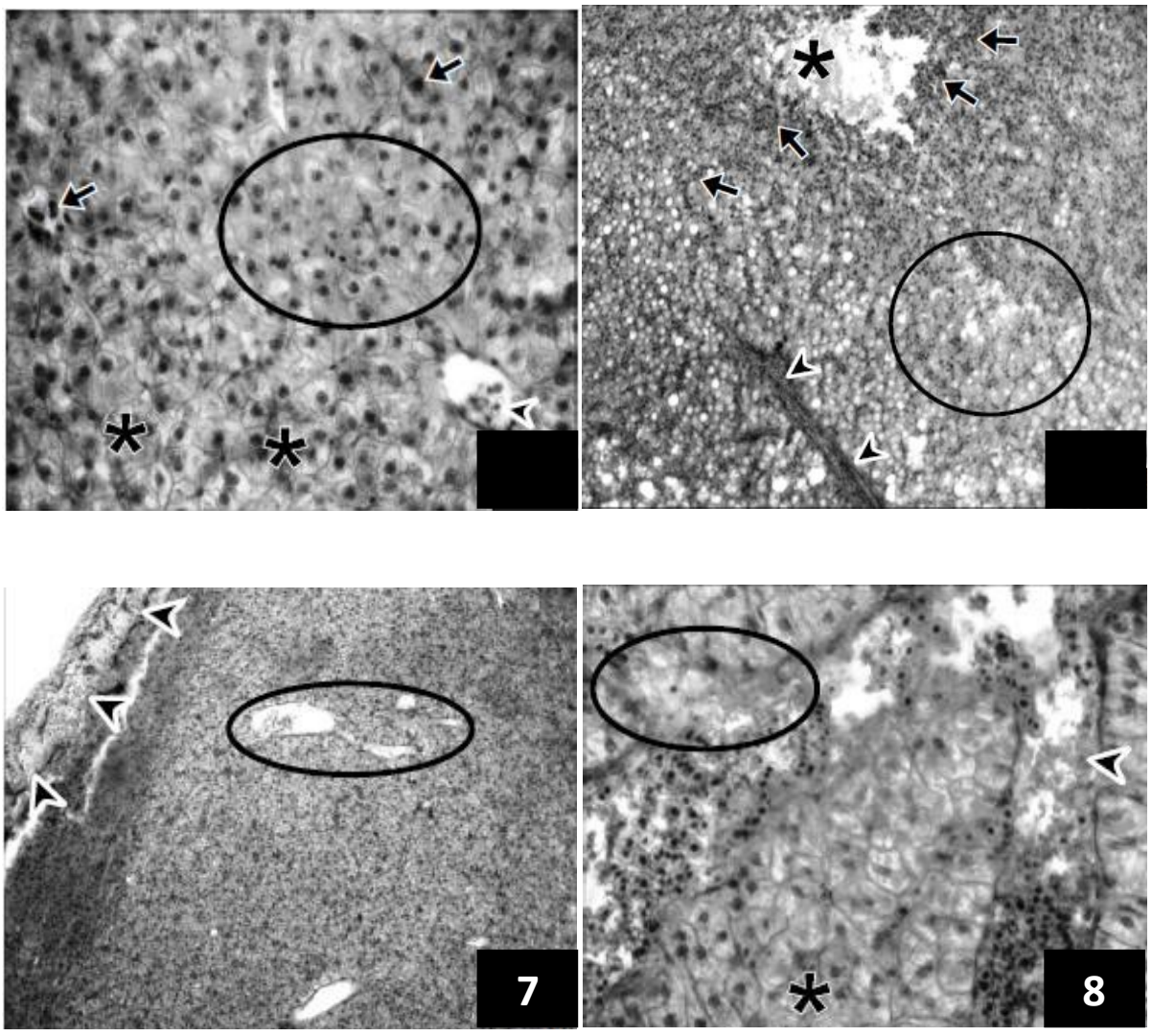
Makinde *et al.* (2015) reported histopathological changes in liver of catfish *Clarias gariepinus* juvenile when exposed to Herbex-D SL, a herbicide containing 2, 4-D amine. Okogwu *et al.* (2015) studied behavioural, haematological and histopathological changes in *Clarias gariepinus* when exposed to 2, 4-Dichlorophenoxyacetic Acid (2,4-D). Srinivasrao *et al*.

(2018) reported degeneration, necrosis, atrophy and rupture of blood cells in *Ctenopharyngodonidella* exposed to Deltamethrin. Necrosis and vacuolization in liver cells of *Channa gachua* exposed to Sedaxane was reported by Kumari *et al*. (2018). Samuel *et al.* (2017) documented histopathological alterations in liver of *Clarias gariepinus* exposed to water pollutant. Elias *et al.* (2018) documented histopathological changed like necrosis, changes in nuclear shape, and formation of vacuoles and atrophy of hepatocytes when liver tissue of *Clarias gariepinus* was exposed to sublethal concentration of the herbicide Thiobencarb. Similar histopathological changes in various intensities are found in *Clarias gariepinus* exposed to Endosulfan at different sublethal concentrations for different time period in the present study.

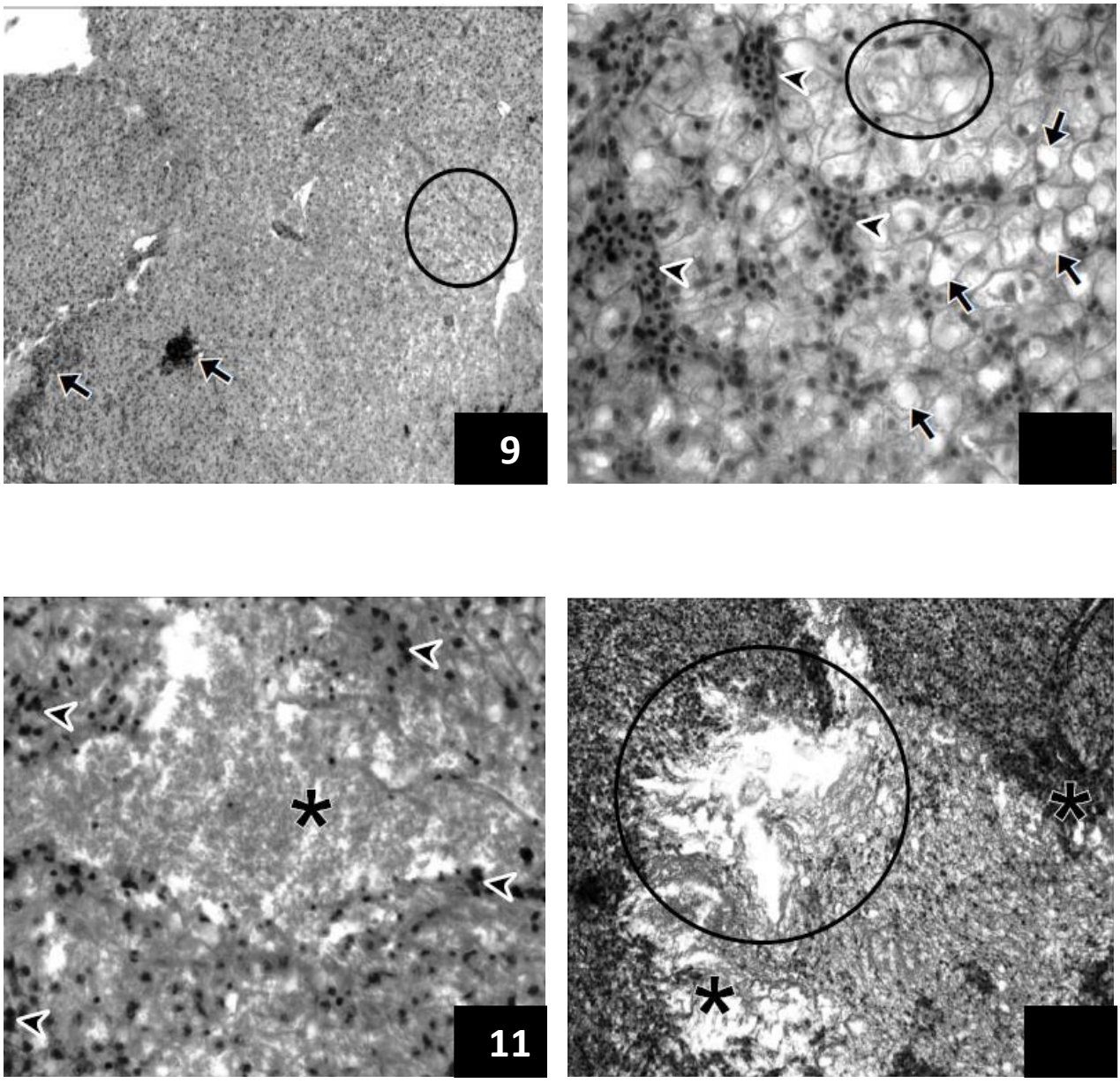


Figs. 1-4. Section of Liver of control and treated fish, *Clarias gariepinus* (Haematoxyline-Eosine stain). Fig. 1 Section of control fish showing normal histological structure of the portal vein (arrow) with hepatocytes (arrowheads) (x400). Fig. 2. Fish exposed to 0.215µg/l Endosulfan for 5 days showing mild necrosis (encircled) and destruction of hepatic cords resulting in damage of tissue (arrows) (x200). Fig. 3. Fish exposed to 0.43µg/l Endosulfan for 5 days showing accumulation of pyknotic nuclei (arrowheads) and cytoplasm vacuolation

(arrows) (x250). Fig. 4. Fish exposed to 0.86µg/l Endosulfan for 5 days showing increased vacuolation in hepatocytes (arrowheads) and nuclear pleomorphism (encircled) (x400).



Figs. 5-8. Section of Liver of treated fish, *Clarias gariepinus* (Haematoxyline- Eosine stain). Fig. 5. Fish exposed to 0.25µg/l Endosulfan for 10 days showing pyknotic nuclei, hydropic degeneration (asterix), nuclear pleomorphism (encircled) and infiltration of leukocytes (arrowhead) (x400). Fig. 6. Fish exposed to 0.43µg/l Endosulfan for 10 days showing mild necrosis (asterix), pyknotic nuclei aggregation (arrows), vacuolar degeneration (encircled) and hemorrhage (arrowheads) (x150). Fig. 7. Fish exposed to 0.86µg/l Endosulfan for 10 days showing necrotic areas (arrowheads) and damaged central canal (encircled) (x100). Fig. 8. Magnified view of Fig. 7 showing rupture due to necrosis of hepatocyte (encircled), cell atrophy (arrowhead) and oedema of the tissue (asterix) (x40).



Figs. 9-12. Section of Liver of treated fish, *Clarias gariepinus* (Haematoxyline-Eosine stain). Fig. 9. Fish exposed to 0.25µg/l Endosulfan for 15 days showing accumulation of pyknotic nuclei (arrows) and vacuolation of hepatocytes (encircled) (x100). Fig. 10. Magnified view of Fig. 9 showing accumulation of pyknotic nuclei (arrowheads), damaged hepatocyte (encircled) and cell cytoplasm vacuolation (arrows) (x400). Fig. 11. Magnified view showing cell atrophy due to necrosis (asterix) and accumulation of pyknotic nuclei (arrowheads) (x400). Fig. 12. Fish

exposed to 0.86µg/l Endosulfan for 15 days showing focal area of necrosis (encircled), extreme damage and destruction of hepatocytes (asterix) (x100).

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