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RESEARCH ARTICLE

THE EFFECT OF QUINALPHOS ON HISTOPATHOLAGICAL CHANGES IN THE LIVER OF FRESH WATER FISH, *ANABAS TESTUDINEUS*

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ABSTRACT

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organ including liver that are responsible for vital functions. Alternations found in these organs are normally easier identifying than functional ones. Quinalphos is one of the organophosphate insecticides represent one of the most widely used classes of pesticide with high potential for human exposure in both rural and residential environments The fresh water fish, *Anabas testudineus* was selected as the test animals. 1/10th of 96 hrs LC_{50} was taken as sublethal concentration of Quinalphos pesticide. After the stipulated period of exposure (24, 48, 72 and 96 hrs) fishes were sacrificed and liver was isolated and used for histopathological studies.

INTRODUCTION

Industrial, agricultural and domestic effluents generally contain a wide variety of organic and inorganic pollutants such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids which are invariably discharged into rivers, canals and streams without scientific treatment. Such contaminants usually change water quality and may cause many diseases, structural alterations and functional changes in the organs of the animals Industrial discharges containing toxic and hazardous substances, including heavy metals and pesticides contribute hugely to aquatic ecosystem (Ghem *et al*., 2001). Indiscriminate use of different pesticides in agriculture to prevent crop damage from pests has been increasing over two decades especially in developing countries. These pesticides through surface run off reach unrestricted areas like ponds and rivers and alter the physico-chemical properties of water and consequently affecting aquatic organisms. Pesticides ultimately find their way into aquatic habitats such as rivers, lakes and ponds, and have been found to be highly toxic not only to fish but also to the organisms, which constitute the food chain. Moreover, Agriculture, as the largest consumer of freshwater and as a major cause of reduction of surface and groundwater resources through erosion and chemical runoff directly correlates with the loss of water quality. Pesticides in general, are used very extensively in agriculture, forestry, public health and in veterinary practices. Hence, it is necessary to study the immediate and chronic effects of pesticides on fish, which form a part of human diet. These compounds have a tendency to accumulate in small quantities in lower fish food organisms and ultimately biomagnify in the fish species.

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A broad-spectrum organophosphate used heavily throughout the world for agriculture and domestic purposes. The frequent occurrence of organophosphate pesticides has been regarded as a serious global public health problem and a major environmental issue. To a lesser extent, they can also absorb the toxins directly from the water. Therefore, it would be pertinent to study the effect of such organophosphate pesticides on long-term exposure by chronic studies to ascertain the residual toxicity (Ramesh Raju Chamarthi *et al.*, 2014)

MATERIALS AND METHODS

Fresh water fish, *Anabas testudineus* were exposed to 24 hrs, 48 hrs, 72 hrs and 96 hrs to a sublethal concentration of Quinalphos pesticide. At the end of exposure period, fish were randomly selected for histopthological examination. They were collected from the Aliyar fish farm, pollachi stocked and acclimatized for a time period of `10-15 days in the laboratory conditions in glass aquaria containing dechlorinated water. The water of the aquarium was aerated continuously through stone diffusers connected to a mechanical air compressor. Water temperature ranged between 26±50°C and the pH was maintained between 6.6 and 8.5. Fish were fed twice daily alternately with rice bran and oil cakes. For the present study, matured adult fishes were exposed to 1/10th concentrations of LC_{50} of quinalphos for 24, 48, 72 and 96 hrs continuously.

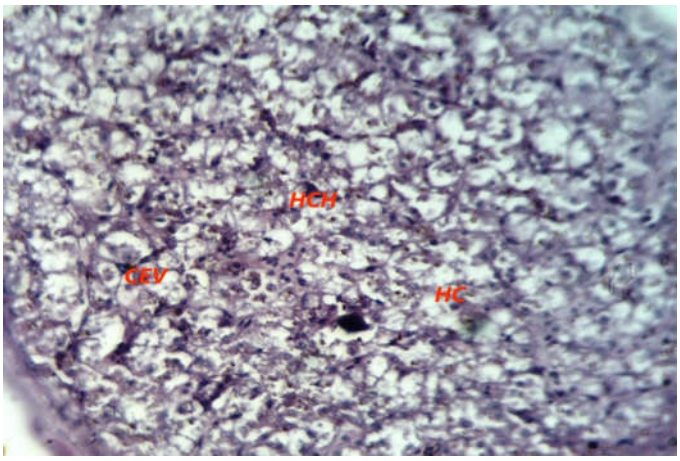
Three replicates of ten fishes for each exposure of the pesticides were used. In these aquaria water was replaced daily with fresh treatment of pesticides. Each experiment was accompanied by its respective control. Three groups of fishes were exposed to 1/10th of the pesticide 'quinalphos' for24, 48, 72 and 96 hrs. Another group was maintained as control. All

the groups received the same type of food and other conditions were maintained similarly. At the end of exposure period, fish were randomly selected for histopathological examination. Tissues of liver was isolated from control and experimental fish. Physiological saline solution (0.85% Nacl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution for 48 hrs, processed through graded series of alcohols cleared in xylene and embedded in paraffin wax. Livers alone were processed by double embedding technique. Sections were cut at 6µ thickness stained with Haemotoxylin Eosin, dissolved in 70% alcohol (Humason, 1962) and were mounted in Canada Balsam. The photographs at 200x magnification were taken with computer aided microscope (Intel play Qx3, Intel Corporation, Made in China).

RESULTS AND DISCUSSION

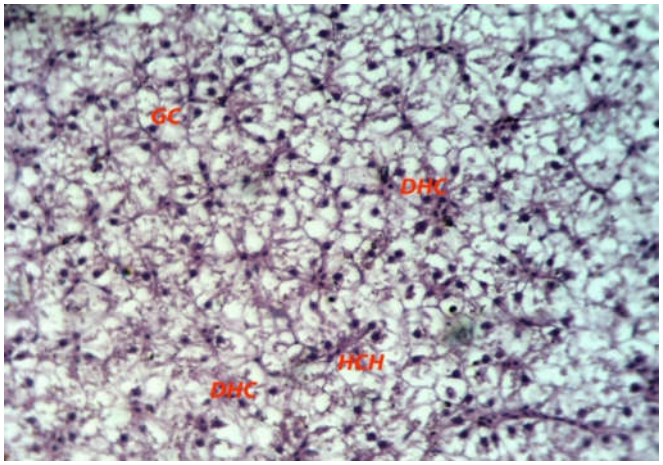
Liver consisted of hepatic cells and connective tissues called lattice fiber which supported the hepatic cells.

HISTOPATHOLOGY OF THE LIVER OF *Anabas testudineus*



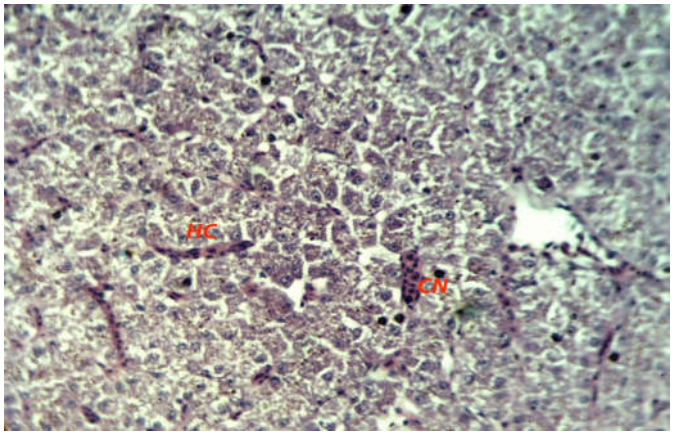
A. Control liver section of *Anabas testudineus*

HC-Hepatocyte cells
HCH-Hepatic cords
CEV-Central Efferent vein



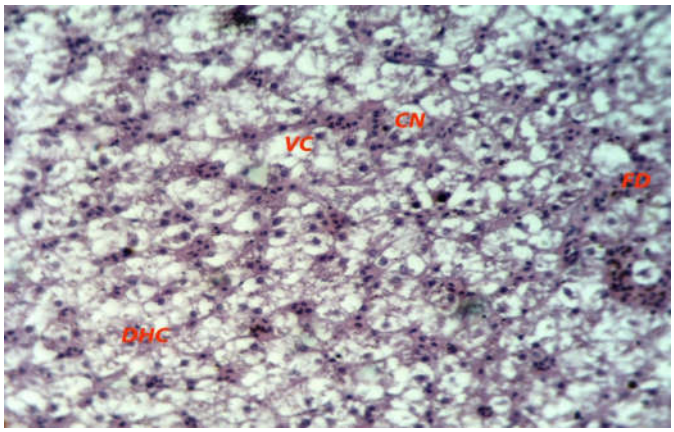
B. Liver section of fish exposed to 24 hours of quinalphos

GC-Gilssen's capsule
DHC-Degenerated Hepatocyte Cells
HC-Hepatocyte cells
HCH-Hepatic cords



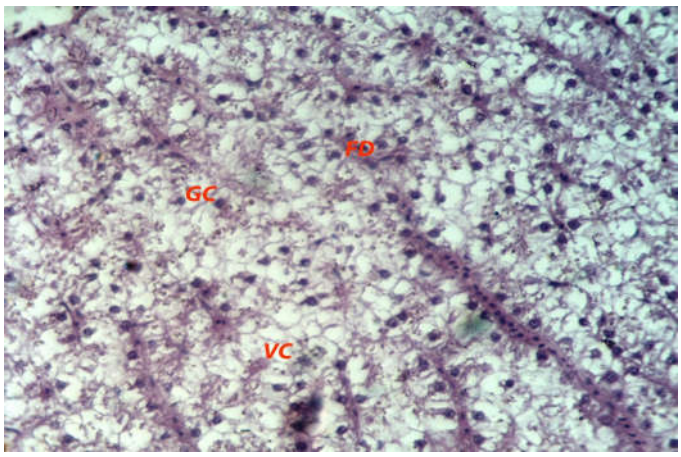
C. Liver section of fish exposed to 48 hours of quinalphos

HC - Hepatocyte cells
CN - Clumping of nucleus



D. Liver section of fish exposed to 72 hours of quinalphos

CN-Clumping Nucleus
DHC-Degenerated Hepatocyte Cells
FD-Fatty Degeneration
VC -Vacuoles



E. Liver section of fish exposed to 96 hours of quinalphos

GC -Gilssen's capsule
FD -Fatty Degeneration
VC -Vacuoles

Hepatocytes were located among the sinusoids and they formed cord like structure, the hepatic cell cords. Bile canaliculus was centrally located in each cord. Fairly large quantities of lipids and glycogen were observed in the hepatocyte cytoplasm. When the fish was exposed for 24 hour to the short term

exposure of quinalphos the liver showed symptoms of general necrosis and degeneration of hepatocytes. After 48 hours of exposure, clumping of nucleus, fatty degeneration was noted. After 72 hours of exposure, hepatic cords showed evidence of cloudy swelling. The central efferent vein and sinusoids were dilated .After 96 hours of exposure; the parenchyma cells showed cloudy swelling. The Glissen's capsules were not thickened. Dilation and congestion in blood sinusoids and intravascular haemolysis in hepatic blood vessels and hepatoportal blood vessels were observed. Moreover focal areas of coagulative necrosis and fibrosis were seen. In liver necrosis and degeneration hepatocytes can be seen in 24 hours of observation. In 48 hour clumping of nucleus will occur followed by fatty degeneration. In 72 hours hepatic cords showed evidence of cloudy swelling and central efferent vein and sinusoids were dilated. 96 hours lot of changes occurred, parenchyma cells showed cloudy swelling, Glissen' capsule were not thickened. Dilation and congestion in blood sinusoids were observed and intra vascular hemolysis in hepatic blood vessels necrosis and fibrosis were also seen. A number of pathological changes occurred in the liver. Since it was the first organ to face any foreign molecule that is carried through portal circulation. In the present study, the fish *Anabas testudineus* after the short term and long term exposure to the quinalphos showed severe damages in liver cells similar to the Rana and Raizada, (1999) recorded histopathological damage associated with vacuolation and necrosis of liver.

The liver made up of hepatocytes that not oriented into distinct lobules but arranged in branched laminae two cells thick, separated by sinusoids. Hepatocytes are polygonal cells with a central spherical nucleus and a densely stained nucleolus (Figueiredo-Fernandes *et al*., 2007). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors (Velmurugan *et al*., 2009). It was observed that histopathological lesions of liver are not specific to pollutants. For example, exposure to organic pollutants and pesticides increases the presence of liver lesions such as foci of cellular alteration (FCA), megalocyticheptoses (MH), hepatocellular nuclear polymorphism (NP), hydropic vacuolation, non-neoplastic proliferative lesions and nonspecific necrotic lesions significantly increased(Myers *et al.*, 1993) Fanta *et al*. (2003) reported abnormalities such as irregular shaped hepatocytes, cytoplasmic vacuolation and nucleus in a lateral position in the siluriform *Corydora spaleatus* exposed to organophosphate pesticides.

Pachew and Santos, (2002) described increased vacuolization of the hepatocytes as a signal of degenerative process that suggest metabolic damage, possibly related to exposure to contaminated water. Tilak *et al*. (2001) reported on the effect of pesticide on the *Ctenopharyngodon idellus* liver and found degenerative of hepatocytes formation of vacuoles, rupture in blood vessels, necrosis and disappearance of the hepatocyte wall and disposition of the hepatic cords. These findings support the present study very much. Hyperplasia, vacuolation disintegrated blood vessels, disrupted hepatocytes, focal coagulative necrosis disorganized hepatic canaliculi in *Ananbas testudineus* exposed to cypermethrin (Sarkar *et al*., 2005). Atif *et al*. (2009) have found rapid and continued destruction of erythrocytes with increased haemolysis and damage of the iron metabolism.

Conclusion

In liver necrosis and degeneration hepatocytes can be seen in 24 hours of observation. In 48 hour clumping of nucleus will occur followed by fatty degeneration. In 72 hours hepatic cords showed evidence of cloudy swelling and central efferent vein and sinusoids were dilated. 96 hours lot of changes occurred, parenchyma cells showed cloudy swelling, Glissen' capsule were not thickened. Dilation and congestion in blood sinusoids were observed and intra vascular hemolysis in hepatic blood vessels necrosis and fibrosis were also seen. From the present study, it is concluded that the above histopathological parameters are the one important and specific biomarkers with regard to effects of toxicants on organisms. So it is also suggested that adequate care should be taken to neutralize and detoxify the toxicants present in the agricultural effluent and follow the treatment procedure before let out into aquatic systems. And we should decrease the pesticide consumptions in agricultural fields. Indiscriminative usage of pesticide will leads into bioaccumulation and biomagnification in humans and other vertebrates it will leads to sever histological and physiological changes. It also effects the ecology of nature.

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