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HISTOPATHOLOGICAL CHANGES IN THE LIVER AND KIDNEY OF *Anabas testudineus* EXPOSED TO SUB-LETHAL CONCENTRATIONS OF PHOSPHAMIDON

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with both the authors. Author BTS conducted the experiment, managed the analyses of the study, wrote the protocol and the manuscript. Author TVA designed the study and managed the literature searches. Both the authors read and approved the final manuscript.

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ABSTRACT

Pesticides usage in agricultural fields to control pests is extremely toxic to non-target organisms like fish and affects fish health through impairment of histological structure, sometimes leading to mortality. The histopathological study reveals the potential adverse effects of Phosphamidon, an organophosphate pesticide, on a fresh water fish, *Anabas testudineus* and it document a dose- dependent reaction of liver and kidney histology. The histological changes observed in liver included fatty-vacuolation and the displacement of nuclei to the periphery of the hepatocytes, congested and constricted liver sinusoids, condensed hepatocytes, destructed cell membrane, necrosis and neoplasm. The major histological alterations identified in the kidney were shrinkage of renal tubules and glomeruli, presence of melano-macrophage centres and degeneration of renal tubules. Histopathology can be used as a tool for assessing the sublethal conditions of water quality and it gives a "rapid early warning system".

Keywords: Histopathology; phosphamidon; kidney; liver; *Anabas testudineus*.

1. INTRODUCTION

Developing countries are faced with the acute need of increasing agricultural output to meet the need of increasing populations. The public awareness in these countries regarding dangers of pesticide pollution is extremely poor. Pesticides have become a potential hazard of the manufacturer, consumer and the environment. Air, water and food have become contaminated with pesticides as a result of their extensive misuse. In the case of aquatic animals such as fish, pollution leads to morphological and cytological changes in the kidney and liver [1].

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Kuttanad, the 'rice bowl of Kerala', is a region where there is overdose application of pesticide during the punja cultivation periods. The freshwater ecosystem of Kuttanad, had sustained a rich and diversified fish fauna. In recent years, human interventions have brought about major changes in these aquatic ecosystems. Though pesticides are applied to enhance agricultural production, the deleterious effects of them are often noticed in non-target organisms like the fish. Most of the pesticides used are highly toxic and are remaining in the environment for a prolonged period, causing pollution. Moreover, due to the repeated applications of the pesticides, their toxic residues in environment and biota have reached an alarming concentration. Phosphamidon, monocrotophos and malathion are the major pesticides being widely used in Kuttanad. As a result of the exposure to pollutants, the histological structure may change and physiological stress may occur. This stress can cause some changes in metabolic functions. A number of studies revealing the changes in functions are initiated by changes in cellular level and tissues [2,3,4,5].

Anabas testudineus is an important species of fish contributing to the fishery wealth of Kuttanad. Phosphamidon is a widely used organophosphate pesticides in Kuttanad. Histology is an integrated parameter for providing an effective monitoring of the effects of water pollution and a better picture of the fish's health is provided by histology [6,7]. Induced histological alternation data were correlated to the severity of environmental degradation in order to determine whether this biological system can be used as a tool for environmental monitoring programs.

In the present study, a laboratory study had been conducted to test the histological changes of liver and kidney due to phosphamidon. The liver and kidney are important for the maintenance of a stable internal environment with respect to water and salt excretion and partially for the metabolism of xenobiotics [8]. The histopathological changes give an early warning of the damage caused in the fish at the histological level before their mortality. Furthermore, the data generated could be useful in the environmental risk assessment of freshwater and marine organisms.

2. MATERIALS AND METHODS

The experiments on the sublethal toxicity of phosphamidon on the juveniles of *Anabas testudineus* (Fig.1) was conducted for a period of 30 days during the investigation based on the LC50 value (39.34ppm) obtained [9]. Five nominal concentrations of the pesticides were selected for sublethal toxicity studies. Maximum and minimum sublethal concentrations were chosen based on [10,11] concentrations of pesticide used for sublethal exposure were 0.5ppm, 1.0 ppm, 2.0ppm, 5.0ppm and 10.0ppm in addition to the control group. The experiment was done in a static system where water and pesticide medium were renewed every 24 hr to obtain the desired pesticide concentration. Ten fishes each in triplicate were exposed in respective toxicant concentration in cement cisterns. The fishes were fed once a day on fresh clam meat *ad libitum*. The dissolved oxygen, pH & temperature were measured immediately before and after the pesticide inoculation.

After 30 days, liver and kidney was dissected out and prepared for the histological analysis. The organs fixed in Bouin's fluid were washed, dehydrated, cleared and embedded in paraffin. Serial sections of the organ was taken at 3 to 5µ thickness and stained with Hematoxylin-eosin staining procedures. Detailed histological observations were carried out with the help of a binocular microscope.

3. RESULTS

3.1 Physico-chemical Parameters

Weekly mean temperature, pH and DO values ranged from 27.8 to 28.35oC, 7.26 to 7.4 and 6.2 to 7.01 mg.l-1 respectively.

3.2 Histopathology of Liver

Control: This liver of *Anabas Testudineu*s consisted of parenchymatous, homogeneous, polygonal cells formed from double layers of liver cells separated from each other by capillary blood spaces called liver sinusoids. The hepatocytes were compact and carried centrally placed nucleus with distinct nucleolus. The hepatocytes and the liver sinusoids of fishes in control groups were observed as intact (Fig. 2 A).

0.5 ppm: Most of the hepatocytes of *A. testudineus* treated with 0.5 ppm phosphamidon were normal in appearance but the liver sinusoids exhibited slight constriction in some fishes (Fig. 2B).

1.0 ppm: Slight vacuolation of hepatocytes and constricted liver sinusoids were the common and conspicuous change observed in this exposure (Fig. 2C).

2.0 ppm: Most of the hepatocytes in this concentration were similar to the hepatocytes of 1.0 ppm treated fishes. Most of the liver sinusoids could be seen as constricted. The vacuoles forced the nuclei towards the periphery in certain fishes (Fig. 2D).

5.0 ppm: Severe congestion of liver sinusoids and vacuolisation of hepatocytes could be compared to the other lower concentrations and control. The vacuoles forced the nuclei towards the periphery of the hepatocytes. As the density they merged each other and thereby increased in size (Fig. 2E). In some fishes, constricted liver sinusoids were also observed (Fig. 2F).

10.0 ppm: Most of the 10.0 ppm treated hepatocytes exhibited fatty vacuolation and the vacuoles were frequently coalesced. Constricted liver sinusoids were a common and conspicuous change in this concentration (Fig. 2G). In addition to this, the destructed hepatocytes were observed around the constricted liver sinusoids (Fig. 2H). Necrosis was identified in some regions of a few fishes (Fig. 2 I). Occurrence of vacuolated hepatocytic region alternating with non-vacuolated hepatic cells could be observed in certain regions. This non-vacuolated region is considered as neoplasm (Fig. 2J).

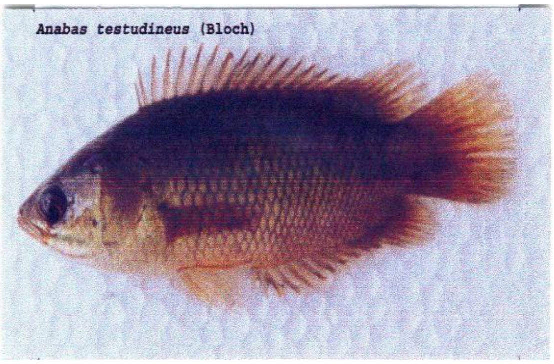

Fig. 1. ***Anabas testudineus***

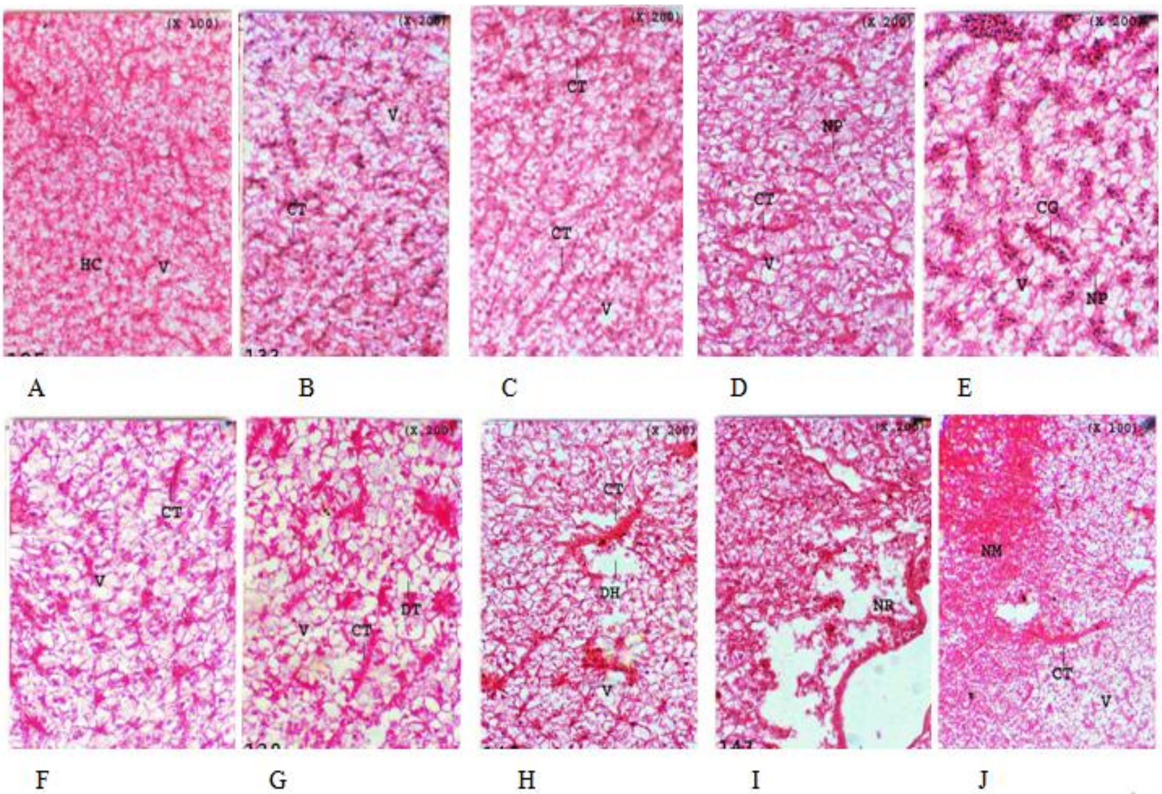

Fig. 2. Liver tissue of *Anabas testudineus* treated with phosphamidon (A) Control - Section of liver of fishes maintained in phosphamidon treatment as control. Hepatocytes (HC), Vacuoles (V). H+E x 100. (B) 0.5ppm - constriction of the liver sinusoids (CT). H+Ex200. (C) 1.0ppm - vacuoles (V) and constricted liver sinusoids (CT). H+Ex200. (D) 2.0ppm - vacuoles (V), peripherally located nuclei (NP) and constricted liver sinusoids (CT). H+Ex200. (E) 5.0 ppm - vacuoles (V), peripherally located nuclei (NP), congested liver sinusoids (CG) and merged vacuoles. H+Ex200. (F) 5.0 ppm - vacuoles and coalescence of vacuoles (V), constricted liver sinusoids (CT) H+Ex200. (G) 10 ppm - vacuoles and coalescence of vacuoles (V) and constricted liver sinusoids (CT). H+Ex200. (H) 10ppm -vacuoles (V), constricted liver sinusoids (CT) and destructed hepatocytes (DH). H+Ex200. (I) 10ppm - necrosis (NR). H+Ex200. (J) 10ppm - vacuoles (V), constricted liver sinusoids (CT) and neoplasm (NM). H+Ex100

3.2 Histopathology of Kidney

Control: The histological structure of kidney of *A. testudineus* maintained in the control consisted of intact renal corpuscles containing vascularised glomeruli, renal tubules and hematopoietic cells except a few exhibited slightly shrunken renal tubules (Fig. 2A).

0.5ppm: *A.testudineus* exposed to 0.5ppm phosphamidon showed slight shrinkage of glomerulus (Fig. 2B).

1.0 ppm: the fishes in this concentration exhibited moderate shrinkage of renal tubules (Fig. 2C).

2.0ppm: Melano-macrophage centres and moderately shrunken renal tubules were frequently observed in most of the fishes exposed to 2.0ppm phosphamidon (Fig. 2D).

5.0ppm: Shrinkage of renal tubules, melano-macrophage centres and severe shrinkage of glomeruli were seen in the kidney tissue after 5.0ppm exposure (Fig. 2E).

10.0ppm: In addition to shrinkage of renal tubules and accumulation of melano-macrophage centres (Fig. 2F), degenerated renal tubules and severely shrunken glomeruli were common and conspicuous changes in 10ppm treated fishes (Fig. 2F,G).

4. DISCUSSION

The liver of *A. testudineus* treated with 1.0, 2.0, 5.0 and 10.0 ppm phosphamidon exhibited vacuolation. As the concentration increased, the size of the vacuoles has also increased due to coalescence of small vacuoles. This extensive intra-cellular vacuolization resulted in the displacement of the nucleus to the cell margin. [12] suggested that the pesticide has induced cytoplasmolysis and karyolysis leading to the formation of vacuoles. [13,14] reported that hepato- cellular vacoulation is a variant of hydropic degenration. But most of the scientists considered this vacuolation as the accumulation of fat in the hepatocytes [15,16,17,18,19].

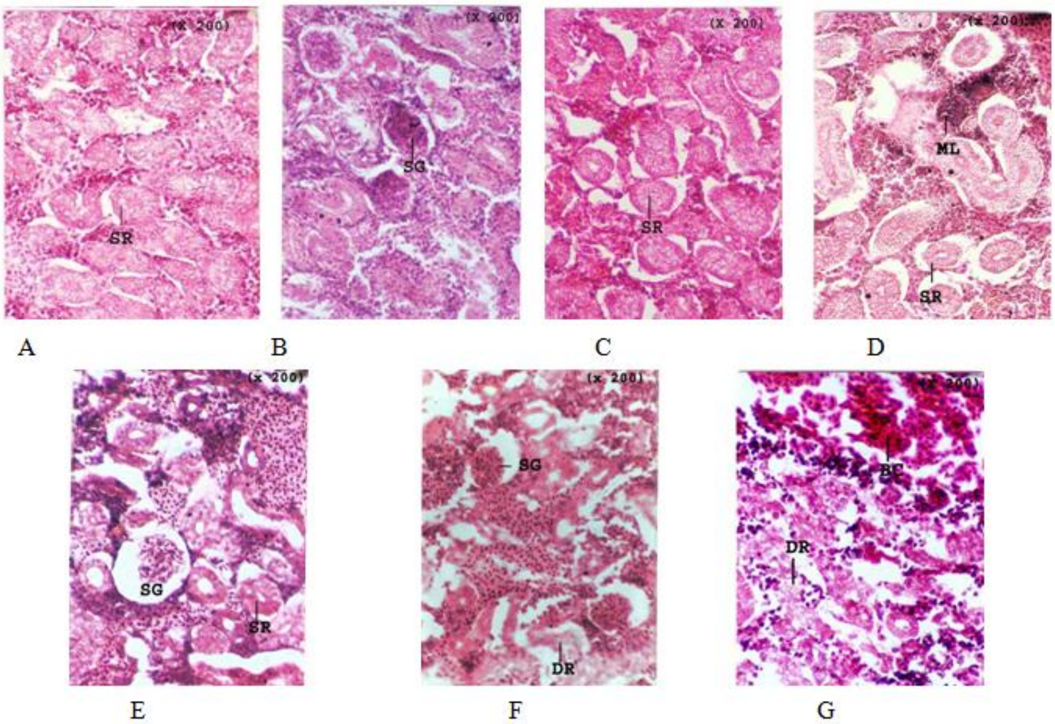

Fig. 3. Kidney tissue of *Anabas testudineus*- (A) Control - Shrunken Renal tubules (RT). H + E x 200. (B) Exposed to 0.5 ppm phosphamidon - shrunken glomerulus (SG). H + E x 200. (C) Exposed to 1.0 ppm phosphamidon - shrunken renal tubules (SR). H + E x 200. (D) Exposed to 2.0 ppm phosphamidon - shrunken renal tubules (SR) and melano-macrophage centers (ML). H + E x 200. (E) Exposed to 5.0 ppm phosphamidon - shrunken glomerulus (SG) and shrunken renal tubules (SR). H + E x 200. (F) Exposed to 10 ppm phosphamidon- shrunken glomerulus (SG) and degenerated renal tubules (DR). H + E x 200. (G) Exposed to10 ppm phosphamidon - Melano-macrophage centers (ML) and degenerated renal tubules. H + E x 200

According to [18,19], accumulation of fat gave the fish modicum of protection from its toxic effects. However, accumulation and sequestration of contaminants can only be effective as long as the capacities of the organ involved are not overloaded. In the present study 5.0 and 10.0 ppm phosphamidon treated *A. testudineus* exhibited the coalascence of vacuoles. The size of the vacuoles in almost all fishes varied between cells [20] and as fat deposition increases the vacuoles become merged. As reported by [1,2,3,4], the hepatocytes of almost all the fishes treated with phosphamidon showed the displacement of nuclei towards the periphery of the cells.Several scientists have reported this type of displacement of nuclei [20,21].

According to [22], coagulative necrosis is a sudden cessation of blood flow to an organ. With coagulative necrosis, shape of cells and their tissue arrangement are maintained, facilitating recognition of the organ and tissue. Necrotic changes occur after cell death and represent the sum of degradative process. Acute and extensive necrosis of liver cells occur in toxic conditions but focal necrosis is more common [23]. *A. testiduneus* treated with 10.0 ppm phosphamidon exhibited the cell death along with rupture of plasma membrane (degenerative necrosis) as reported by [15,17,23,24].

All the concentrations of phosphamidon treated *A. testudineus* exhibited the constricted liver sinusoids. This constriction of liver sinusoids might be due to the pesticide reaction in the wall of the blood vessel during the detoxification process of the pesticides.While studying the pathological conditions in the liver of ruffe, *Gymnocephalus cernua* from the highly polluted Elbe estuary [20] also observed similar constriction of sinusoids in fish.

10.0 ppm phophamidon treated *A. testudineus* showed a destruction of hepatocytes. This destruction of hepatocytes might be due to the adverse reaction of the pesticide on the hepatocytes. Since this lesion was found only in the higher concentrations, it might be assumed that it is a severe damage that occurs when the fish is exposed to higher concentrations [25,26].

10.0 ppm phosphamidon treated *A. testudineus* have exhibited an early neoplastic condition. In the case of *A. testudineus* the hepatocytes appeared normal, retaining their normal architecture [27] in the neoplasmic area. All these fishes remained healthy during the study period even after being affected with neoplasm [28,29]. Reported a similar condition in channel cat fish treated with aflatoxin B1. In the present study this might be due to a carcinogenic action of the pesticide, which might be attributed to the cumulative toxicity as reported by [30].

In the case of kidney, the lesions are non-specific and hence it is not possible to attribute the occurrence of a given type of lesion to a particular pollutant but an attempt is made here to discuss the pathological changes in relation to concentrations between pesticideand specific kidney conditions in the fishes under study. Number of scientists reported histopathological alternations in the kidney structures of fishes [31,32,33,16]. In response to stressors such as pesticide exposure, the fish undergo a series of biochemical, physiological and histological changes in an attempt to compensate the challenge imposed on them and thus cope with the stress.

The present study showed that the sublethal exposure to relatively high concentrations of phosphamidon could negatively affect the health status of *A. testudineus*, which is connected with the changes in histological structure of kidney cells of *A. testudineus*. Accumulation of melano- macrophage centres were observed in higher concentration. Many studies have suggested that the general functions of these aggregates are the detoxification or recycling of endogenous and exogenous materials [34,35]. Melano-macrophage centres have an important role in the initial responses of fish to foreign material, [36]. However, it is the clear indicator of stressful environmental conditions as reported by [37,38,39].

Histopathological changes in the glomerulus and renal tubules have been reported in many disease conditions. In the present study the shrinkage of glomeruli and renal tubules and the degeneration of renal tubules were observed in higher concentrations of phosphamidon. This results substantiate the findings of [3,4,5,40,41,42].

5. CONCLUSION

Liver exhibited a dose dependent degeneration in histology when it was exposed to various sublethal concentrations of phosphamidon. Since the liver has a regenerating capacity, it is not much affected by the exposure to toxicant medium. If better environmental conditions are provided the liver can regain its original functions. But chronic exposure to the toxicant medium will definitely damage the organ.

The damage of the kidney cells increased as the concentration of phosphamidon increases. The melano-macrophage centres are the clear indicator of stressful environmental conditions due to the pesticide. Regular monitoring of the histological conditions of the liver and kidney will be helpful in the water quality assessment of the medium and hence histopathology can be used as a tool for assessing the sublethal conditions of water quality. It gives a "rapid early warning system". Furthermore, the data generated could be useful in the environmental risk assessment of freshwater and marine organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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