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DOSE DEPENDENT EVALUATION OF HISTOPATHOLOGICAL ALTERATIONS CAUSED BY DICHLORVOS ON LIVER AND KIDNEY OF *CIRRHINUS MRIGALA*

Histopathology

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ABSTRACT

Organophosphorous pesticides (OPs) are the most widely used pesticides due to their low persistence and high effectiveness in pest eradication. Dichlorvos is used against pests of various domestic animals, stored grains and to control ectoparasitic infections of various organisms. This pesticide often ends up producing both lethal and sub lethal effects on the fishes and various other non target aquatic organisms. Present study is aimed to investigate histopathological effects of dichlorvos on the liver and kidney of Indian carp viz. Cirrhinus mrigala. Carp was divided into five groups containing three fishes in each group. Group 1 fishes were designated as control. Fishes of group 2, 3, 4, 5 were placed in 1.0ppm, 1.4ppm, 1.7ppm and 2ppm concentrations of dichlorvos for the period of 10 days. Various histopathological alterations were observed after the exposure of different concentrations of dichlorvos. In liver infiltration of blood cells, hemorrhage, nuclear degeneration, hypertrophic nuclei, pyknotic nuclei, congestion of nuclei, infiltration of nuclei and enucleated areas at some places was depicted after dichlorvos treatment. Kidney exhibited hypercellular glomeruli, tubular cell degeneration and irregularity in position of nucleus in renal cell observed. Interstitial tissue showed degeneration of cells, nuclear hypertrophy, glomerular hypertrophy, blood vessel enlargement and areas of hemorrhage.

KEYWORDS

Dichlorvos, Indian carp, liver, kidney, histology.

INTRODUCTION

Environmental pollution caused by the use of chemical pesticides is a serious problem of global dimension. In developing countries like India, the use of chemical pesticides has become necessary to protect crops from a wide variety of pests. Pesticides originating from the agricultural practices are discharged directly or indirectly into the water bodies like rivers, ponds, lakes etc and cause harm to the aquatic fauna. Many of these compounds present in surface and ground water are considered as major threat to aquatic organisms and also degrade the water quality (Katsumata *et al*., 2005). Pesticides and other related chemicals can cause adverse effects by interfering with the enzymatic systems and causing destruction to various tissues of the body. The three major pesticides are herbicides, insecticides, and fungicides. Among these different groups of pesticides organophosphorus compounds (OPs) have been widely used insecticides due to their relatively low persistence under normal environmental conditions and high effectiveness for insect and pest eradication (Qian and Lin, 2015). These insecticides are used for the control of wide variety of pests which would otherwise diminish the quantity and quality of food production. Contamination of surface water by different concentrations of insecticides causes various harmful effects on growth, survival and reproduction of aquatic animals. Hence insecticides are threatening the long term survival of major ecosystems and causing loss to biodiversity by disruption of ecological relationships between the organisms. OPs interfere with the energy production or release of stored metabolic energy of fishes in severe stress conditions and ultimately leads to the death of the fish. Histopathological changes in gills, liver, heart and renal tubules of kidney, haemopoietic tissues such as spleen and different genetic and behavioral disorders are some of the biological indicators of exposure of fish to insecticides. Among different OPs, Dichlorvos is widely used pesticide in agricultural practices throughout the world. Dichlorvos or 2,2 dichlorovinyl dimethyl phosphate (DDVP) is widely used insecticide which is effective against various insects like aphids, mushroom flies, spider mites, caterpillars, thrips etc. It is commercially available since 1961 and its use has been the matter of concern due to its ill effects to other non target organisms. Dichlorvos acts as both contact and stomach poison. Like other organo phosphosphate insecticides, it also acts on acetyl cholinesterase hence affects the nervous system of insects. It is claimed to damage the DNA of the insects (Espeland *et al.,* 2010). fish provide an important tool to monitor the quality of aquatic environment and thereby the health status of various organisms inhabiting those diverse aquatic ecosystems.. It is extremely toxic pesticide to the aquatic organisms such as fish through impairment of metabolism or sometimes led to the death. Acute toxicity or sub lethal effects of dichlorvos in fish includes oxidative damage, inhibition of AchE activity and histopathological changes in different organs like liver, kidney and gills. . At only 1ppm concentration dichlorvos show both acute and chronic toxicity. Various workers have determined sub lethal and lethal concentrations of dichlorvos in different species of fishes. For freshwater and estuarine fishes, the 96 hrs LC_{50} value ranged from 0.2 mg/L to 12 mg/L and in case of marine fishes it was estimated to be more than 4 mg/L (Das, 2013). In case of *Cirrhinus mrigala* 96 hrs LC_{50} values of dichlorvos were 20 mg/L (Srivastava *et al*., 2014) and 9.1 ppm (Velmurugan *et al*., 2009). In case of *Cyprinus carpio* 96hrs LC_{50} values were 0.95 mg/L (Tak *et al*., 2014), 2.51 mg/L (Gündue and Yerli, 2012) and 0.34 ppm (Verma *et al*., 1981). Liver is the major site of metabolism, digestion and detoxification of various substances from the body. Liver is the principal organ that is capable of converting drugs into form that can be readily eliminated from the body (Bloom and Fawcett, 1968). It makes many of the chemicals required by the body to function normally, it breaks down and detoxifies substances in the body and also acts as a storage unit. Kidneys involved in excretion and water balance are major osmoregulatory organs in fishes occupying significant portion of trunk. Within freshwater, kidney is responsible for preventing excessive solute loss due to presence of more salts in their blood as compared to water present outside their body. Dichlorvos causes various histopathological changes in liver and kidney of *Cirrhinus mrigala*.

MATERIALS AND METHODS

Present investigation was carried on the liver and kidney of *Cirrhinus mrigala* which was obtained from the Fish farm, Deoli (Ghagus), Bilaspur, Himachal Pradesh which is relatively free from the pollutants. The experimental set up was done on fish farm Bilaspur for 40 days. The fishes of desired species of carp were collected and then transferred to cemented tanks of capacity of approximately 1700L. The physiochemical properties of water were according to the environmental conditions of the fish farm. All experimental procedures were conducted after the approval of Department of Fisheries, Himachal Pradesh.

GROUPING OF ANIMALS

Carp was divided into five groups containing three fishes in each group.

1. Group -1 fishes were designated as control.
2. Group-2, 3, 4, 5 fishes were kept in 1.0ppm, 1.4ppm, 1.7ppm and 2ppm concentrations of dichlorvos for 10 days.

DOSE ADMINISTRATION

Dichlorvos was administrated by mixing it in water at regular interval of ten days. Experiment was done in the still water. For the fish of group 2, 3, 4 and 5 the 1.0ppm, 1.4ppm, 1.7ppm and 2.ppm concentrations of dichlorvos were added in the water respectively. The fishes of each group were sacrificed at the fish farm after 10 days. The liver and heart of treated and control fishes were excised and then transported from Deoli to the laboratory of Himachal Pradesh University, Shimla in ice

box. The desired tissues were then kept in -70^0 C (Lab cool) and then processed for different investigations.

HISTOLOGICAL STUDY

Liver and kidney of *Cirrhinus mrigala* were excised immediately after sacrificing the fishes. Tissues were fixed in Bouin's fixative for 24 hours. After that tissues were washed in running tap water until the entire yellow color disappeared. Tissues were dehydrated serially in different grades of alcohol (30%, 50%, 70%, 90%, 100%) and cleared in xylene. Tissues were then embedded in paraffin wax (58-60˚C). Sections of about 5-6µm thickness were cut on the rotary microtome and subjected to hematoxylin-eosin staining.

RESULTS AND DISCUSSION

Various histopathological alterations were observed after the treatment of dichlorvos.

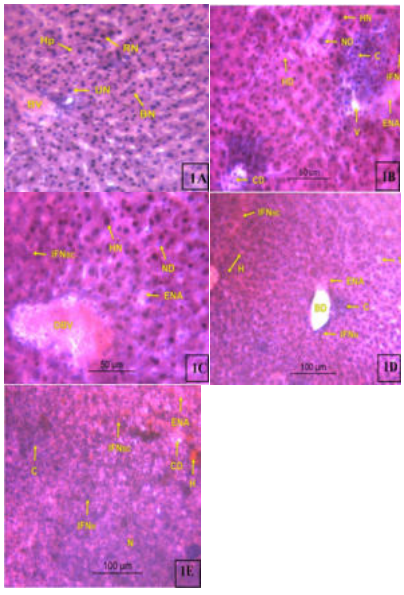

Figure 1: Fig 1A showing control liver. Fig 1B, 1C, 1D, 1E showing liver at 1ppm, 1.4ppm, 1.7ppm and 2ppm concentrations of dichlorvos. polygonal hepatocytes (HP), uninucleate (UN) cells, binucleate (BN) cells, Blood vessel (BV), degenerated nuclei (ND), hypertrophic nuclei (HN), Cytoplasmic degeneration (CD), hepatocyte degeneration (HD), vacuolization (V), congestion (C) of nuclei, infiltration of blood cells (IFNBC), enucleated areas (ENA) and dilation of sinusoids (DS), hemorrhage (H)

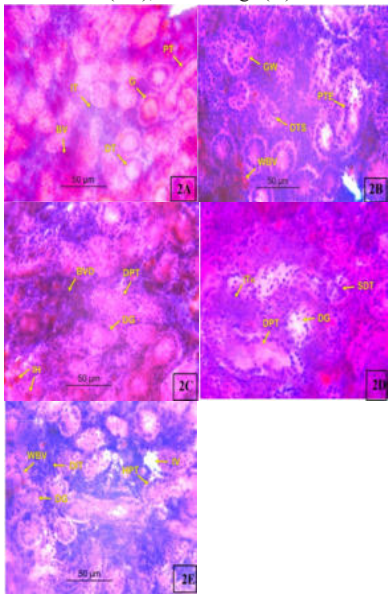

Figure 2: Fig 2A showing control kidney. Fig 2B, 2C, 2D, 2E showing kidney at 1ppm, 1.4ppm, 1.7ppm and 2ppm concentrations of dichlorvos. distal tubule (DT), proximal tubule (PT), interstitial tissue (IT), glomeruli (G) and blood vessels (BV), proximal tubule enlargement (PTE), widening of blood vessels (WBV), distal tubule shrinkage (DTS) and glomerular widening (GW), damaged proximal tubule (DPT), blood vessels degeneration (BVD), degenerated glomeruli (DG), internal hemorrhage (IH). Shrunken distal tubule (SDT), internal vacuolization (IV)

Use of chemical pesticides is increasing day by day in developing countries like India. Their use has been a matter of major concern because of their harmful effects to various non target organisms. Their widespread use in agriculture to control various kinds of pests is one of the major reasons of aquatic pollution. The pesticides and their residues mostly reach into aquatic ecosystems through surface runoff (Jergentz *et al*., 2004). Organochlorine pesticides were mostly used earlier but various ecological concerns arising out of high persistence of organochlorines have favoured extensive use of organophosphates for controlling household, agricultural and public health pests due to their less persistence and high efficacy (Jyothi and Narayan, 1999). This shift has resulted into increased concentration of organophos phates into water bodies causing acute and chronic toxicity to various aquatic organisms including fish fauna (Aker *et al.,* 2008*;* Pandey *et al.,* 2009). Aquatic organisms like fish accumulate pollutants directly from contaminated water and indirectly through food chain (Ashraf, 2005). Once the toxicant enters the body of fish that may affect the organs leading to physiological and pathological disorders. Fishes are considered as one of the most significant indicators in fresh water systems for the evaluation of metal pollution (Rashed, 2001).

Dichlorvos is an organophosphorus pesticide widely used in agriculture, animal husbandry, horticulture, food storage and even to control fleas on domestic pets. Dichlorvos or formulations containing dichlorvos appear under trade names of "vapona", "nuvan"," nogos" and "phoracide" etc. This pesticide often ends up producing both lethal and sub lethal effects on the fishes and various other aquatic organisms (Gupta *et al*., 2008). It induces many significant changes in fish biology out of which neurotoxicity, behavioral changes, alterations in biochemical parameters, bioaccumulation in tissues, undesired changes, chromosomal changes and various histopathological changes are the major ones. Histopathological studies are potential tools to analyze the effects of toxicants on target organisms of fish in laboratory experiments and in field investigations (Kori- Siakpere *et al.,* 2005).Histopathology of liver is increasingly being considered as biomarker of xenobiotic exposure (Fernandes *et al*., 2008) Liver of *Cirrhinus mrigala* showed various changes after treatment with different concentrations of dichlorvos which included congestion of nuclei, nuclear and cellular degeneration, nuclear and cellular hypertrophy, infiltration of blood cells and nuclei, dilation of sinusoids and pyknotic nuclei at some places.

Various researchers have similar results of liver histopathology after the treatment of various chemical pesticides. After exposure of organophosphorus pesticides for 96hrs alterations like irregular shaped hepatocytes, cytoplasmic vacuolization and laterally placed nucleus was observed in siluriform *Corydoras paleatus* (Fanta *et al*., 2003). Peebua *et al*., (2008) reported lipid vacuoles, hepatocyte swelling and pycnotic nuclei in *Oreochromis niloticus* after the exposure of alachlor for 96 hrs. After the exposure of organophosphorus pesticide, dimethoate to *Cyprinus carpio* various alterations like disruption of regular arrangement of hepatocytes, congestion and rupture of vein, extensive hemorrhage, cytoplasmic vacuolization, indistinct cellular boundaries, pyknotic nuclei, nuclear degeneration and necrosis were noticed. Hemorrhage and cytoplasmic vacuolizations were earliest effects in liver of exposed fish (Singh, 2013). Sublethal concentrations of diazinon leads to hypertrophy, necrosis and pyknosis in various fish species (Rahman *et al*., 2002). Liver of *Cyprinus carpio* exposed to 0.448 and 0.976 mg/L diazinon showed dilation of sinusoids and veins, blood cells infiltration and pyknotic nuclei (Körkmaz and Dönmez, 2017).

Kidney showed various histopathological changes after the treatment of pesticide. normal structure of kidney was disrupted and deformities in proximal tubules, distal tubules and glomeruli were observed. Histopathological changes in the kidney tissue such as severe necrosis, cloudy swelling,cellular hypertrophy and granular cytoplasm were reported by Tilak et al., (2007) in *Channa punctata* exposed to sublethal concentration of Butachlor and Machete, an Herbicide.Greeshma *et* al., 2021 studied Quinalphos induced effects in kidney of *Oreochromis mossambicus.* Results were similar to our findings.

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