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ORIGINAL ARTICLE

A STUDY ON HISTOPATHOLOGICAL IMPACT OF 2, 4 –DICHLOROPHENOXYACETIC ACID ON GILLS AND LIVER OF *CHANNA STRIATUS*

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ABSTRACT

An investigation on the effect of the pesticide, 2, 4-Dichlorophenoxyaceticacid on the gills and liver of *Channa striatus* was carried out. The fish were exposed to sublethal concentrations (100 mg/l and 200 mg/l) of 2, 4-D for 30 days. The gills and liver of fish were removed for histopathological examination. The major histopathological changes in liver includes loss of cellular architechure, necrosis in hepatocytes and accumulation of fat in parenchymal cells. The gills showed lamellar degeneration, epithelial lifting and necrotic changes in epithelial cells.

Keywords: 2, 4-Dichiorophenoxyaceticacid, *Channa striatus*, histopathology, gill, liver.

1.INDRODUCTION

India is primarily an agro-based country with more than 60-70% of its population dependent on agriculture. However, 30% of its agricultural products is lost owing to pest infestation. In the absences of a better alternative, deployment of pesticides becomes inevitable despite their known hazardous effects. Utilization of pesticides in India is about 3% of the total world consumption and is increasing at the rate of 5% per annum (Bhadbhade *et al.*, 2002). Environmental pollution is one of the undesirable side effsects of industrialization and an important aspect of environmental degradation. The pollutants associated with the industrial effluents are organic matter, inorganic dissolved solids, fertilizers, thermal constituents in the form of heat suspended solids, microorganisms and pathogens. Among these pollutants, organic pollutants decrease the level of dissolved oxygen in the water bodies. The disposal of these waste materials or waste water leads to contamination of rivers, lakes, chronically affecting the flora and fauna (Mathivanan, 2004). Fish is good indicator of aquatic contamination because its biochemical stress response are quite similar to those found in mammals (Mishra and Shukla, 2003). Presence of pesticide in streams and lakes is largely due to the runoff from agricultural fields and outfall from pesticide manufacturing factories (Poonam Tyagi *et al.*, 2000). These kinds of chemicals have their short-term benefits, though are undeniable, pesticides are hazardous because of their interference with the environment. Pesticides wherever applied, they found their way into water bodies ultimately affecting aquatic fauna in general and fish in

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Histopathological alterations can be used as indicators for the effects of various pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular changes in the affected organism (Fatma, 2009).

Among the different pesticides that contaminate aquatic ecosystems, the phenoxyacetic herbicides attract special attention because they constitute one of the largest groups of herbicides used around the world. In particular, 2,4-dichlorophenoxyacetic acid (2,4-D) has been the most widely used pesticide for over 60 years and is present in more than 1500 formulations of 2,4-D-based products currently on the market (Tayeb *et al.*, 2011) Pesticide toxicity is highly dependent on concentration, frequency, intensity of exposure, and target organism susceptibility, which in turn, is influenced by age, sex, health state and genetic variations (Fent 2003, 2004).

2,4-dichlorophenoxyacetic acid (2,4-D) is a common herbicide that was introduced into commerce in 1946 and rapidly came to be used worldwide. It is employed for post emergence foliar spray and is also used for weed control of wheat, rice, maize and aquatic weeds (Farah *etal.*2004). However, the widespread and intensive use of 2,4-D may give rise to several toxicological and environmental problems and has led to the emergence of herbicide resistant weeds (Watanabe et al., 1997; Bradberry *et al.*, 2000; Toyama *et al.*, 2003). Hence, it is useful to have an insight into histological analysis regarding the extent of damage in the gills and liver when 2,4-d enters the body of *Channa striatus.*

2.MATERIALS AND METHODS:

Experimental Fish:

Healthy adult fish *Channa striatus* were collected from local area around Chidambaram. Fishes were washed with 0.1% of potassium permagnate solution to avoid dermal infection. They were acclimatized to the laboratory conditions for two weeks in 100L capacity of fish tank. If mortality occurred during this period, dead fish were removed immediately. During acclimatization fish were fed with pieces of live earthworm on alternate days. Water in the tank was also changed once in 24h.

Experimental design:

The fish were divided into three groups of 10 specimens each. The specimens were transferred from the initial acclimation tank to exposure tanks (10 L capacity). The first group acts as a control group. The second and third groups were exposed to 100 and 200 mg/l 2, 4-Dichlorophenoxyacetic acid of water respectively for 30 days. Physicochemical condition of water during experimental period were recorded.

Histopathological analysis:

After treatment, both the experimental and control fish were sacrificed at the end of 30 days. Gills and liver tissues were removed and put in 10% formalin. After fixation for 24-30 h, tissues were dehydrated through a graded series of ethanol, cleared in xylene and infiltrated in the paraffin. Sections of 5 μm were prepared from paraffin blocks by using a rotary microtome. These sections were then stained with Hematoxylin-Eosin for histopathological analysis and photographed using Leica photomicroscope.

3.RESULT:

Control group of Gills:

Gills of control fish (*Channa striatus*) show a histological picture similar to that of a normal fish with the lamellae lined by squamous epithelium composed by pavement and nondifferentiated cells. In control fish, the secondary gill lamellae (SGL) appeared as finger-like structures. The SGL was thin, slender and attached on either side of the primary gill lamellae (PGL) (Figure-1)

Treated groups of Gills:

After 30 days of expose to 100mg/l of 2,4-D, the gills exhibited fusion and shortening of lamellae, excessive secretion of mucous in the intercellular space, hypertrophy, fusion of secondary gill lamellae, primary gill lamellae and necrosis were found in the gills of 2, 4- D treated (*Channa striaus)* (Figure-2). After 30 days, the gills exhibited cytoplasmic vacuolization, necrosis and hypertrophy which were some of the observable changes. The severe pathological changes of gill lamellae were noticed to 200 mg/l for a week (Figure-3)

Control group of Liver

Liver of fish is responsible for digestion, filtration and storage of glycogen. The liver produces many enzymes. The liver functions to store food energy. The normal liver is made up of continuous mass of hepatocytes with large number of blood sinusoids **(**Figure-4**).**

Treated group of Liver:

After 30 days, the histopathological analysis exhibits liver enlargement of hepatocytes, aggregation of nucleus and hypertrophy(100 mg/l). Additionality, disintegration of central vein were also observed in the liver. (Figure-5).The fish were exposed to the sublethal concentrations of 200mg/l of 2, 4- D for 30 days. After 30 days fishes exhibited rupture of hepatocyte, necrosis, space formation and vacuolization of the liver, enlargement of hepatocytes and displacement of nuclei **. (Figure -6)**

4.DISCUSSION:

Histopathological results indicated that gill was the primary target organ affected by dichlorvos. Gills are generally considered as good indicator of water quality (Rankin *et al*., 1982), being models for studies of environmental impact (Mallat, 1985; McKim and Erickson, 1991; WenderlaarBonga and Lock, 1992), since the gills are the primary route for the entry of pesticide. In fish, gills are critical organs for their respiratory, osmoregulatory and excretory functions. Rao *et al*. (2006) observed uncontrolled degeneration of the primary lamellae and secondary lamellae, hypertrophy, hyperplasia, necrosis of the epithelial cells, epithelial lifting, dilation of the blood sinuses of the secondary lamellae, lamellar aneurism, hemorrhages in the gill of fish exposed to profenofos. Gills are the first route of entry for pesticides into fish, and, in response to environmental changes, they may present adaptive strategies to preserve physiological function (Menezes-Faria *et al*. 2007). Histopathological anomalies in the gills due to contamination by pesticides and other toxic agents have been revealed by many investigators (Abdel-Moneim *et al*. 2012, Menezes-Faria *et al*. 2007, Simonato *et al*. 2008). Lamellar fusion is a similar defense mechanism that decreases the gas exchange surface vulnerable to the action of the herbicide. Similar changes were described by Zodrow *et al*. (2004) in investigation using acute exposure.

Sarma *et al*., (2012) observed marked necrosis,vacuolisation and loss of hepatocytes in the liver. Shastry and Agarwala, (1975) also repoted binucleated and balloon shaped cells and necrosis in liver of *Heteropneustes fossilis*. The toxic effects of pesticides on various aspects of liver of fish were reported by many authors, (Konar, 1970;Bhatia, 1977). (Ammnikutty and Rege, 1977) noted disturbances in the arrangement of hepatic cords, swelling of hepatocytes in the liver of thiodan exposed *Gymnocorym busternetzi* at .0004 ppm and .00053 ppm concentration. The present study about the acute toxicity of 2,4-D herbicide represents an important tool for assessing possible risks to similar species in the aquatic environment and for determining ecotoxicological, morphological and biochemical standards in case of contamination with toxic substances. According to Ortiz *et al*. (2003), after exposure to

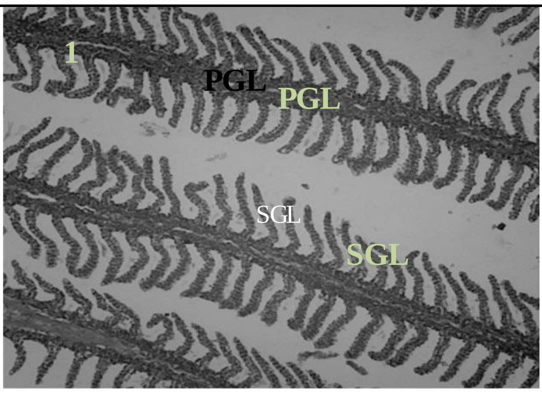

Fig.1:Gills of *Channa striatus* exposed in the control tank shows no pathological lesion.

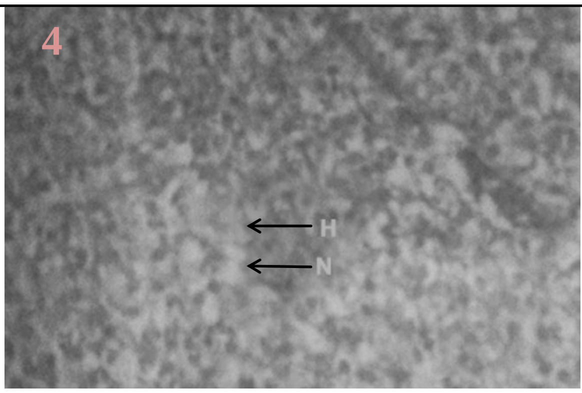

Fig.4:Liver of *Channa striatus* exposed in the control tank shows no pathological lesion.

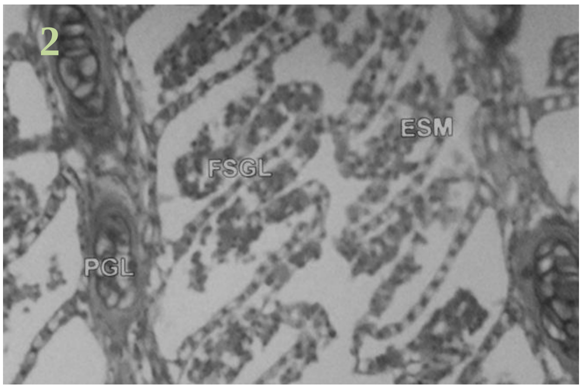

Fig.2:Gills of *Channa striatus* exposed to 100 ml of 2,4-D shows fusion of PGL and SGL.

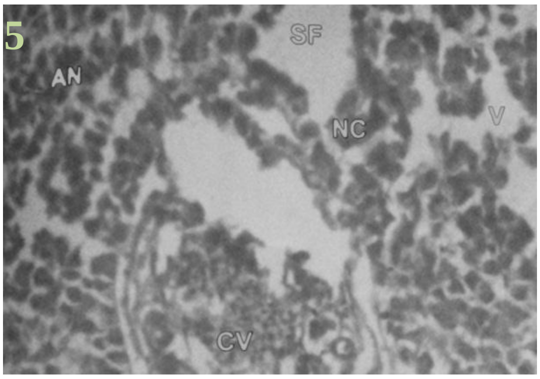

Fig.5:Liver of *Channa striatus* exposed to 100 ml of 2,4-D shows enlargement of hepatocytes, .

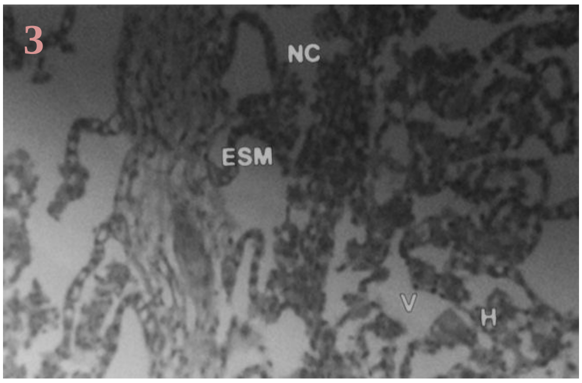

Fig.3:Gills of *Channa striatus* exposed to 200 ml of 2,4-D shows change of ESM and NC

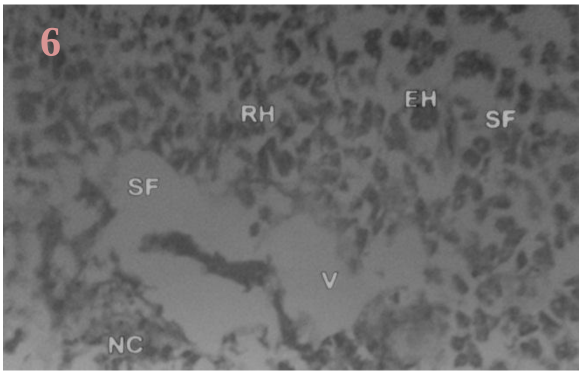

Fig.6:Liver of *Channa striatus* exposed to 200 ml of 2,4-D shows rupture of hepatocyte, necrosis and SF.

toxic substances, hepatocytes showed vacuoles that presented as clear vesicles occupying the entire cytoplasm, thus indicating the toxic potential of 2,4-D herbicide. The vacuolated aspect of hepatocytes found in fish exposed to 10 and 20µl/L of 2,4-D implies that spongiosis can evolve tumor formation, which was confirmed by Hinton and Laurén (1990) and Santos *et al*. (2010). Histopathological hepatic alterations such as hepatocyte vacuolization in the 10 and 20µl/L groups and swollen nuclei in the 40µl/L group suggest a physiological response to cellular stress upon exposure to a toxic agent. In accordance with the studies of Hinton & Laurén (1990) and Menezes-Faria (2009) these alterations likely occurred because the liver is important for many nutritional functions such as lipid and carbohydrate storage. Histological effects of xenobiotic on organs of fish have been studied by several authors, in view of the studies cited above, and it is apparent that in the present investigation, 2,4-Dichlorophenoxyacetic acid at sublethal concentrations caused considerable histological damages to the organs studied. It is concluded that more or less similar pathological changes are induced in the gills and liver of different fishes by different toxicants, damage varies depending upon the dose of chemical, duration of exposure, toxicity of chemical and susceptibility of the fish.

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