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RESEARCH ARTICLE

A STUDY ON HISTOLOGICAL ALTERNATIONS IN GILL AND LIVER OF *Heteropneustes fossilis* UPON EXPOSURE TO HERBICIDE 2, 4 DICHOLOPHENOXYACETIC ACID

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ABSTRACT

This study was carried out to evaluate the effects of 2, 4 D on gill and liver tissue of *Heteropneustes fossilis* in order to understand its toxicity. The experimental fishes were exposed to two sublethal concentrations 3.00 ppm ($1/10^{th}$ of LC_{50}) and 0.60 ppm ($1/50^{th}$ of LC_{50}) doses of 2, 4 D for duration of 30 days. Histopathological analysis showed damage to liver and changes in gill architecture which were characterized by vacuolation, hepatic necrosis, disruption of gill lamellae. Alternation or change in gill structure could impair oxygen intake which eventually might cause death of the fish. The damage in liver tissue might be an indication of poor physiological activity as different important enzymes are secreted by it. Hence the application of herbicides in agricultural field and aquatic body should be monitored as it can reach humans through food chain.

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INTRODUCTION

The effect of different chemicals has serious impact on the health of humans as well as animals of both aquatic and terrestrial habitat. Herbicides are chemicals which are commonly used to control undesirable herbs and vegetation. It is applied in water bodies to control aquatic weeds. It can also enter the aquatic ecosystem through surface runoff. However, high concentration of herbicides could lead to significant losses of aquatic vegetation and algal biomass, which could ultimately hamper the aquatic fauna. As pesticides are not fully selective, it is found from Environment Toxicity studies that these substances affect non-target species in the environment (Rao, 2006). When the concentration of herbicide exceeds tolerant level it causes damage to important vital organs and reduces the survival, growth and the reproduction performance of aquatic organisms (Rahaman *et al.,* 2002). Fish is good indicator of aquatic contamination because its biochemical stress response is quite similar to those found in mammals (Mishra and Shukla, 2003). 2, 4 Dichlorophenoxyacetic acid, usually referred to as 2, 4 D, is a chlorinated phenoxy herbicide, often used in agricultural fields. Commercially it can be found in various forms such as acids, amines, various salts and ester derivatives, which vary in chemical properties and toxicity.

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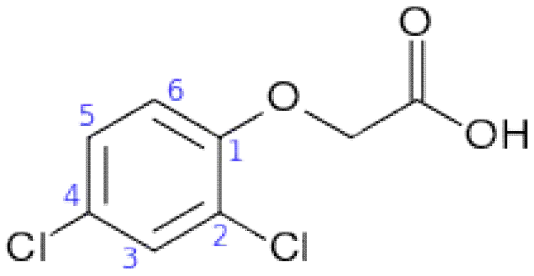
It is used for weed control of rice, wheat, maize, aquatic weeds and also employed for post-emergence foliar spray (Farah *et al.,* 2004).



Chemical structure of 2, 4-D

The intensive and widespread use of 2, 4 D exhibit deleterious physiological, behavioural, neurological, immunological, histological and hematological alternations as reported by many observers at different times (Tuschl and Schwab, 2003; Stürtz *et al.,* 2008; Uyanikgil *et al.,* 2009; Kubrak *et al.,* 2013) and has led to the emergence of herbicide resistant weeds (Watanabe *et al.,* 1997; Bradberry *et al.,* 2000; Toyama *et al.,* 2003). Thus, this study was undertaken to establish the effect of sublethal concentrations of 2, 4 Dichlorophenoxyacetic acid (a herbicide) on histopathological changes in gills and liver of catfish, *Heteropneutes fossilis*.

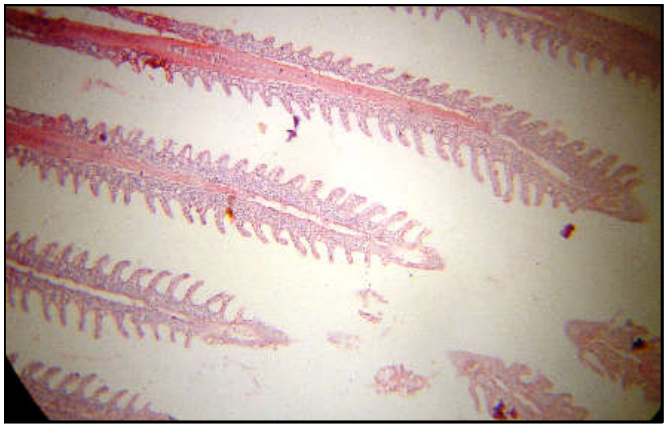


Fig. 1.Gill structure of normal control fish. (Magnification X100)

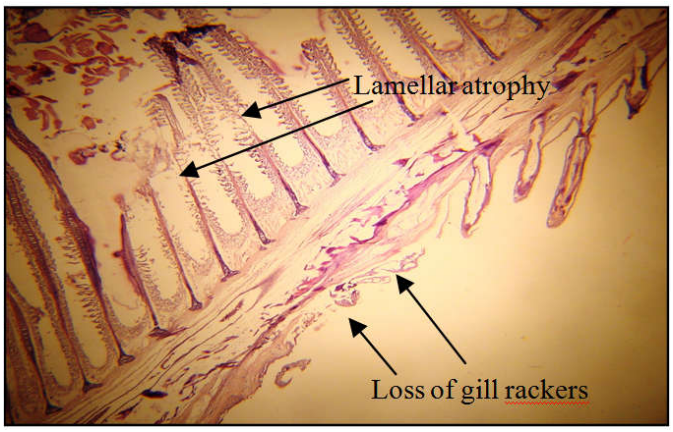


Fig. 2. 3.00 ppm (1/10th of LC_{50}) 2, 4 D treated Gill. (Magnification X100)

Fig. 3. 0.60 ppm (1/50th of LC_{50}) 2, 4 D treated Gill. (Magnification X400)

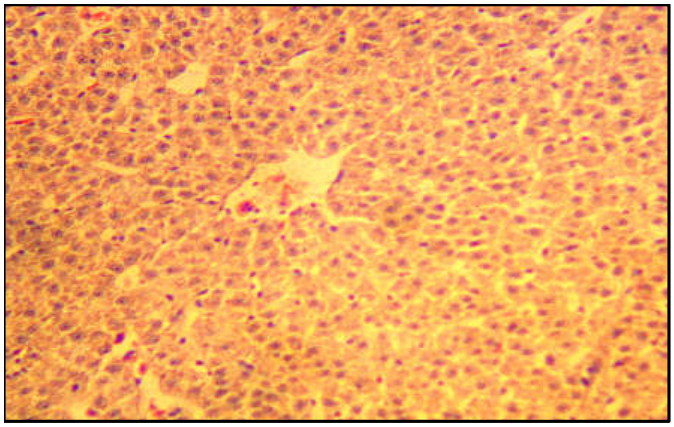


Fig. 4. Liver of normal control fish. (Magnification X100)

Fig/ 5. 3.00 ppm (1/10th of LC_{50}) 2, 4 D treated Liver. (Magnification X400)

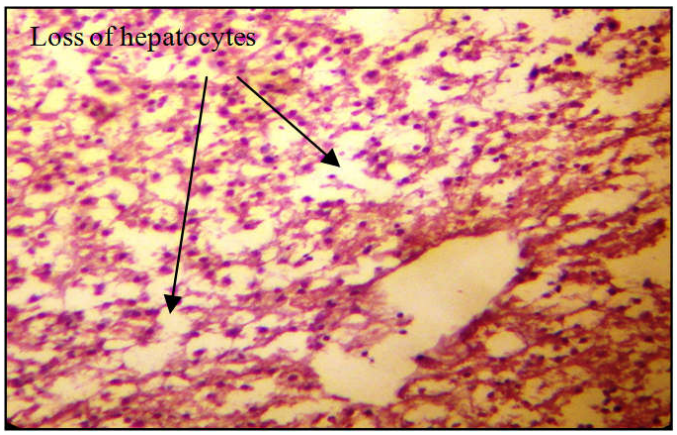


Fig 6: 0.60 ppm (1/50th of LC_{50}) 2, 4 D treated Liver. (Magnification X100)

MATERIALS AND METHODS

The experimental fish, *Heteropneustes fossilis* weighing about 10 gm and length of about 11 cm were purchased from local fish market of Dhubri town, Assam. Prior to the start of the experiment, the fishes were acclimatized for about a week in an aquarium under suitable laboratory conditions. Sublethal dose of 2, 4 D (3.00 ppm i.e., 1/10th of LC_{50} and 0.60 ppm i.e., 1/50th of LC_{50}) were administered separately in the experimental fishes for a period of one month (i.e., 30 days). The experiments were carried out in three different aquaria, one of which was left untreated as normal control group and the other two each containing the different treated group.

The fishes were grouped in three different groups each containing 5 fishes:

Group-I represent normal control group,
Group-II represent 2, 4 D (3.00 ppm) treated group and
Group-III represent 2, 4 D (0.60 ppm) treated group.

Histological method

Fishes were collected from each group randomly after the exposure of 30days and dissected to collect the liver and gills. The specimens were taken in normal saline solution and fixed in 10% formalin. It was then hydrated in graded alcohol, then cleared in xylene and finally embedded in molten paraffin

block. Using rotary microtome, the specimens were sectioned at 4µm thickness. Standard H & E method was used for staining and finally mounted in DPX. The slides as prepared were examined under microscope for histopathological analysis.

RESULTS

Histopathological changes

Gills: The gill is made up of filaments of primary lamellae from which secondary lamellae arise. Gill arches and gill rays are clearly observed in Group-I fishes (Fig. 1). Comparing with the normal control set, it was found that the fish treated with different sublethal dose of 2, 4 D shows various histopathological changes. The gill of 3.00 ppm 2, 4 D treated group shows degenerative changes with loss of gill rackers as well as atrophy of lamellar structure in the gill filaments (Fig. 2). Mild congestions of blood vessels were observed in the primary lamellae of 0.60 ppm 2, 4 D treated *H.fossils*. The epithelial layer was also seen to be swollen (Fig.3).

Liver: No histological changes were observed in the liver tissue of normal control group (Group-I) of experimental fish. The liver appears as continuous mass of hepatic cells or hepatocytes having cord like arrangement with the central vein (Fig. 4). Result of the present study demonstrated that the sublethal concentration of 2, 4 D produces large histopathological alterations in the liver of treated fishes. Focal and diffuse area of haemorrhage, extensive necrosis with loss of hepatocytes was observed. Thickening of the endothelium of the central vein was also observed in experimental fishes treated with 3.00 ppm concentration of 2, 4 D (Fig.6). Whereas in lower dose of 2, 4 D (0.60 ppm) treated fishes, the cytoplasm of liver tissue showed increased granularity with disintegration of hepatocytes. Focal areas of necrosis and pyknosis of the nuclei were observed in the liver tissue (Fig.5). Moreover in some areas complete loss of hepatic structures were seen.

DISCUSSION

Histopathological anomalies due to contamination of different toxic chemicals have been revealed by many researchers. Moreover the frequency of pathological changes increases with increase in the concentration of toxicants. Fish gill is an organ with a large surface area, highly sensitive to the effect of toxicant (Reiser *et al.,* 2010). The work of Anusuya and Hemalatha, (2015) showed that gills of *Channa striatus* treated with 2, 4 D (100mg/l) has hypertrophy, fusion and shortening of lamellae and necrosis in gill structure. Samanta *et al.,* (2016) also observed similar findings in glyphosate (17.20 mg/l) exposed *H.fossilis*. Literature review showed that histopathological changes such as fusion of secondary lamellae accompanied by hypertrophy or hyperplasia of primary filaments occurred in *Tilapia zilli* treated with aluminium (Hadi and Alwan, 2012). However in 2, 4 D amine treated *C.gariepinus* for 96hr showed mild desquamation of the epithelium, vacuolization and lifting of epithelial layer as observed by Makinde *et al.,* (2015). The liver of bluegill showed rapid shrinkage of parenchymal cells and loss of vaculation as examined by Cope *et al.,* (1970). 15 mg/kg of 2, 4 D treated rat showed disrupted hepatic cords at many areas and upon exposure to 150mg/l 2, 4 D, the liver showed pycnotic nucleus, congestion of hepatocytes and necrotic cells at the end of 4 weeks of treatment (Tayeb *et al.,* 2010). Histopathological alternations were also observed in Rhamdia quelen liver upon exposure to 700mg/l of 2, 4 D for 96 h (Cataneo *et al.,* 2008). The parenchymal cell of liver underwent rapid shrinkage and loss of vacuolation as observed by Cope *et al.,* (1970) upon exposure to 2, 4 D. 96 hr exposure to acute concentration of 2, 4 amine showed hepatitis (Makinde *et al.,* 2015). Degeneration and disintegration in most cytoplasmic contents, necrosis along with pyknosis and rupture of hepatocytes were observed in the work of Pandey and Dubey, (2015) on *H.fossilis* upon exposure to pentachlorophenol (PCP) for 21 days. Deka and Mahanta (2012) were also observed pyknotic nuclei in liver of malathion treated *H.fossilis.* Thus the present investigation showed somewhat similar resemblence with the above findings. The study showed that 2, 4 D at different sublethal concentration causes significant histomorphological changes in the liver and gill of *H.fossilis*. The histopathological changes were seen to be more pronounced in sublethal concentration at higher dose of 2, 4 D than lower dose level. Moreover, the induced histopathological changes in the gills may lead to several physiological stresses in the fish. Hence, we conclude from the results of the present study that the low concentration of 2, 4 D used in this research shows potent histopatological effects while the 2, 4 D toxicity was found to be significant at high dose level causing degenerative changes in tissue architecture.

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