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Research Article

Study of Induce Histological and Biochemical Alterations in *Hypophthalmicthyes molitrix* after Acute Exposure to Deltamethrine

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Authors' Contributions
WA performed the experiments. AK concieved the study design. MI helped in biochemical analysis. HAS did histological analysis. CM and IA helped in fish sampling. ZS did literature survey.

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Deltamethrine, Acute exposure, Silver carp, Serum, Histology.

Abstract | Pesticides not only brought great economic benefits but also cause serious ecological harms like non-target toxicity of organism. Synthetic pyrethroid (deltamethrine) is widely used due to its less toxicity and easy degradation in soil. In this study, fresh water fish *Hypophthalmicthyes molitrix* were exposed to sub lethal concentrations of deltamethrine. Fish were divided into 4 groups and each group contains six fish. Experimental groups *viz.* A, B and C were exposed to 25, 50 and 75% of LC_{50}, respectively for four days. Blood samples for biochemical analysis (blood glucose, cholesterol and total protein) and gills for histology were taken after 96 hrs. After acute exposure to pesticide, significant changes were observed in serum biochemistry and histology. Serum glucose and cholesterol were increased while total protein was decreased. Histopathological result reveled that gills of experimental fish was damage severely resulting Necrosis, Damaged nuclei , rupturing of epithelial cells, Mucous cells and severe lamellar fusion. It was concluded that, deltamethrine is highly toxic for non-target aquatic organisms like silver carp (*Hypophthalmicthyes molitrix*).

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Introduction

Industrial effluents, domestic sewage and pesticides are the important sources of aquatic pollution. Aquatic bodies that run through agricultural or industrial areas have high vulnerability of being polluted by pesticides through surface runoff and leaching. Aquatic ecosystems are greater part of natural environment. Aquatic ecosystems are continuously being polluted with a wide variety of environmental pollutants such as pesticides from industrial, agricultural and domestic activities (Okuku and Peter, 2011).

Environmental pollution is a universal problem in a present society. Pesticides are used to get better crop production and other benefits. Extensivly used pesticides has raised the questions about human health and non-target animals. Unfortunately, the application of these pesticides is highly toxic even at very small concentration to a number of non-target aquatic organisms such as fish (Begum, 2005; El-Sayed *et al.*, 2007). Extensive use of insecticides has contaminates the ecosystems with toxicant. Pesticides are present in different concentrations in groundwater, crop samples and agricultural streams (Mahboob et al., 2015; Sharma *et al.*, 2015; Yadav *et al.*, 2015).

Due to short biodegradation period and lack of the accumulative tendency in organisms, pyrethroid deltame-

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thrine, is extensively used (Laskowski, 2002). Their poisonous effect is in the parts per billion for non-target organisms (Bradbury and Coats, 1989b). Fish are considered to be indicators of environmental pollution because it is very sensitive to the presence of toxicant (Prusty *et al*., 2011). Fish during their life cycle may be exposed to a wide range of pesticides. When pesticides enter into the organs of fish they may considerably alter certain biochemical and physiological processes (Banaee *et al*., 2011). Pyrethroids are absorbed directly through gills into the circulation (Aydin *et al*., 2005). Pyrethroids cause 1000 times' greater toxicity to fish as compare to other groups of birds and mammal (Bradbury and Coats, 1989a).

In biological monitoring program It has been reported that biochemical changes are useful for observing water pollution, environmental quality and the health conditions of aquatic organisms (Kohler *et al*., 2007; Kori-Siakpere *et al*., 2008). Deltamethrine (Pyrethroid) is extensively used to control pest (Bradbury and Coats, 1989a). Sediment samples taken from the lake have confirmed the presence of deltamethrine (Balint *et al*., 1995). Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish during lab experiment (van der Oost *et al*., 2003; Boran *et al*., 2012). Gills are the first target of water borne pollutants due to the constant contact with the external environment (Perry and Laurent, 1993). Since gills in fishes are considered the main passage for entrance of pollutants to the internal body organs (Takashima and Hibiya, 1995). Alterations found in this organ are normally easier to identify than functional ones (Fanta *et al*., 2003) and serve as warning signs of damage to animal health (Hinton and Laurén, 1990). This study was designed to check out acute toxic effect of pyrethroid deltamethrine on fish *(Hypophthalmicthyes molitrix*). In this experiment histopathology of gills and biochemical variable (glucose, cholesterol and total protein) was observed after acute exposure to deltamethrine.

Materials and Methods

Experimental animal

Live silver carp **(***H. molitrix***)** were purchased from Himalayan Fish Hatchery Muredke, Sheikhupura. The average weight and length of fish specimen was 160.33±5.27 g and 9.96±0.138 cm, respectively. Fish were acclimatized with uninterrupted air supply with air pump in aquarium containing 40 liter tap water (without chlorination) for duration of two week at room temperature. After it fish were exposed to toxicant for 96 h. During this period dead fish were removed instantaneously. This study is performed at lab of Department of Zoology, Government College of Science, Wahdat Road, Lahore, Pakistan.

Pesticides

Commercially synthesized deltamethrine (Parathyroid) was used as toxicant and the concentration applied on experimental groups were described in our earlier reports (Karim *et al*., 2016a, b). Fishes were divided into four groups. One of them was a controlled group and A, B, C were experimental groups. Each group contain six fish (n=6). According to LC_{50} values three sub-lethal concentrations of deltamethrine were prepared. Three doses used for the experiment groups A, B, and C was as follows: Group A= 0.4 µg/ L (25% of LC_{50}), Group B =0.8 µg/ L (50% of LC_{50}), Group C=1.2 µg/L (75% of LC_{50}).

Biochemical and histological analysis

After 96 h fish blood was drawn directly in sterilized syringes through cardiac puncture for biochemical studies of blood glucose, cholesterol and total protein. 2ml of blood was taken in serum vacutainer (devoid of any clotting factor) to separate the serum. At 4000rpm blood was centrifuged for twenty minutes. Then separated serum was stock up at -20°C until study. Small piece of gills was fixed in 10% formalin after rinsing with saline solution for histological studies. The process of gills fixation was done by following standard protocol (Mumford, 2007).

Statistical analysis

The data ware statistically evaluated by One Way ANOVA test with SPSS 13 Statistical program and data were reported as Mean ± SEM with n=6.

Results

Glucose

After 96 h exposure, the serum glucose level significantly increased. The concentration of glucose in control group was 54.2±0.80 mg/dl, when fish exposed to sub lethal concentrations of deltamethrine, glucose values increased significantly (P<0.000) in group A (62.8±1.15 mg/dl), group B (74.6±1.53 mg/dl) and group C (63±1.30 mg/dl) in acute exposure, respectively (Figure 1A).

Cholesterol

The concentration of cholesterol significantly increased in experimental groups as compared to control group. The concentration of cholesterol in control group was 177±5.08 mg/dl, when fish exposed to sub-lethal concentrations of deltamethrine, cholesterol values increased significantly (P>0.000) in group A (212±4.06 mg/dl), group B (227.6±3.26 mg/dl) and group C (229±1.81 mg/dl) in acute exposure, respectively (Figure 1B).

Total protein

The concentration of total protein significantly decreased in experimental groups after 96 h exposure to deltamethrine. Concentration of total protein in control group was 6.64±0.23 mg/dl, when fish exposed to sub lethal concentrations of deltamethrine, total protein values decreased significantly (P<0.000) in group A (5.25±0.27 mg/dl), group B (4.4±0.20 mg/dl) and group C (4.22±0.23

mg/dl) in acute exposure, respectively (Figure 1C).

Histopathology of gills

Histopathological investigations are reliable biomarkers to check toxicity induce by pesticide. Normal aspect of control group gills showed primary lamella, secondary lamellae, mucous cell, chloride cell, pillar cell and epithelial cell. Histopathology of group A gills after acute exposure showed necrosis, damaged secondary lamella, damaged primary lamellae. Deltamethrine induce alteration in gills of group B after acute exposure resulting necrosis, degeneration, damaged nuclei, and rupturing of epithelial cells. Gills texture of group C was altered severely causing necrosis, hypertrophy of mucous cells and lamellar severe fusion. These results reveled that deltamethrine badly effect the gills of experimental groups (A, B and C) as compare to control group (Figure 2).

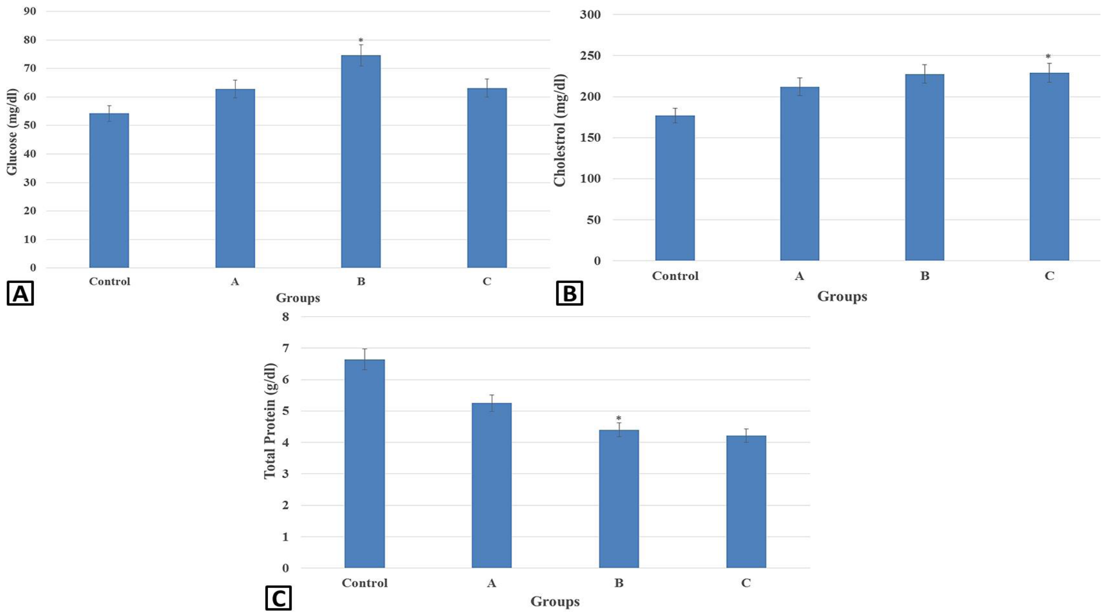

Figure 1: Glucose (A), cholesterol (B) and total protein (C) concentrations in control and experimental groups.

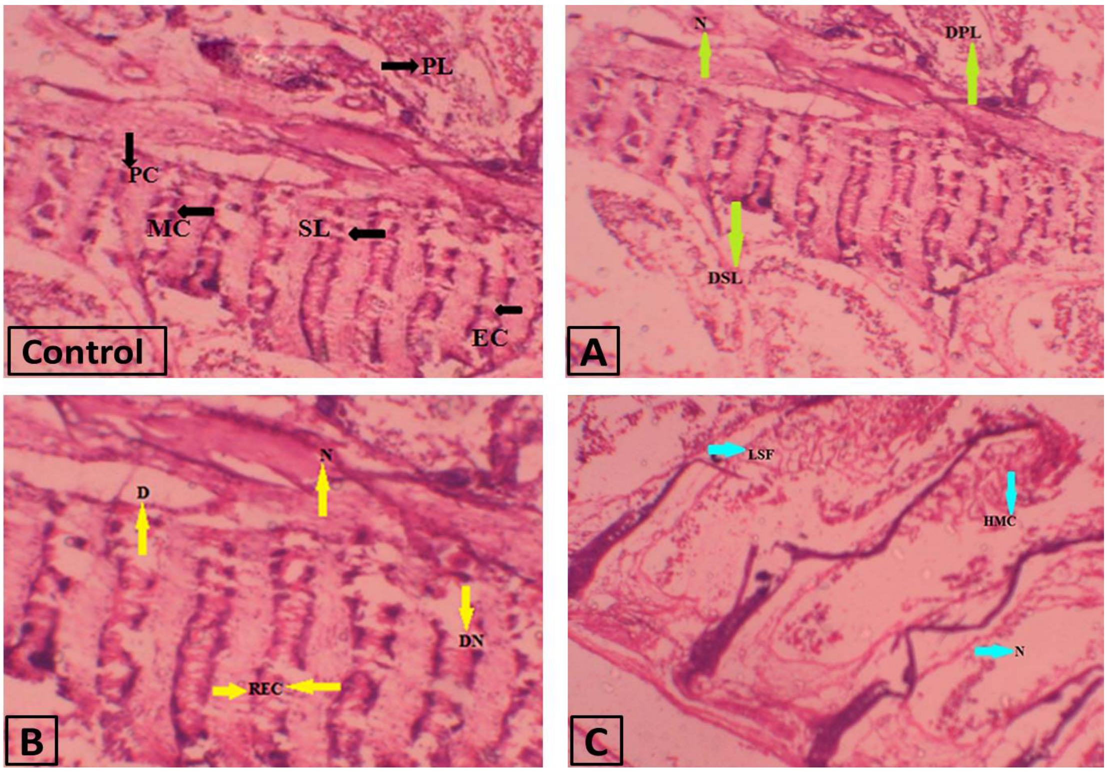

Figure 2: Photomicrograph of Gills of *Hypophthalmicthyes molitrix*. section of control group showing primery lamella (PL), secondary lamellae (SL), mucous cells (MC), epithelial cells (EC) and pillar cells (PC). Group A showing necrosis (N), damaged secondary lamella (DSL), damaged primary lamellae (DPL). Group B showing necrosis (N), degeneration (D), damaged nuclei (DN) and rupturing of epithelial cells (REC). Group C showing necrosis (N), hypertrophy of mucous cells (HMC) and lamellar severe fusion (LSF).

Discussion

The estimation of serum characteristics of blood plasma in fish has become a diagnostic tool to study the toxicological and pathological impacts of pollutant like pesticides (Suvetha *et al*., 2010). Glucose provides energy to organism but hyper-glycemia in blood due to pesticide exposure is a common marker of environmental stress in fish (Sepici-Dinçel *et al*., 2009). In agreement with our results (Ceron *et al*., 1997) observed significant hyperglycemia in common eel (*Anguilla anguilla*) after exposing it to sub-lethal concentrations of diazinon .Glycogen in hepatic tissue of fish was reduced and caused the Hyperglycemia against toxicant insult. After cypermethrin (pyrethroid) exposure to Indian major carp *Labeo rohita*, Increased blood glucose level was reported (Das *et al*., 2003) which are similar to our results. The low level of blood protein may be due to degradation or increased proteolytic activity or reduced protein production (Shakoori *et al*., 1990; Sulekha and Mercy, 2011). The important reason for low synthesis of total protein, immunoglobulin and albumin by the liver was exposure of diazinon which damaged the hepatocyte (Gokcimen *et al*., 2007). In agreement with our result a low level of serum total protein was reported in fish *Oreochromis niloticus* (One *et al*., 2008) and in *Rhamdia quelen* (Borges *et al*., 2007) in response to Cu and cypermethrin exposure, respectively.

The increase in blood cholesterol level was due to less excretion of cholesterol in the bile duct and the large amount of cholesterol produced Kohler by liver as a result of stress. Similar increase in serum cholesterol was observed in fish after exposure to pollutants (Goel and Garg, 1980; Ghazaly, 1991). In *Rhamdia quelen* after cypermethrin exposure hypercholesteremia was observed (Borges *et al*., 2007). In agreement with our result, Yousef *et al.* (2003) reported that aggregation of pesticides in the liver increased the permeability of hepatic cells resulting into hypercholesteremia and disrupt lipid metabolism.

Histopathology of gill is the appropriate bio indicator to pollution monitoring because gills come in immediate contact with the environment, tissue damages brought about by water borne pollutants can be easily observed (Dutta *et al*., 2003). Deltamethrine induce alteration like damaged nuclei, rupturing of epithelial cells, mucous cells and severe lamellar fusion. These types of lesions were seen (Das and Mukherjee, 2000; Cengiz and Unlu, 2006; Sahoo *et al*., 2017).

Conclusion

Result of this study concluded that deltamethrine induce serological and histological changes in silver carp so there is need to use biological control method in replacement of pesticide as alternative method to control pests.

Conflicts of interest

The authors declare no conflicts of interest.

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