Research Article

Neemax Induced Histological Changes in the Liver of *Cyprinus carpio*

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Abstract

Objective: The aim of this study, was to evaluate the toxicity effect of Neemax on liver of common carp (*Cyprinus carpio*) as histological biomarkers through acute and chronic exposure with different concentrations. **Material and methods:** The toxic effects of Neemax on liver of fish, *Cyprinus carpio* were studied. Fishes were starved for first 24 h and then fed with rice bran mixed mustard oilcake in the ratio of 2:1 during acclimatization. There are 6-10 specimens exposed to the sub lethal dose for the 15, 30, 45 days with a control group simultaneously. After a particular time, fishes were sacrificed and liver tissue was muscle dissected for histology. **Results and conclusion**: The result showed remarkable effect of Neemax toxicity as compared to the control group. Liver appeared with multiple markers such as hepatocytes degeneration, increased sinusoids and marked degeneration with necrosis and none of these morphological changes were found in control fish.

Keywords: Toxicity, *Cyprinus carpio*, Histological markers, Neemax

Introduction

Pesticides have been one of the most effective weapons discovered by man to protect agricultural products from pests. However, they are the major cause of concern for aquatic environment due to their toxicity, persistency and tendency to accumulate in the organisms (Joseph and Raj, 2010) and it is difficult to remove them from any aquatic ecosystem. Pesticides applied in the environment can find their way into water bodies either from the air or by runoff or by percolation to groundwater. There are four major routes through which pesticides can reach the water bodies: it may drift outside of the intended area when it is sprayed, it may percolate, or leach, through the soil, it may be carried to the water as runoff, or it may be spilled, for example accidentally or through negligence. They may also be carried to water by eroding soil. Factors that affect a pesticide's ability to contaminate water include its water solubility, the distance from an application site to a water body, weather, soil type, presence of a growing crop, and the method used to apply the chemical. Once pesticides enter water bodies they have a potential to cause harmful effects on human health, aquatic organisms and can cause disruptions of the aquatic ecosystems. This may result into a loss in fish production in streams and large water bodies especially where fishing is one among the major economic activities of a particular community (Zacharia, James Tano et al. 2011).

Fishes are considered good indicators of environmental contamination because they are aquatic organisms that bio-accumulate several classes of pollutants in their tissues from water and food sources. In particular, the common carp *C. carpio* is an economically important fish spread worldwide in cultures in Asia, Europe and Latin America and is easily maintained in laboratory.

The studies on histopathological changes in fishes have been carried out by Das and Mukerjee, (2000), Cengiz and Unlu, (2002), Parashar and banerjee, (2002), John et. al. (2007). (Kunjamma et al. (2008), Velmurugan et al. 2009, Rani and Venkataramana, (2012). In view of above it was felt that it would be worthwhile to study the histological changes in the liver of the fish which would throw a clear light on the extent of effect that it causes. Hence in the

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present work, we studied the toxic effects of Neemax on histological changes in liver of fish, *Cyprinus carpio*.

Materials and methods

Fish were collected from ponds of local government hatchery with the help of fisherman, Fishes were caught by fishing net and carefully packaged into aerated polythene bags filled with water. Fishes were brought to laboratory and immediately given 0.05% potassium permanganate treatment for two minutes for disinfecting them. After disinfectant treatment they were transferred into plastic pools of 500 litres capacity for two weeks acclimatization to laboratory conditions. Fishes were starved for first 24 hrs and then fed with rice bran mixed mustard oilcake in the ratio of 2:1 during acclimatization. Water of the pool was changed daily and dead fishes whenever located were removed immediately A few mortality occurred and fishes appeared normal and healthy.

The experiment was conducted under natural photoperiod and temperature. The water quality was measured as per APHA (2005). The temperature of the experimental water was 25±1.5^{0}C, pH was 7.4±0.4, and dissolved oxygen was 6.4±0.6 mgl^{-1}and total hardness as calcium carbonate was 148±3.2 mgl^{-1}

Common carp *Cyprinus carpio* fish individuals of size, 10-15 cm, and weight, 50-65 gm are sorted and starved for 24 hrs before starting the experiment. There are six to ten specimens exposed to the sub lethal dose for the 15, 30, 45 days and a control was run simultaneously. At the end of each exposure period, fishes were sacrificed and tissues such as liver, muscle dissected and removed. The tissue 10mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 min and the clear supernatant was used for the analysis of total protein.

Fishes were sacrificed at 15, 30 and 45 days of exposure. Fishes were first immobilized in ice and then dissected out carefully; liver were removed and fixed in bouins fluid for 24 hr and then processed and embedded in paraffin for block preparation. The sections were cut at 5-6 micron and stained in haematoxylin and eosin. The slides were examined under light microscope and photographed for histopatholgical effects.

The organ most associated with the detoxification and biotransformation process is the liver and due to its function, position and blood supply (Van der Oost et al., 2003). It is also one of the organs most affected by contaminants in the water (Rodrigues & Fanta, 1998).

Results and discussion

The physical and chemical properties of water used in the present study were determined. The measured values were always within the maximum permissible limit of (APHA, 1992) indicating an unpolluted nature of the water. The values are represented in table 1.

Table 1. Water Quality of Upper Lake, Bhopal

Sr. No.	Parameters	Results
1	pH	7.4
2	Total dissolved Solid	214 mg/l
3	Turbidity	11.4 NTU
4	Alkalinity	124 mg/l
5	Total Hardness	148 mg/l
6	BOD	6.2 mg/l
7	COD	16.6 mg/l
8	Nitrate	0.41 mg/l
9	Phosphate	0.038 mg/l
10	Chloride	34.99 mg/l
11	Sulphate	11.2 mg/l
12	Dissolved Oxygen	6.4 mg/l

Liver is the first organ to face any foreign molecule through portal circulation and is subjected to more damage. Liver is an important organ of detoxification which breaks down toxic substances and metabolites of administered substances (Shoeiba Tasneem et al., 2014).

The LC50 value of (Neemax) 700ppm 1/10th LC50 value i.e 70 ppm was taken for neemax to see the histopathological changes in the liver after completion of 15, 30 and 45 days respectively as mentioned in table 2 and figure 1.

The liver is the primary organ for metabolism, as well as detoxification of pollutants like pesticides, and discharge of harmful substances. The major functions of the liver involve protein, lipid and carbohydrate metabolism, as well

Table 2. Histopathological parameter changes in liver

Test (with wet Muscle)	Control	15 days	30 days	45 days	90 days
T. Glucose	45.35±0.650	41.12±0.720	30.275±0.675	27.657±0.720	25.237±0.819
T. Glycogen	7.295±0.356	6.34±0.350	5.252±0.325	4.101±0.345	3.728±0.425
T. Protein	35.370±0.975	36.00±0.930	25.250±0.940	20.050±0.960	16.060±0.860
T. Lipid	7.585±0.515	7.680±0.592	6.275±0.625	6.768±0.415	5.820±0.512
T. Free Amino acids	25.450±0.550	42.10±0.980	51.25±0.535	56.210±0.610	61.410±0.820

as detoxification, which could lead to histological damage. The changes in biochemical parameters such as carbohydrates, proteins and lipids are important to indicate the susceptibility of organ systems to pollutants by altering their function. Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism (Remia et al., 2008).

Protein can be expected to be involved in the compensatory mechanism of stressed organism. The result of the present study showed that when the fish were exposed to Neemax (0.05ppm) the protein content were found to have decreased (Table 2). The present decrease was found to be greater in all exposures in liver tissue. The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress (Remia et al., 2008).

The results of the present findings showed a significant decrease in carbohydrate content in all the tissues studied (Table 2). The decrease in carbohydrates contents may result in impairment of carbohydrate metabolism due to toxic effect (Thenmozhim et al., 2010). The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition. Many workers reported a similar trend of decrease in carbohydrate (Venkatramana et al., 2006; Thenmozh et al., 2010).

Various authors studied similar reduction of lipids in various tissues (Srinivas et al., 1991) has showed decreased lipid content in *T. mossambica* on exposed to atrazine. Gradual depletion in lipid content of liver and muscle when exposed to malathion was analysed by (Mishra et al., 2004). The results of the present findings showed a significant decrease in lipid content in all the tissues studied (table 2).

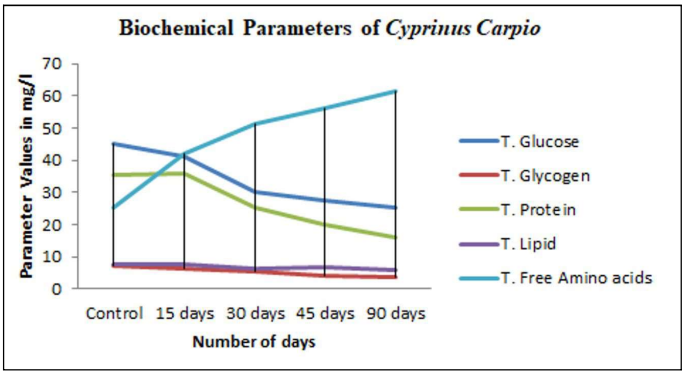

Figure 1. Histopathological parameter changes in liver

Conclusion

The present study has produced a better perception of the possible disruptions to the aquatic biota exposed to low levels of Neemax over a long period in laboratory in such a way to reflect that what might happen in the field conditions. Therefore the use of the pesticides in farm lands should be controlled to prevent possible contamination by reaching into the aquatic environment to protect the aquatic organism from toxic chemicals.

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