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RESEARCH ARTICLE

SUBLETHAL HAEMATOLOGICAL EFFECTS OF DICHLORVOS ON THE FRESH WATER FISH, *CYPRINUS CARPIO* VAR. *COMMUNIS*

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ABSTRACT

The acute toxicity of an organophosphorous pesticide dichlorvos on haematological parameters of an economically important fresh water teleost fish *Cyprinus carpio* var. *communis* was evaluated under static conditions. The median lethal concentration or Lc_{50} value of dichlorvos was found to be 0.95 mg/l indicating the high toxicity of the pesticide. Fishes were exposed to different concentrations of dichlorvos and the alterations of the different haematological parameters were studied at the end of 24 hrs and 96 hrs of exposure. Reduction in the number of red blood cells, packed cell volume, mean corpuscular haemoglobin indicated anemia and also a decrease in white blood cells was indicated a decrease in the immunity of the exposed fish.

INTRODUCTION

In India, the use of pesticides in agriculture has significantly increased during the past three decades. The agricultural runoff in the aquatic environment has lead to massive killing of fish and hence warrants close attention. To avoid such damage it is imperative to test the toxicity of pesticide before they are applied to the agricultural field on a large scale. Dichlorvos, also known as DDVP (o:o-dimethyl =2,2- dichlorovinyl phosphate) is also an organophosphorous pesticide which has been produced and used all over the world since 1961 and is employed as an insecticide in agricultural products, in controlling the internal and external parasites of farm animals, house hold insects, insects of public health importance such as flies and mosquitoes in open places, for the control of insects of stores and food processing plants such as flies and moths in mills, bakeries, dairies etc. In Kashmir markets, it is sold in the market under the trade name of Nuvan. Fish is one of the important dietary constituents of the food of the people of Kashmir. The valley being land locked, bowl like, people are fond of eating fish in harsh weathers when the valley remains cut off for days together with rest of the world. The people of Kashmir, mostly due to climatic conditions are non vegetarians and fish forms their choicest food from the time immemorial. However, during the last few decades due to indiscriminate use of pesticides in agricultural and horticultural practices, the fish production has decreased significantly (Yousuf, 1996 and Bhat *et al*., 2012). Kashmir valley being bowl type, all these harmful pesticides and other toxic substances find directly their way directly into the river Jhelum, which is the lone drainage water body of Kashmir and have polluted the river Jhelum significantly. Fish being at higher trophic level has been affected badly, its population and density in the river Jhelum has decreased drastically (Bhat *et al*., 2010). Haematological values of fish have also been used as probes in connection with pollution and its effects. Recently, pesticide pollution of the aquatic environment has received wide spread attention. Bouck and Ball (1966) stated that haematology may be a useful tool in monitoring stress levels of aquatic pollution on fish. Johnson (1968) suggested the use of fish blood parameters as best in studies and detection of pesticide toxicity. In recent years fish haematology has became an increasingly important tool of fishery biologists and research ichthyologists. Its uses are many and varied and many offer a reliable indication of physiological well being. Haematological research is the major means to learn a toxicants mode of action. Haematological parameters can be detected rapidly and hence can be used for prediction and diagnosis of pesticide toxicity. It is with this background that the present study was taken to evaluate the toxicity of various sub lethal doses of Dichlorvos in *Cyprinus carpio* var. *communis* based on the results of haematological investigation.

MATERIALS AND METHODS

Alive, healthy and disease free specimens of *C. c.* var. *communis* used in the present study were collected from the local water bodies and acclimatized in the laboratory for 15 days in 50 litre glass tanks before they were used for experimentation . The average length and weight of the fish were 15±1.5cm and 105±6.5gms respectively. Fishes were then put into four separate glass aquaria keeping one aquarium as control. Each aquarium containing fresh dechlorinated water was fitted with artificial aerators to ensure proper aeration. During acclimatization fishes were fed with a commercial fish food once daily. Left over food in the aquarium was removed daily when water of the aquarium was changed. Dead fish if any was removed immediately to avoid fouling of water. After two weeks of acclimatization, fishes were starved for 24hrs prior to exposing them to different concentration of pesticide. The three sub lethal concentrations 50%, 60% and

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70% of Lc_{50} was calculated for experimental studies. Four aquaria each filled with about 40 ltrs of fresh dechlorinated water and 10 acclimatized fishes were taken. The four groups of fishes were treated for 96 hrs as follows.

Group 1 Control
Group 2 Exposed to 50% Lc_{50} value
Group 3 Exposed to 60% Lc_{50} value
Group 4 Exposed to 70% Lc_{50} value

The water of all the aquaria was changed after 24 hrs and different volumes of pesticides were added to obtain the required concentrations. A control set with the same number of fish and same volume of water was run for comparison. Blood samples were collected directly from the heart of the experimental fish with the help of heparinized syringes. For blood collection, the experimental fishes were divided in two groups, each comprising of 5 fish in group I and group II. Blood from the first group was collected at 24 hr exposure and from the 2nd group after 96 hrs of exposure for the estimation of various parameters like total RBC Count, Packed Cell Volume, Mean Corpuscular Haemoglobin and total WBC Count. After blood collection, the samples were processed immediately for the estimation of these parameters. The results were processed stastically using analysis of variance (ANOVA) and are presented as mean±SD.

RESULTS

Mortality studies showed that the sub-lethal level Lc_{50} of *C. c.* var. *communis* for 96 hr exposure was 0.95 mg/l for dichlorvos. The minimum effective doses 50% of Lc_{50} (0.48mg/l), 60% of Lc_{50} (0.66mg/l), 70% of Lc_{50} (0.85mg/l) were calculated for experimental purposes .

Haematological estimation like total RBC Count, Packed Cell Volume, Mean Corpuscular Haemoglobin and total WBC Count of control as well as treated *Cyprinus carpio* var. *communis* were carried out. The results were as follows.

Haemoglobin and Haematocrit

Initial mean value of haemoglobin in control fishes was found to be 8.7g%.The normal value significantly ($p<0.01$) changed to 8.1g%, 7.6g% and 7.2g% after 24hrs exposure to 0.48mg/l,0.66mg/l and 0.85mg/l of dichlorvos respectively. At the end of 96hrs of exposure, the haemoglobin concentration significantly ($p<0.01$) decreased to 7.7g%, 7.3g% and 6.8g% after exposure to 0.48mg/l,0.66mg/l and 0.85mg/l of dichlorvos respectively. The mean value of Haematocrit found in the control fish recorded was 31%. After 24 hrs of exposure to three sub lethal concentrations of dichlorvos i.e. 0.48mg/l, 0.66mg/l and 0.85mg/l, the normal value significantly ($p<0.01$) changed to 27.5%,26% and 24.5% respectively. At the end of 96hrs of exposure to above three sub lethal concentrations of dichlorvos, the value of haematocrit significantly ($p<0.05$) changed from 31% in control fish to 28%, 25.5% and 23% respectively.

RBC and WBC count

During the present study both RBC and WBC count showed reduction in number after exposing to different concentrations and period of exposure of pesticides (Table 1). The normal mean value of RBC count in *C. c.* var. *communis* was recorded $1.86\times10^6/mm^3$, which significantly ($p<0.01$) dropped to $1.65\times10^6/mm^3$,$1.58\times10^6/mm^3$, and $1.48\times10^6/mm^3$ upon 24hrs exposure to 0.48mg/l,0.66mg/l and 0.85mg/l of dichlorvos respectively. At the end of 96hrs exposure to above three sub lethal doses of dichlorvos i.e. 0.48mg/l,0.66mg/l and 0.85mg/l, the normal value significantly ($p<0.05$) changed from $1.86\times10^6/mm^3$, to $1.61\times10^6/mm^3$, $1.50\times10^6/mm^3$, and $1.38\times10^6/mm^3$, respectively. The mean value of WBC count found in the control fish was recorded as $2.98\times50/mm^3$ which significantly ($p<0.01$) dropped to $2.86\times50/mm^3$, $2.82\times50/mm^3$ and $2.76\times50/mm^3$ upon 24hrs exposure of 0.48mg/l,0.66mg/l and 0.85mg/l of dichlorvos respectively. At the end of 96hrs of exposure, the WBC count reduced significantly ($p<0.01$) from $2.98\times50/mm^3$ in control fish to $2.87\times50/mm^3$,$2.80\times50/mm^3$ and $2.76\times50/mm^3$ after exposure to 0.48mg/l,0.66mg/l and 0.85mg/l of dichlorvos respectively. + =($P<0.05$);++ =($P<0.001$),when students 't' test was applied between control and treated groups Values in the parenthesis are percent change with control taken as 100%.

Table 1 Change in certain haematological parameters in fish *C. c.* var. *communis* after dichlorvos exposure. Values are mean± standard deviation of four replicates

Exposure	**Total RBC Count($\times10^6/mm^3$)**			
Time(hr)	**Control**	**0.48mg/l**	**0.66mg/l**	**0.85mg/l**
24 hrs	1.86±0.03 (100)	$1.65\pm0.02^{++}$ (89)	$1.58\pm0.03^{++}$ (85)	$1.48\pm0.03^{++}$ (80)
96hrs	1.86±0.04 (100)	$1.61\pm0.05^{+}$ (86)	$1.50\pm0.02^{+}$ (81)	$1.38\pm0.05^{+}$ (74)
Exposure	Packed cell volume **(g %)**			
Time(hr)	**Control**	**0.48mg/l**	**0.66mg/l**	**0.85mg/l**
24 hrs	31±1.3 (100)	$27.5\pm0.81^{++}$ (89)	$26\pm0.44^{++}$ (84)	$24.5\pm0.36^{++}$ (79)
96hrs	31±1.7(100)	$28\pm0.74^{+}$ (90)	$25.5\pm0.71^{+}$ (82)	$23\pm0.66^{+}$ (74).
Exposure	**Concentration of haemoglobin (g %)**			
Time(hr)	**Control**	**0.48mg/l**	**0.66mg/l**	**0.85mg/l**
24 hrs	8.7±0.60 (100)	$8.1\pm0.40^{++}$ (93)	$7.6\pm0.32^{++}$ (87)	$7.2\pm0.27^{++}$ (83)
96hrs	8.7±0.52 (100)	$7.7\pm0.45^{++}$ (88)	$7.3\pm0.38^{++}$ (84)	$6.8\pm0.32^{++}$ (78)
Exposure	**Total WBC count ($\times50/mm^3$)**			
Time(hr)	**Control**	**0.48mg/l**	**0.66mg/l**	**0.85mg/l**
24hrs	2.98±0.09(100)	$2.86\pm0.07^{++}$ (96)	$2.82\pm0.08^{++}$ (95)	$2.76\pm0.10^{++}$ (93)
96hrs	2.98±0.13(100)	$2.87\pm0.06^{++}$ (96)	$2.80\pm0.09^{++}$ (94)	$2.76\pm0.08^{++}$ (93)

DISCUSSION

Haematoloigical indices are of different sensitivity to various environmental factors and chemicals (Vosyliene, 1999). Haematology and clinical chemistry analysis, although not often used in fish medicine can provide substantial diagnostic information. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of haematological parameters (Van Vuren, 1986). Thus, water quality is one of the major factors responsible for individual variation in fish haematology since they are sensitive to slight fluctuation that may occur within their interval milieu (Fernades and Mazon, 2003). On the basis of haematological studies, it would be possible to predict the physiological state of fish in natural water bodies. Haematological studies in teleosts have indicated that haematocrit values might be useful as a general indicator of fish health, since fish given iron

deficient diets or those exhibiting anaemia, all posses reduced haematocrit (PCV) values (Gatlin and Wilson, 1986). Previous haematological studies of nutritional effects (Rehulka,1989), infectious diseases (Rehulka, 2002a) and pollutants (Rehulka, 2002 b) brought knowledge that erythrocytes are a major and reliable indicator of various sources of stress (Rainza-paiva *et al.*, 2000). Erythrocytes reflect the state of the organism over a prolonged period of time (Sniezsko, 1961). High concentration of pesticides or long term exposure of fish to their sub lethal concentrations usually decreases erythrocyte indices.

In the present investigation significant decrease in the various parameters of blood were observed in *C. c.* var. *communis* due to the treatment of different doses of dichlorvos for short durations. The total number of RBC'S and WBC'S, the haemoglobin content and the haematocrit value registered significant decrease. This is an indication of severe anaemia. The decline in blood cells and haemoglobin content was found to be concentration and time dependant indicating the haemotoxicity of dichlovos to fish. The low levels of haemoglobin indicating anaemic conditions in fish may be due to stress caused hemolysis (Panigrahi and Mishra, 1978) and inhibition of aerobic glycolysis curtailing denova synthesis of haemoglobin (Lewis, 1970, Koundinya and Ramamurthi, 1979, Bielinska, 1987). Matkovics *et al.*, (1981) also observed a quick decrease in haemoglobin content in response to Paraquat toxicity in *Cyprinus carpio.* The lower haemoglobin levels of treated fish in the present study might also be due to disruption of the iron synthesizing machinery (Beena and Vishwarajan, 1987).

Haematocrit (PCV) values of *C. c.* var. *communis* exposed to three sub lethal concentrations of dichlorvos for two exposure periods i.e. 24 hr and 96hr followed the same pattern as for hemoglobin content. The hematocrit values decrease when a fish loses its appetite, is diseased (Blaxhall, 1972) or poisoned by pesticides (Gill and Pant, 1985). The reduction in packed cell volume (PCV) of *C. c.* var. *communis* may also be due to decreased rate of erythropoiesis as well as hemolysis as observed in the *Flounder pleuronectus flesus* when subjected to cadmium toxication (Larson,1975). In addition an alteration in the fish metabolism would have also led to decreased values of haematocrit (Srivastava and Mishra, 1979) in *C. c.* var. *communis.* The reduction in the red blood cell count in the present investigation may be attributed to the disruptive action of the pesticides on the erythropoietic tissues as a result of which the viability of the cells might be affected. In consistence with our data, other researchers have shown a reduction in these parameters in fish exposed to various toxic environments. Decreased erythrocyte count and haemoglobin content in *Cyprinus carpio* after acute exposure to diazinon was reported by Svoboda *et al.*, (2001). Other effective substances of organophosphorus pesticides also induce changes which give evidence for decreased hematopoiesis followed by anaemia induction in fish. Changes in erythrocyte profile induced by acute effect of dichlorvos in *Clarias batrachus* (Benarji and Rajendra Nath, 1990), trichlorphon in *Piaractus mesopotamicus* (Tavaries *et al.*, 1999), melathion in *Cyprinion wabsoni* (Khattak and Hafeez, 1996), formothion in *Heteropneustes fossilis* (Singh and Srivastava ,1994). Seth and Saxena (2003) reported reduction in RBC and Haemoglobin in *Channa punctatus* exposed to sub lethal concentrations of fenvalerate. A reduction in haematological values indicated anaemia in the pesticide exposed fish and may be due to erythropoiesis , haemosynthesis and osmoregulatory dysfunction or due to an increased in the rate of erythrocyte destruction in haemopoietic organs (Jenkins *et al*., 2003; Seth and Saxena,2003). In the present study, the decrease in RBC count during the acute treatment might have resulted from severe anaemia state or haemolysing power of toxicant (dichlorvos) particularly on the red cell membrane.

The reduction in the leukocyte counts in the present study due to exposure of fish to dichlorvos and dimethoate may be due to the mal functioning of the haemopoietic system. Many researchers have reported low values of leukocyte counts in the blood of fish exposed to different pollutants. Chindah *et al.,* (2004) reported a decrease in white blood cell count in *Tilapia guineensis* after acute exposure to organophosphorus pesticide (chloropyrifos). Lymphopenia as a consequence of diazinon (organophosphate pesticide) was reported by Sibel *et al.,* (2006) in European cat fish (*Silurus glanis L.*). These changes in leukocyte count gives evidence for decreased level of non specific immunity in fish after acute exposure to toxic substances. The reduction in the number of lymphocytes in toxicant exposed fish may occur either due to the fall in the delivery of these cells to the circulatory system through a reduced production or alternatively increased rate of removal and rapid destruction of cells.

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