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Research article

Haematological changes induced in the fish *Ctenopharyngodon idella* (Valenciennes) exposed to organophosphate Dichlorvos both technical and 76% EC (Nuvan)

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Key words: *Ctenopharyngodon idella*, Dichlorvos, 76% EC (Nuvan) RBC, WBC, Hb, Haematocrit, PCV, MCH and MCHC, lethal and sublethal.

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Abstract

Defilement of anthropogenic xenobiotic chemicals Viz., pesticides as organophosphate–the dichlorvos alter the circulatory fluid, blood in the heterotrophic, cold blooded nektonic culturableexotic fish, *Ctenopharyngodon idella*. The fish were exposed to both lethal and sublethal concentrations of technical grade and 76% EC (Nuvan). In both the concentrations the branchial single circuit blood pumped through venous blood, right from the early point showed imbalance of homeostasis with a dire consequence of haemostasis. The alterations studied were RBC, WBC, Hb, Haematocrit, PCV, MCV and MCHC. A decrement and increment of the parameters studied were reported herewith.

Introduction

All pollutants are not toxicants but all toxicants are pollutants. The toxicants cause the death if it is in lethal where as in sublethal concentration; render the animal not suitable to maintain its life, the active buoyant swimmers especially the fish, heterotrophic cold blooded animals. Due to the presence even in low concentrations bring changes and in aquatic toxicology any variations in the blood serve as biomarker. Such rapid changes in the characteristics of the fish blood measured are used as indices of pollution by pesticides. The provide assessment of pollution and will be warning sign of the inhabiting organism.Such alterations either increase or decreases in the blood were studied and reported [1-6] and even mentioned in the review articles [7-11].

Different chemicals of the four classes of pesticides organochlorines, organophosphates, carbamates and synthetic pyrethroids are reported and such changes in the fish are varied being species specific. Howeverpaucity of information is available for the grass carp *Ctenopharyngodon idella,* for the pesticide dichlorvos. Hence the present study is contemplated to assess the effects of the toxicant dichlorvos both in lethal and sublethal concentration using technical grade as well as 76% EC as Nuvan.

Experimental

Materials and methods

Collection and maintenance of test organism

The freshwater fish *Ctenopharyngodon idella* 3 to 5cm in length 4 to 5 gms in weight irrespective of their sex, have been chosen as the test organisms for the present investigation. Healthy and active fish were obtained from Ratna Singh Hatcheries, Nandivelugu, Guntur district, Andhra Pradesh, India. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28±1°C. Water was renewed every day with 12-12h dark and light cycle. The water used for acclimatization and conducting experiments was clear unchlorinated ground water and the hydrographical conditions as physical and chemical properties of water were: Turbidity-8 silica units, Electrical conductivity at 28°C -816 Micro ohms/cm, pH at 28°C-8.1, Alkalinity: Phenolphthalein-Nil mg/l, Alkalinity: Methyl orange-172mg/l, Total Hardness (as $CaCO_3$-232mg/l, Carbonate Hardness (as $CaCO_3$)-232mg/l, Non Carbonate Hardness (as $CaCO_3$)-Nil mg/l, Calcium Hardness (as $CaCO_3$)-52mg/l, Magnesium Hardness-40mg/l, Nitrite Nitrogen (as N)-Nil mg/l, Sulphate (as $SO4_2^-$)-Trace mg/l, Chloride (as Cl^-)-40mg/l, Fluoride (as F^-)-1.8mg/l, Iron (as Fe)-Nil mg/l, Dissolved Oxygen- 8–10ppm, Temperature-28±2°C.

During the period of acclimatization, the fish were fed *(ad libitum*) with groundnut oil cake and rice bran. Feeding was stopped one day prior to the acute toxicity test. All the precautions laid by committee on toxicity tests to aquatic organism [11-13] were followed and such acclimatized fish only were used for experimentation. If mortality exceeded 5% in any batch of fish during

acclimatization, the entire batch of that fish were discarded. Such acclimatised fish were selected for experimentation. The technical grade which was 95-98% pure which was supplied by the Syngenta India Ltd, Mumbai-400 020. The pesticide 76%EC Nuvan is locally purchased manufactured by Hikal Limited 629/630 GIDC Industrial estate Panoli Bharuch Gujarat marked by Syngenta India., 14, J. Tata Road, Mumbai. The organophosphate pesticide was introduced into water from where the pesticide entered into the fish through gill. A total of 50 fish were taken each in sublethal and lethal concentrations for both technical grade and 76%EC Nuvan. The 96hrs LC_{50} values are determined by Finneysprobit analysis [12] as mentioned in APHA [13-15]**.**Haematological changes were determined at the end period of 96hrs LC_{50} value (9.841 ppm for Technical and 11.238 ppm for 76 % EC Nuvan) and $1/10^{th}$ of LC_{50} of 96hrs exposed for 10 days for both technical grade and 76%EC Nuvan.50% of the organisms were dead for lethal concentrations and the remaining organisms were sacrificed for the experimentation.

Ctenopharyngodon and *idella* are both Greek words, meaning "comb-like throat-teeth" and "distinct" respectively. The grass carp is one of the largest members of the minnow family. The body is oblong with moderately large scales, while the head has no scales. There are three simple and seven branched rays on the dorsal fin. Grass carp are silvery to olive in color, lacking the golden hue of common carp, and they have no barbels.

Sampling of blood

Fish were euthanized by an overdose of MS-222 and then weighed and measured. Blood sample was collected by caudal severance from the disease free test fish during early hours of the day and stabilized with 50 IU sodium heparin (anticoagulant)/ml blood.

Haematological examination

The haematological variables analyzed were red blood cells count (RBC), haemoglobin (Hb), white blood cells count (WBC), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin(MCH) and mean corpuscular haemoglobin concentration(MCHC).

Determination of red blood corpuscles (RBC) count

RBC count was determined with an improved Neubauer crystalline counting chamber [16]. The blood was sucked up to 0.5mark on the RBC pipette and immediately, Hayem's solution as a diluent stain was drawn up to 101 mark and the pipette was rotated between the thumb and the forefinger to facilitate adequate mixing of the solution (dilution: 1:200). The counting chamber and the cover glass were cleaned thoroughly and cover glass was placed in position over the ruled area. The fluid from the stem of the pipette was expelled as it contains only the diluting fluid. The pipette was then held at an angle of 45^0 with the tip of the pipette at the junction of the edge of cover glass and the counting chamber. A drop of blood was placed from the tip of the pipette on the central platform near the edge of the cover slip, so that the drop was sucked up between the central platform and cover slip by the capillary force. The cells were allowed to settle for 2 to 3 min. The ruled area of the counting chamber was focused under the microscope and the numbers of RBC were counted in 80 small squares (4 squares of 16 at the four corners and one of 16 at the centre). The cells touching the upper and left hand lines were counted. The cells touching the lower and the right hand lines were omitted.

The numbers of RBC per sq mm were calculated as follows

The area of Small Square: 1/400 sq mm
The depth of the counting chamber: 1/10 mm
Therefore the volume of a small square is: 1/400 x 1/10 = 1/4000 cu mm
The dilution of blood is 1:200
Total number of RBC = n x 4000 x 200/80
n =Number of cells counted in 80 small squares

Determination of white blood corpuscles (WBC) count

WBC count was determined [17]. The blood was drawn up to 0.5 mark of WBC pipette and immediately the diluting fluid was drawn up to the 101 mark above the bulb (the dilution fluid consists of 1.5ml of glacial acetic acid and 1 ml of aqueous gentian violet solution and made up to 100 ml with distilled water). The solution was mixed thoroughly by shaking gently and allowed to stand for 3 min. Cleaned Neubauer counting chamber and cover glass were placed over the ruled area. Excess solution was expelled and a drop of fluid was allowed to flow under the cover slip by holding the pipette at an angle of 40^o and allowed to stand for 2 to 3 min. The WBC was counted in the four corner square millimetres and the number of WBC per cubic millimetre was calculated.

Estimation of haemoglobin (Hb)

Hb concentration in the blood was estimated by cyanmethaemoglobin method [18].Hb is converted into cyanmethaemoglobin by the addition of potassium ferricyanide (KCN) and the colour was read in a spectrophotometer at 540 nm against a reagent blank.

Determination of packed cell volume (PCV) or Haematocrit value

Packed cell volume was determined by micro haematocrit method [19]. The heparinised blood was filled up to the

mark 100 of the haematocrit tube with the help of Pasteur pipette and centrifuged at 3000 rpm for 30min. The relative volume of the height of the RBC's packed at the bottom of the haematocrit tube was recorded as packed cell volume in terms of percentage of total blood column taken in the haematocrit tube.

Determination of mean corpuscular volume (MCV)

MCV indicates the average size of the blood cell in a given sample of blood. MCV was calculated by the following formula and expressed as femtoliter (fL).

MCV = Haematocrit (%) x10/RBC count.

Determination of mean corpuscular haemoglobin (MCH)

MCH represents the average content of the HB in each red blood cell. MCH is influenced by the HB concentration and the number of RBC. MCH was calculated by the following formula and expressed in picogram (pg).

MCH = haemoglobin (g/dL) x 10/ RBC count

Mean corpuscular haemoglobin concentration (MCHC)

MCHC reflects the average concentration of the haemoglobin in the red blood cells in a given of the blood. MCHC was obtained by the following formula and expressed in terms of gram percent (g%).

MCHC = haemoglobin (g/dL) x 100/ haemoglobin (%)

Result and discussion

The blood parameters in both lethal and sublethal concentration of technical grade as well as 76% EC Nuvan are presented in the Figure-1 and represented as graph in Figure-1. Marked changes are observed in exposed fish in the commercial formulation experimented fish resulted more percentage of changes rather than the technical grade, because of the ingredients mixed imparting additive toxicity. The RBC count decreased in both lethal and sublethal concentration and more percentage of decrement in 76% EC. The WBC count increased in both lethal and sublethal more in 76% EC.The haemoglobin content decreased in both lethal and sublethal concentration more in 76% EC. The haematocrit value altered accordingly to the RBC count. Accordingly the calculated values of MCV, MCH and MCHC also showed changes.

The review article mentioned about organochlorines organophosphates, carbamates and synthetic pyrethroids alter the haematological changes as effects in fish [6]. The work of organophosphates was reviewed and gave a thought that the pesticides do effect the biochemical changes in the blood and also parameters of the blood change [20-22]. In the opinion of [7] the review articles on toxicity of pesticides in fish documented the work where pesticide induce alteration in blood parameters and stressed the impact of such changes [23-24]. Haematobiochemical changes induced by pyrethroid insecticides in avian, fish and mammalian species [25]. The effects of pesticides in the blood of fish serve as indices of toxicity. The review on dichlorvos toxicity in fish indirectly opined that the toxic action result in fish alteration are likely [9] and that is mentioned. The dichlorvos toxicity on fish, a review published and commented on the toxic effect of biochemical nature of the circulating fluid [26]. The review mentioned that the haematological parameters are important in toxicological research and indicative of toxic stress. After exposure to two sublethal concentration 203 µg/L and 68.0 µg/L of chlorpyrifos for 1, 3, 7, 14 and 21 days a decreased erythrocyte count, leucocyte count, haemoglobin and haematocrit mean values in *Channa punctatus* (Bloch) [27] was also in support of present research. Toxication altered the haematological parameters in *Cyprinus carpio* particularly ESR count due to chlorpyrifos toxicity which is another organophosphate [28-29]. The same was opined, for the same chlorpyrifos to *Cyprinus carpio* [30]. In bony fish *Tilapia guineensis* exposed to common pesticides produced characteristic alteration in the blood [31]. The review on the toxicity and other effects of dichlorvos an organophosphates pesticides to freshwater fish, documented exposure of *Orieochromis niloticus* to dichlorvos for 96 hours duration marked changes of packed cell volume (PCV), haemoglobin (Hb), Red Blood cell (RBC), neutrophil, monocyte and lymphocytes count [8]. The review also mentioned the work done on the fish *Cyprinus carpio* exposed to dichlorvos [32]. The other works also emphasized the haematological changes are no doubt serve indices of toxicity [33-36].

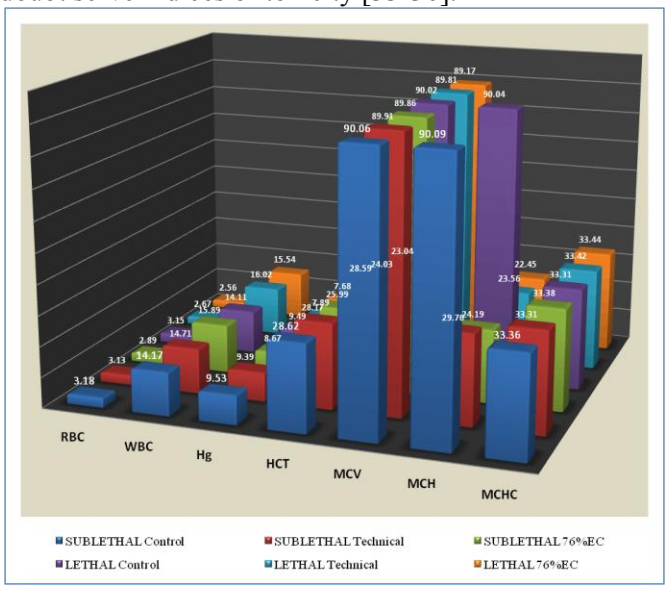

Figure 1. Haematological alterations of *Ctenopharyngodon idella* (Valenciennes) exposed to lethal and sub lethal concentrations of technical grade Dichlorvos and 76% EC (Nuvan).

Haematological changes of silver carp *Hypophthalmichthys molitrix* in response to diazonin pesticide after the exposed to half of the lethal concentration was reported [37]. The results showed that leucocytes (WBC), haematocrit (Ht) haemoglobin (Hb), MCHC and lymphocyte cortisol and glucose increased where MCV and MCH were significantly decreased. They also reported to significant differences in RBC Monocyte and Eosinophils among the fish exposed to the toxicant which are derivative changes. Haematological parameters of *Cyprinus carpio* exposed to Monocrotophosan organophosphate exposed to 4.5 ppm, 6.7 ppm and 13.5 ppm was studied and found that a significant decrease in all the sublethal concentrations and maximum decrease at 12.5 ppm RBC content, ESR PCV and a significant increase in RBC and WBC count was reported [38]. The effect of Glyphosite (Roundup 41%) on the haematology of the freshwater fish *Catla catla* was studied and the RBC, WBC, haemoglobin and haematocrit value showed marked decline while MCV, MCH and MCHC showed fluctuating pattern [39]. The present work can be correlated with this work, but both of them belong to same group of pesticides i.e., organophosphate.

Changes in haematology of the freshwater fish *Channa punctatus* (Bloch) exposed to delta methrin for 45 days was reported [40], where there was a significant decrease in haemoglobin content, total erythrocyte count, PCV, MCV and MCHC. On the other hand a significant increase in total leucocyte count and MCH which coincides the present work. Toxic impact of pesticide trichlorofon on the morphological characteristics of blood cells of fish *Channa punctatus* (Bloch) and opined not only the quantitative aspects of the blood parameters are altered but also the morphological aspects of the fish where compared with control are altered. Anisocytosis deformed erythrocytes and large bulged nucleus, deformed cells are clumped and finally cell lysis, an increase in the size of MCV has been associated in response to stress[41].Haematological changes induced by pyrethroid insecticide fenvalerate in cat fish *Clarias gariepinus* was reported [42], a significant decrease in the haemoglobin content haematocrit value and erythrocytes whereas the leukocyte was increased. The work is similar of the present report even though both of them belong to two different classes of pesticides. Haematological response of grass carp *Ctenopharyngodon idella* after exposed to endosulphan was studied [43]. The fish were exposed to two sublethal concentrations for 15, 30 and 45 days. The RBC, haemoglobin content and hematocrit values showed declined trend and MCH, MCHC also showed declined trend while MCV showed an increasing trend. She opined variation in erythrocyte count is the indices of polluted aquatic environment. The present study also showed similar results but both of them belong to different class of pesticides.

The effects of Deltamethrin on haematological indices of Indian major carp *Cirrhinus mrigala* (Hamilton) was studied by exposing the fish to lethal and sublethal concentrations and the results showed that the RBC, HB and Haematocrit values decreased in both lethal and sublethal concentration. WBC, MCV and MCH were increased consequently [44]. An increase in WBC revealed haematological toxicity coincide the present work. Haematological changes in African catfish *Clarias gariepinus* exposure to mixture of Atrazine and metolachlor in the laboratory was studied [45]. Because of mixture, the two chemicals behaved differently when compared with the present result. A significant reduction in Hb, RBC, PCV and MCV were observed. The MCHC of result showed an increase. The haematological parameters are used in monitoring health status of the fish in the wild and culture medium. Even the present study fish is a culturable one and stringent steps have to be taken not to use the pesticides in disease management of culture practices. Haematological changes in the fish *Channa punctatus* (Bloch) exposed to fenvalerate. The percentage of haemoglobin PCV, RBC showed a gradual increase while WBC showed are increase [46]. The result showed similar trend of the present study. Haematological effects of commonly used fungicide on fish *Clarias batrachus* was studied [47] where they exposed the fish to sublethal concentration and blood was assayed for selected haematological parameters. A decrease in the Hb, MCV, MCHC, RBC levels and an increase in WBC level was observed. The report in strictly different owing to chemical nature of the two toxicants i.e., Chloropyrifos and Mancozeb.

Haematological changes in the fish *Cyprinus carpio* exposed to permethrin, a synthetic pyrethroid of class I type was reported [48]. Where in a decrease in RBC, Hb, PCV was found but an increase in WBC, MCV, MCH, MCHC was observed when the fish is exposed to technical grade and 25% ECin both lethal and sublethal concentrations. Commercial formulation concentration was causing more alteration because of the ingredients present which shows similar result of the present study even though both belong to different classes. A similar report was observed in sublethal effects of dichlorvos on the freshwater fish *Cyprinus carpio* var. Communis [49]**.** The fish were exposed to three sublethal concentrations 50% 60% and 70% and the results showed changes of the RBC and WBC count, Hb and haematocrit and PCV. Since osmoregulatory dysfunction was the cause for increase in the rate of destructions in hematopoietic organs [50]. The same is reiterated [51]. Studies on freshwater fish *Channa punctatus* (Bloch) in sublethal toxicity of Deltemethrin in relation to sex was reported [52]. The fish exposed to different concentrations of Deltemethrin ranging from 0.02ppm and 0.2ppm of 5 exposure periods 24, 7, 15,20 and 30 days in sub lethal concentrations and due to long duration exposure , due to

change in haemostatic mechanisms the fish showing leucocytosis initially and fish might have evoked a changed and neutralized the toxicant effects of deltemethrin. However the present study cannot be compared with this result because of a different methodology. Haematological alterations in *Cyprinus carpio* as biomarkers of cypermethrin toxicity exposed to lethal and 1/10th of 96h LC_{50} values as sublethal for 5, 10, 15 days. RBC count, Hb content, PCV showed decrement at both lethal sublethal concentrations. WBC count and MCHC exhibited increasing trend at sublethal and decreasing trend at lethal concentrations whereas MCH increased in both concentrations [10]. The presence of the toxicant disturbs the balance in the echo systems and the fish is definitely not comfortable.

Conclusion

Hence it may be concluded that, even the *Ctenopharyngodon idella* a grass carp cultured along with the other major carps, when pesticides contaminate the culture medium, alter the blood parameters and such alterations are more severe in EC due to the ingredients mixed. Hence stringent measures have to be taken for quality control before giving pesticide representativeness for environmental usage.

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