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Impact of Endosulfan on Certain Hematological Parameters of *Channa punctatus*

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Abstract

Hematological parameters are routinely used as indicators of the physiological or sublethal stress response to endogenous or exogenous changes in fish. In this work, the sublethal toxic effects of endosulfan (0.3 μg l^{-1}) on certain hematological parameters of Channa punctatus were examined for different exposure periods and the analyses were made on 15, 30, 45, and 60 days. Endosulfan caused significant lower value of red blood corpuscles (RBC) and hemoglobin when compared to the control groups ($p < 0.05$). However, white blood corpuscles (WBC) increased throughout the study period. The results indicated that a low amount of endosulfan alters the hematological parameters of fish, which can be useful in diagnosing the structural and functional status of fish exposed to toxicant.

Keywords: *Endosulfan, Channa punctatus, hemoglobin, RBC, WBC, toxicant*

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Introduction

In the present study, the selected toxicant is endosulfan representing organochlorine group of pesticides. Technical endosulfan consists of a mixture of alpha and beta - isomers in an approximate ratio of 70:30. The major metabolites are endosulfan sulfate and endosulfan diol. Endosulfan is a contact and stomach poison that has been used to control insects; it is used in countries throughout the world to control pests on fruits, vegetables, tea, and on non-food crops such as tobacco and cotton. In addition, to its agricultural use, it also used as a wood preservative and control of the tsetse fly. It can be absorbed following ingestion, inhalation and skin contact. Sign of acute intoxication include neurological manifestations, such as hyperactivity, muscular twitching and convulsions, sometimes followed by death. Fish are extremely sensitive to endosulfan and mortality of fish have been reported as a result of the discharge of endosulfan into rivers.

Loss of commercial fisheries is all the more cause for concern at a time when oceans are being increasingly considered as future suppliers of protein for the growing human population. Its immediate concern is not shown; valuable human food in terms of fish would be lost. Therefore, conservation of Indian fisheries needs well studied and documented investigation of effects of above said toxicant. Hence, keeping all these issues in mind, the present study is undertaken to evaluate some hematological alterations by the pesticide-endosulfan to fresh water edible teleost fish *Channa punctatus.* The test fish *Channa punctatus* is available throughout the year and can easily survive and get acclimatized to laboratory conditions. It is of high nutritive value and is regularly consumed by local people in and around Meerut region, especially by poor section of our society. Also, this fish has the capacity to withstand a wide range of experimental conditions.

Materials and Methods

Live specimens were procured from local freshwater ponds of Meerut or purchased from fish market of Meerut city. Prior to experimentation, fish were disinfected with 0.1% potassium permanganate solution and were maintained for three weeks in well aerated tap water. Thereafter, they were acclimatized to experimental tanks for at least a week. All precautions for the hygiene of fish were taken and the physico-chemical characteristics of water were routinely analyzed. All the fish were grouped according to experiments and control groups were also maintained separately. The experimental toxicant endosulfan was administered to experimental fish and control group was

maintained without the toxicant administration.

Determination of LC50

Preliminary toxicity tests were conducted under laboratory conditions to determine the LC50 value for 96hr of endosulfan according to the 'Standard Methods' of the American Public Health Association (1971). Stock solution of insecticide – endosulfan (in acetone) to obtain the desired degree of concentration based on the progressive bisection of intervals on the logarithmic scale, as given by Duodorff *et al.* (1951). The test fish were examined carefully for pathological symptoms and were transferred from the acclimation tank to the experimental containers. All the precautions prescribed in 'Standard Methods' were followed. Required quantity of water was added to each container ensuring the availability of at least one liter of water for each gram biomass of the fish. Artificial aeration was also provided whenever necessary. After every 24hr, the number of dead fish was also recorded, as they did not respond on being probed with a glass rod and respiration ceased.

Determination of Sub-Lethal Concentration

To observe the chronic effects of endosulfan, sub-lethal concentration were determined. 1/10 concentration of 96hr LC50 values were selected as the sublethal concentrations. It was observed that no mortality occurred at these concentration upto 120 days.

Chronic Exposure

In chronic experiments, healthy fish weighing 60 $\pm$ 4gm and 15 $\pm$ 2cm in length were selected and exposed to sub lethal concentrations of endosulfan (0.3µg/l). A control group was also maintained in tap water free of toxicant. Each group was regularly checked for infections, disease and other unhealthy conditions during the experimentation. After every 24hr, water was renewed and toxicant added. At the expiry of each experiment period (15days, 30days, 45days and 60days); control and toxicant exposed fish were processed simultaneously.

Food and Feeding

The fish were conditioned to feeding on pelleted diet (prawn powder, fish powder and minced liver in 2:2:1 ratio) @ 2% of body weight. For chronic exposure or long term experiments, the fish were fed twice daily at 7:00 AM and 7:00PM.

Hematological study

The study was conducted on following parameters as Hemoglobin (Hb), Total Erythrocyte Count (TEC), Packed Cell Volume (PCV), Total Leucocyte Count (TLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC).

Hemoglobin

Blood from caudal vessel of control and experimental fish was drawn with the help of heparinized needles. Hemoglobin, hematocrit (PCV), erythrocyte and leukocyte count were made in whole blood. hemoglobin content of the blood was estimated by acid haematin method by *hemometer*. The hemoglobin content was expressed as g/dl.

Total Erythrocyte Count (TEC)

The number of erythrocytes per cubic millimeter of blood was calculated with the help of a hemocytometer using a Neubaur's counting chamber as given by Dacie and Lewis (1977).

Total Leucocytes Count (TLC)

The method given by Dacie and Lewis (1977) was followed to count the number of leucocytes. The diluted blood was transferred to Neubaur's chamber (counting chamber) and counting the number of WBC in corner most four squares, following the usual precautions.

Hematocrit

Hematocrit was determined by the method given by Dacie and Lewis (1977). Blood was collected with the help of sterilized needles and was filled in Wintrobe tubes. It was carefully mixed by inversion and the tubes were centrifuged at 2500 x g for 30 minutes. The height of the column of red blood cells was recorded in each case and this is taken as the packed cell volume (PCV).

Mean Cell Volume (MCV)

The mean cell volume was calculated by dividing the hematocrit value with the total number of erythrocytes per liter of blood. It is expressed in *femtoliters* (fl).

Mean Cell Hemoglobin (MCH)

Mean cell Hemoglobin was calculated by dividing Hemoglobin concentration with the total number of erythrocytes present in one liter of blood. The MCH value is expressed in *pictograms.*

Mean Cell Hemoglobin Concentration (MCHC)

Mean Cell Hemoglobin Concentration was calculated by dividing hemoglobin concentration with packed cell volume. MCHC value is expressed in gm per dl of blood.

Results

On exposure to endosulfan, increase in Hb and TEC was observed after 30 days but decrease was observed after 15, 45 and 60 days in comparison to respective control. In case of PCV, decrease noted after all the exposure days over the control (Fig. 1). In case of TLC and MCV decreased was recorded upto 60 days (except increase in MCV value at 45 days exposure). The value of MCH and MCHC was recorded increase at 15 and 30 days exposure but

decrease at 45 and 60 days prolonged exposure period in comparison to respective control group (Fig. 2).

Increase noted in the levels of Hb, PCV and RBC count on exposure to endosulfan may be due to polycythemia produced by the pesticide effects on fish, due to internal hypoxia conditions (Dheer and Mahajan, 1977) or asphyxia (Mahajan and Juneja, 1979) and decreased rate of erythropoiesis or accelerated destruction of red cells (Mc Leay, 1973; Narain and Srivastava, 1979 and Cameron and Wohlschlag, 1969). Physiologically, this type of erythrocytic response seems to be secondary to a typical stress situation caused by the presence of endosulfan in the medium. Possibly, the fish face respiratory difficulty when they confront a toxic environment and try to compensate for the reduced oxygen uptake at the gill detergentace by increasing the level of blood constituents concerned with oxygen uptake and delivery. However, prolonged exposure to endosulfan exhausts the hematopoietic potential as revealed by the lowered RBC counts and hemoglobin noted in the fish on further exposure upto 60 days. The hemolytic and destructive effects of the pesticides on blood cells were supported by Robert (2001).

In the present study decrease in TLC and MCV in *Channa punctatus* after exposure to endosulfan is line with those recorded in *Colisa fasciatus* and *Macrones keletius* (Gunther) and *Oreochromis mossambicus* after exposure to lead, tannery effluents and paper pulp mill effluents (Srivastava and Mishra 1983; Subramanian 1988) respectively. Decrease in WBC count was observed in *Tilapia sparrmanii* on exposure to manganese and iron at neutral and acidic pH (Wepener et al., 1992). In concern to increase of MCH and MCHC in initial stages of exposure and decrease in prolonged exposure period may be due to dehydration and shrinkage of red blood cells as revealed by the decreased MCV. Such kind of results has also reported by Munni kumari and Yadav (1992). The study concerned with the pathologic and clinicopathologic findings due to chronic exposure to the organophosphate fungicide edifenphos on Nile tilapia *Oreochromis niloticus*. Eight weeks exposure to 1/10 96 hours LC50 (0.1 ppm) led to adverse effect on some serum parameters (Gaafar *et al.*, 2010).

References

American Public Health Association, 1971. 13th ed. New York, American Public Health Association.

Cameron, J.N. and Wohlschilag, AG, D.E. 1969. **J. Exp. BioI. 50, 307-317.**

Dheer, J. and Mahajan, C.L. 1977. In: 64th Annual Indian Science Congress, Bhubaneshwar.

Doudoroff, P., Anderson, B.G., Burdick, G.E., Gallsoff, P.S., Hart, W.B., Patrick, R., Strroma, E.R., Surbes, E.W. and W.M. Vanhou 1951. Sew. Ind. Wastes. **23,** 130-139.

Gaafar, A.Y., El-Manakhly, E.M., Soliman, M.K., Soufy, H., Mona S. Zaki, Safinaz G. Mohamed And Shahenaz M. Hassan, 2010. **J. Of American Science, 6.10-17.**

Mahajan, C.L. And Juneja, C.S. 1979. **J. Of Environmental Health. 21, 169-172.**

Mcleay, D.J. 1973. **Gen. Camp. Endocrinol. 21, 431-440.**

Munni Kumari And Yadav, S.C. 1992. **Him. J. Env. Zool. 6, 20-23.**

Narain, A.S. And Srivastava, P.N. 1979. *Arch.* **Bioi. Bruscelles 90, 1401-159.**

Robert, R.J. 2001. *Fish Pathology*. 3rd Ed., Bailliere Tindall, London, Philadelphia, Sydney, Tokyo, Tornonto.

Srivastava, A.K. And Mishra, J. 1983 **J. Comp. Path. 93, 27-31.**

Subramanian M.A., Hameed, M.S. And Varadaraj, G. 1988. **The Indian Zoologist 12, 71-74.**

Wepener, V., Van Varen, J.H.J. And Dupreez, H.H. 1992. **Bull. Environ. Contam. Toxicol.** 49, **613-619.**

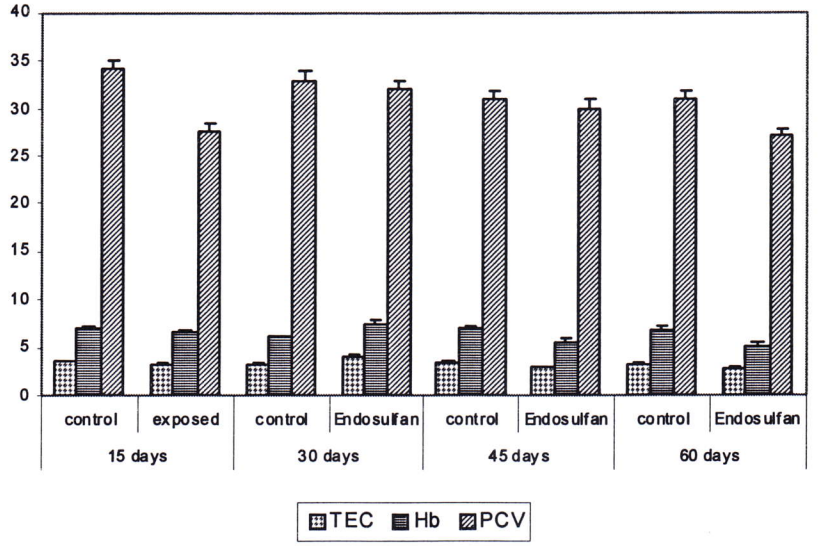

Figure 1. Showing the post exposure effect of endosulfan on total erythrocytes, Haemoglobin and PCV.

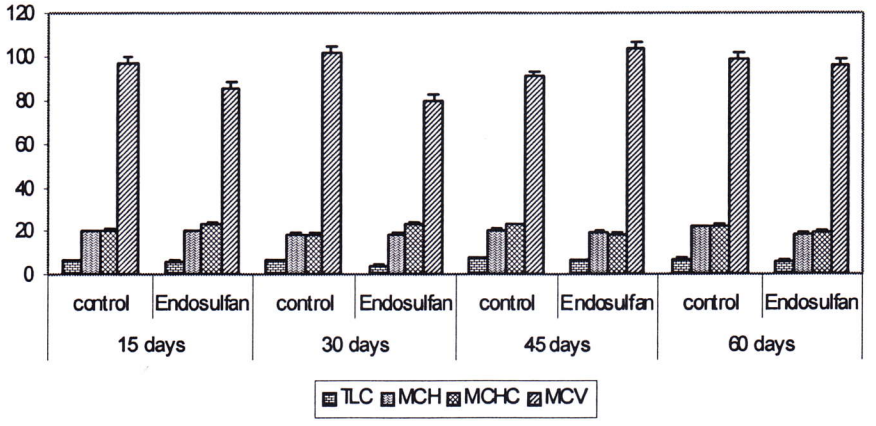

Figure 2. Showing the post exposure effect of endosulfan on leucocytes & blood indices.