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RESEARCH PAPER

Effect of endosulfan on air-breathing fresh water teleost, *Clarias batrachus* (Linn.) a haematological and biochemical response

AMIT KUMAR SINGH, J.K. DUBEY, D.B. MISHRA, G. SINGH AND V.B. SINGH

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ABSTRACT

Clarias batrachus (Linn.), air-breathing fresh water teleost fish of commercial importance, was exposed to median lethal concentration (5.38 ppm) of endosulfan. The haematological and biochemical parameters of fresh water fish were evaluated under static condition. Fishes were exposed to predetermined LC_{50} (5.38 ppm) and studied at the end of 24 hrs period, Blood samples were taken from the control and experimental fishes. Blood was assayed for selected haematological and biochemical parameters (heamatocrit, haemoglobin, red blood cell, counts, white blood cell counts, differential white blood cell, counts, erythrocyte sedimentation rate, and total plasma protein and plasma glucose concentration). Sub-lethal concentrations of endosulfan caused a dose dependent decrease in the hemoglobin values, haematocrit values, red blood cell counts, plasma proteins; where as the plasma-glucose, erythrocyte sedementation rate, total white blood cell count were increased. The differential white blood cell counts were decreased except for the lymphocytes in which there was a slight increase. The above observation may show the significance of haematological and biochemical assaying the pesticide hazards to the fishes and can be used as an indicator of pesticide related stress in field of environmental toxicology.

See end of the article for authors' affiliations

Correspondence to :
AMIT KUMAR SINGH
Department of Zoology, T.D.P.G. College, JAUNPUR (U.P.) INDIA

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Extensive use of pesticides has facilitated increase in agricultural productivity, despite a decrease in the total average of land cultivated. Mass mortality of aquatic organism has often been caused by pesticide exposure, especially from accidental or direct spraying of water bodies. More commonly aquatic organisms are subjected to long term stresses from exposure to sub-lethal concentrations. However, in the long run, these sub-lethal concentrations may also prove to have deleterious effects as do lethal concentration, because sublethal and small effects on aquatic organisms may alter behaviours, feeding habits, reproduction rates etc. In addition, pesticides that do reach the water body can accumulate in fish and other aquate fauna which are then harmful to humans when ingested.

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a- hexahydro 6, 9- menthano-2, 4, 3-benzadioxathiepin -3-oxide) is a polycyclic chlorinated hydrocarbon, a hazardous chemical. In India, it has been identified as one of the main pesticides found in waters of major rivers. Endosulfan run off from the agricultural field into the river, many fish kills have been reported. In case of mammals, there is lesser susceptibility to endosulfan toxicity than the aquatic animals.

Therefore, in the present investigation an attempt has been made to study the effect of endosulfan on alterations in haematological parameters of *Clarias batrachus* (Linn.) with particular references to the concentration of the pesticide and duration of exposure.

MATERIALS AND METHODS

Specimens of *Clarias batrachus* (Linn.) were obtained from the river Gomati in Jaunpur city, U.P. and acclimated to laboratory conditions for 21 days, prior to experiment. During acclimatization period, fish were kept in sand post of 40 litres and were fed goat libitum with rice bran, once daily. Water was replaced every 24 hrs after feeding in order to maintain a healthy environment for the fish, during both acclimation and experimental period. The size of fish varied from 18-20 cm in standard length and 38-40 gram in weight. Fish of both sexes were used without discrimination.

The water quality parameters of the diluting water used in the tests experiment and determined by standards methods (as per APHA, 1998) are presented in Table 1.

For acute toxicity studies, five circular plastic tubes were taken and each was filled with 20 litre of water and 5.38 ppm of endosulfan. 10 fish which were already tested for 24 hrs were introduced into each tub. Control was

Table 1 : Water quality parameters estimated as per APHA, 1998

Parameters		Values
Temperature	-	26.0 ± 2.0 ^{0}C
pH	-	7.6 ± 0.1
Dissolved oxygen	-	5.22 ± 1.04 mg/L
Free carbon di-oxide	-	5.30 ± 0.09 mg/L
Total alkalinity	-	43 ± 6.00 mg/L
Hardness	-	60 ± 9.18 mg/L
Turbidity	-	0.318 ± 0.07 mg/L

maintained into 2 circular plastic tubs with 10 fish per tubs. At the end of 24 hrs period exposure, blood was drawn from control and endosulfan treated fish by cardiac puncture using a hypodermic syringe previously rinsed with heparin, which was previously rinsed with heparin.

The whole blood was used for estimation of haematocrit, hemoglobin, red blood cells, white blood cells, differential white blood cell count and erythrocyte sedimentation rate. The reminder of blood sample was centrifuged at 10,000 ppm for 20 minute to separate the plasma; which was used for plasma glucose and protein-estimation. The micro haematocrit method of Snieszko (1960), was used to determine the haematocrit. Hemoglobin concentration was measured by the cyanmethnomoglobin method (Larsen and Snieszko, 1961). Red and white blood counted under light microscope with an improved Neubauer haemocytometer Differential Leucocyte counts were obtained from Leishman/Giemsa stained blood smears. Erythrocyte sedimentation rate was determined with microhematocrit tubs filled with blood and allowed to stand for 20 minutes. Plasma-glucose was estimated by Enzymatic-colorimetric method (Trinder, 1969) and Plasma-protein estimation was done according to the method of Lowry *et al.* (1951).

The mean values of the various haematological and biochemical parameters for the control and experimental fish were analysed for statistical significance using the student's t-test. The calculations of statistical significance were made by the student's t-test at 0.01-0.05 levels.

RESULTS AND DISCUSSION

The changes in haematological and biochemical parameters of fish, *Clarias batrachus* (Linn.) exposed to acute toxicity of Endosulfan are presented in Table 2 and 3. During treatment (5.38 ppm) significant decrease in haematocrit and haemoglobin concentration and non-significant decrease in red blood cell counts and erythrocyte sedimentation rate were observed in the

Table 2 : Changes in haematological parameters in air-breathining fresh water fish, *Clarias batrachus* (Linn.) treated with acute concentration of Endosulfan

Parameters	Control	Exposed with Endosufan
Haematocrit (%)	45.80 ± 2.08	36.18 ± 1.38
Haemoglobin (g/dl)	7.32 ± 0.81	6.67 ± 0.53
RBCc (million/cu mm)	3.83 ± 0.12	1.44 ± 0.08
WBCc (thousand/cu mm)	7.44 ± 0.60	9.82 ± 0.13
ESR (nm/hrs)	28.24 ± 2.13	23.97 ± 1.49
Plasma protein (g/ml)	7.850 ± 0.563	6.305 ± 0.28
Plasma glucose (mg/100ml)	108.84 ± 1.98	123 ± 1.38

The values are expressed as the mean ± SE (n=5)
Significance at 0.05 – 0.01 level.

Table 3 : Changes in differential white blood cells counts as a result of exposure of Endosulfan a fresh water fish, *Clarias batrachus* (Linn.)

Parameters	Control	Exposed with Endosulfan
Lymphocytes	35.18 ± 0.98	46.03 ± 1.38
Basophils	11.66 ± 0.45	10.18 ± 0.48
Neutrophils	10.78 ± 0.42	8.89 ± 0.93
Eosinophils	11.98 ± 0.32	10.66 ± 0.18
Thrombocytes	29.18 ± 0.33	24.41 ± 0.64

composition as seen from the differential white blood cell counts, while a decreases in the total plasma protein was recorded in fish exposed to elevated pesticide concentration, where as total WBC and plasma glucose level increased in the pesticide treated fish.

In the total white blood cell count, there was a slight decrease in the percentage of neutrophils and eosinophils, when compared to control. A slight statistically insignificant decrease in percentage was recorded in the basophils and thrombocytes. Finally, a sharp increase in percentage of total WBC counts of fish exposed to pesticide-endosulfan.

Fish exposed to pesticide, showed abnormal behaviour changes like, fast swimming activity, hypersensitivity, darkening of skin on whole body surfaces, etc. indicating the high toxicity of Endosulfan.

Pesticides usually get into the aquatic environment through run-off from treated land, spray drift during treatment, washing of spraying equipments in water ways or even from air, through the control of aquatic weed and the control or the elimination of unwanted fish. Many aquatic organisms were killed in various parts of the world had been attributed to the indiscriminate use of pesticide in agriculture, when not used according to the manufacturer specification.

Contamination of aquatic environment by pesticides whether as a consequence of acute and chronic events constitute additional source of stress for aquatic organisms. The toxicants and pollutants have significant effect, which can result in several physiological dysnfuction in fish. Dysfunction in the fish may change the haematological and biochemical parameters.

The tissues of fish have been known as the water exchanges tissue with blood. Haemoconcentration and haemodilution have been described (Mishra and Srivastava (1979). It has been observed haemoconcentration after copper exposure and haemodilution following zinc exposure in *Colis fasciatus*. In the present study, the decrease in haematocrit following endosulfan exposure in *Clarias batrachus* (Linn.) may be an indication of haemodilution. Tort and Torres (1988) reported decreases in haematocrit following 24 hrs exposure of dog fish, *Scynohinus canicula* to cadmium contamination. They attributed this decreased to haemodilution. They observed depletion in the haemoglobin and haematocrit values in the fish which could also be attributed to the lysing of erythrocytes due to pesticide exposure. Similar reductions have been reported by Samprath *et al.* (1993) and Musa and Omoregie (1999), when they exposed fish to polluted environment under laboratory conditions. Thus, the significant reduction in these parameters is an indication of severe anemia caused by exposure of the experimental fish to heavy metals/pesticides in water.

There was no significant change in erythrocyte count and erythrocyte sedimentation rate; may be attributed to the swelling of red blood cells. Flos *et al.* (1987) reported that the swelling of the red blood cells may be, due to an increase in protein and carbon di-oxide in the blood.

Plasma protein content was found to decrease with pesticide exposure in the present study. This could be attributed to renal excretion or impaired protein synthesis or due to lie disorder (Kori-Siakpere, 1995).

On the other hand, the observed decrease of plasma protein could also result from the break down of protein into amino acids first and possibly into nitrogen and other elementary molecules.

In the present study, the significant increase of plasma glucose level might have resulted from gluconeogenesis to provide energy for the increased metabolic demands imposed by pesticidal stress, particularly in osmo-regulations which may contribute to the restoration of plasma osmolarity in the face of failing blood levels of Na^+ and Cl^-. Blood glucose has been employed as an indicator to environmental stress. The increase in the blood glucose is usually correlated with the mobilization of glycogen and development of status of hyperglucaemia. The hyperglycaemia response varies with the nutritional status of the fish (McLeay, 1977).

The white blood cells in fish respond to various stressors including infections and chemical irritants (Christenson Faindt -Poeschi, 1978). Thus, increasing or decreasing number of white blood cells is a normal reaction to a chemical such as pesticides in the present study. The increases in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to lindane and malathion (Joshi *et al.*, 2002).

In the differential white blood cells count, a sharp decrease was observed in the percentage neutrophils and eosinophilis. The reduction in the percentage neutrophils and eosinophils here are in agreement with the findings of Sharma and Gupta (1984), when juveniles of mudfish, *Clarias batrachus* were exposed to carbon tetrachloride. This was attributed to tissue damage. Statistically significant increase of lymphocytes was recorded in this investigation. This is in agreement with the findings of Samprath *et al.* (1993) when they exposed the *Nile tilapia, O. niloticus* to a toxic environment. This may attribute to stimulation of the immune mechanism of the fish to eliminate the effects of the pollutants.

In conclusion, the changes in the haematological parameters indicate that they can be used as indicators of pesticide related stress in fish on exposure to elevated Endosulfan levels in the water.

Authors' affiliations

J.K. DUBEY, D.B. MISHRA, G. SINGH AND V.B. SINGH, Department of Zoology, T.D.P.G. College, JAUNPUR (U.P.) INDIA

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