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A Unique Haematological Response of Tilapia, *Oreochromis mossambicus* (Peters), to Sublethal Concentration of the Insecticide, Ekalux EC-25

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Exposure of tilapia, *Oreochromis mossambicus* (Peters), for 24 hr to a sublethal concentration of an organophosphorus insecticide, Ekalux EC-25 (25% w/w Quinolphos), elevated the erythrocyte count, haemoglobin content and haematocrit. This enabled the fish to effectively compensate for the insecticide-induced reduction in gas exchange through the gills. However, the compensation seems to have been effected by a unique mechanism of decreasing the size of the erythrocytes (microcytosis) but, maintaining the haemoglobin saturation unaltered, so that, the total surface area for oxygen absorption per unit volume of blood increased. The mechanism may be conceived as an economy measure in the synthesis and incorporation of haemoglobin under the stress induced by the insecticide. The present results thus suggest that microcytosis could be an effective adaptive mechanism for increasing the total oxygen carrying capacity of blood in the fish.

Key Words: Ekalux EC-25, Haematology, Insecticide toxicity, *Oreochromis mossambicus*, Organophosphorus compound, Tilapia

Introduction

Peripheral haematology is a reliable indicator of the physiological status of fishes and an effective parameter to assess the background effects of environmental contamination (Malte 1986, van Vuren 1986). Haematological changes in fishes induced by sublethal concentrations of various biocides are reported by many workers (Shammi & Qayyum 1978, Joshi et al. 1979, Koundinya & Ramamurthi 1979, 1980, Verma et al. 1979, 1981, 1987, Mathiessen 1981, Mishra & Srivastava 1983, van Leeuwen et al. 1986 Vig et al. 1987). But no such information is available on the effect of Ekalux an insecticide widely used in agricultural practices in India. The present paper reports the results of a study on the sensitivity of the peripheral haematological make up of tilapia, *Oreochromis mossambicus* (Peters) to a sublethal concentration of this pesticide.

Material and Methods

Specimens of *O. mossambicus* collected from a local freshwater pond were transported alive to the laboratory in large polythene containers. Fishes were acclimatised for one day to laboratory conditions by keeping them in large aquarium tanks containing filtered well-water.

Apparently healthy fishes, ranging 35-120 mm in standard length were selected for the study. Ten fishes were maintained as control and 10 others were used for the experiment. Both the control and experimental fishes were not fed during the experimental period.

Ekalux EC-25 (Pesticide Corporation of India Ltd.) is a wide-spectrum contact and stomach insecticide with a quick 'knock-down' effect. It contains 25% (w/w) Quinolphos—an organophosphorus acid ester. It is widely used in agricultural practices in India.

For TL_m determination and for experimental studies, circular polythene troughs of 16 l capacity (36 cm diameter and 16 cm height) were used. Filtered well-water was used throughout the experiment. TL_m concentration (TL_m-24 hr) was determined by adopting the procedure suggested by Doudoroff et al. (1951) for sewage and industrial wastes. The TL_m-24 hr of the insecticide formulation was 2.1 mg/l; the sublethal dose used was 1.9 mg/l at which no mortality occurred and the fish showed no apparent stress signs. The exposure time was 24 hr.

Haematological analyses were carried out by standard methods suggested by Blaxhall and Daisley (1973). Blood samples were collected by severing the caudal peduncle. Total erythrocyte count (TEC), haemoglobin content (Hb), haematocrit (Ht) and erythrocyte sedimentation rate (ESR) were determined and from the results, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), volume index (VI), colour index (CI), and saturation index (SI) were computed. The results were statistically tested employing Student's 't' test.

Results and Discussion

Results of the haematological analyses of the control and experimental fish are presented in table 1.

Exposure of *O. mossambicus* to sublethal concentration of Ekalux caused significant increase in total erythrocyte count ($P < 0.01$) and haemoglobin content ($P < 0.05$). Even though haematocrit of the exposed fish registered higher mean value than of the control, the observed difference between the two groups was not statistically significant.

In fish, erythrocytosis and increase in Hb and Ht following stress are regarded to be the result of impairment of gas exchange by the gills (Grizzle 1977, Larsson et al. 1980, Haniffa et al. 1986) and the consequent "excitation" or "stimulation" of erythropoiesis (Joshi et al. 1979) or "compensatory erythropoiesis" (Larsson et al. 1980, Sharma & Joshi 1986). However, this type of compensatory reaction usually stimulates erythropoiesis, thereby leading to the release of immature erythrocytes into the circulating blood (see Joshi et al. 1979, Joshi 1987a).

Based on his studies on acid-aluminium-exposed *Salmo gairdneri*, Malte (1986) concluded that increase in erythrocyte volume resulting in dilution of Hb within the cells, and consequent increased affinity of Hb to oxygen, is an important response that improves oxygen loading in the gills of fish suffering respiratory impairment under the stress of toxicants.

TEC, Hb and Ht increased in Ekalux-exposed *O. mossambicus* indicating that compensation of respiratory stress occurred in them. But the significant observation has been the remarkable reduction in the size of erythrocytes (microcytosis) of this fish. A large number of microcytes appeared in the peripheral blood of the insecticide-exposed fish; the frequency of occurrence of microcytes had been as high as 1-6 cells per oil-immersion field of TLM (10 × 100 magnification). Though microcytes are known to occur in the peripheral blood of teleosts, their abundance in normal, healthy fishes is very negligible (Joshi 1987b), and these cells are generally regarded as abnormal or aberrant forms of erythrocytes. The mechanism by which compensation of respiratory stress was achieved in *O. mossambicus* under the stress of Ekalux, therefore, seems to be unique. The evidence available from the present study is enough proof to hypothesize that compensation of respiratory

Table 1 *Haematological variables (mean ± S.E.) of control and Ekalux-exposed Oreochromis mossambicus*

Haematological variables	Control (n =10)	Ekalux-exposed (n = 10)	't' values
TEC (X 10^5/mm^3	2.076 ± 0.077 (1.720 – 2.595)	2.40 ± 0.053 (2.095 – 2.585)	3.113 **
HB(g%)	11.64 ± 0.18 (10.2 – 12)	12.07 ± 0.04 (11.8 – 12.2)	2.214*
Ht(%)	31.4 ± 0.57 (28 – 34)	32.8 ± 0.86 (28 – 38)	1.224*
ESR (mm/hr)	4.6 ± 0.88 (1 – 10)	2.9 ± 0.26 (1 – 4)	1.657*
MCV((µm^3)	153.06 ± 5.53 (115.61 – 186.05)	136.83 ± 2.81 (122.27-152.74)	2.356*
MCH(pg)	56.67 ± 1.8 (45.47 – 67.44)	50.56 ± 1.21 (47.20-58.23)	2.536*
MCHC(%)	37.12 ± 0.51 (34.71 – 40.00)	37.06 ± 1.01 (32.11 – 43.57)	0.048
VI	1.00	0.89 ± 0.02 (0.82 – 0.99)	-
CI	1.00	0.90 ± 0.28 (0.84 – 1.07)	-
SI	1.00	1.00 ± 0.32 (0.87 – 1.17)	-

Ranges are indicated in parentheses * $P < 0.05$; ** $P < 0.01$.

impairment in Ekalux-exposed tilapia was achieved through microcytosis—release of smaller erythrocytes, which in the present case were saturated with Hb, as evident from the saturation index and MCHC of the erythrocytes of the exposed fish. This mechanism will not only considerably increase the surface area for oxygen exchange in unit volume of blood, but will also help disperse hemoglobin in blood. Malte (1986) contented that swelling of RBC enhances oxygen affinity of Hb, because Hb gets diluted within the cells. In the present case, it may be conceived that microcytosis helped 'dilution' of Hb in unit volume of blood by its dispersal through the small units of microcytic erythrocytes. The physiological efficiency of this mechanism will have to be confirmed, however.

Many toxicants are known to lower total erythrocyte count, haemoglobin content and haematocrit, or to cause anaemia in fish (Fletcher & White 1986, Torres et al. 1986). Such a decrease in these haematological variables is accounted for as a general non-specific stress response, resultant of haemodilution (Torres et al. 1986)—a mechanism that "... reduces the concentration of an irritating factor in the circulatory system" (Smit et al. 1979). The slower ESR of the Ekalux-exposed fish in the present study indicates that haemodilution did not occur in them. Further, colour index of the RBCs of the exposed fish was below unity. Their MCV and MCH were also significantly lower than those of the control fish. Theoretically, this situation is a typical case of microcytic-hypochromic anaemia. But, the fact that in the exposed fish, TEC, Hb and Ht were higher than in the control (the low MCH and CI of the RBC of these fish are attributable to the smaller average size of their RBCs) overrules the occurrence of anaemia in its true sense. This is further substantiated by the fact that the saturation index of both the control and exposed fishes remained at unity indicating that, though smaller in size, the RBCs of the exposed fish were fully saturated with Hb. Moreover, the unaltered MCHC of the exposed fish shows that there was no significant reduction in the average haemoglobin concentration in unit volume of blood of Ekalux-exposed fishes.

The present results, in addition to suggesting that the observed mechanism of compensation may be an effective measure of economy in the synthesis and incorporation of haemoglobin in fish under stress, also suggest that microcytic erythrocytes may not be simple abnormal/pathological or aberrant cells as hitherto believed but, a variation of some adaptive significance that would help vertebrates to circumvent probable impairment/reduction of their normal respiratory efficiency. However, more detailed work would be required to prove this hypothesis unequivocally.

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