Effect of Intoxication by Some Heavy Metals on Blood Characteristics of *Siganus Rivulatus* (Siganidae, Teleost)

By

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Introduction:

Blood alterations in fish or damage of hemopoietic tissue organs may be associated either with changes in environmental conditions or with water born pollutants (McCay, 1929, Dawson, 1935 & Gradner and Yevich 1969b).

Most of the previous studies on the effect of intoxication with pollutants on blood characteristics, were based on differential counts of W.B.C. & R.B.C., as well as the relative concentration of hemoglobin content and hematcrit values (Khadre, 1990, Amin, 1992, Houston et al 1993, Zia & McDonald 1994, Adak, 1995 & Abdel Magid, 1997). In present study morphological characteristics of red blood cells were studied beside the other hematological parameters on *S. rivulets* exposed to sublethal concentrations of some heavy metals (Cu, Pb & Cd).

Material & Methods:

A group of rabbit fish *Siganus rivulatus* was collected from the catch of beach seine at EL-Mex region. Size of the fish was varied between 7.9 to 14.3 cm and weight ranged from 7.1 to 12 gm. They were then put in glass aquaria (60 liters capacity) for about two weeks for acclimatization. Then the tested media were prepared. Experimental fish after this acclimatization were transferred one hour after preparation of the media, into the experimental aquaria. These were glass aquaria of 36-liter capacity each. They

were provided with electric aerators, with plastic tubes.

Experimental glass aquaria were cleaned carefully & washed with water & acetone then with running tap water several times and finally with sea water, before running the experiments.

Pollutants:

1000 ppm stock solutions of the three metals were prepared. Cadmium as cadmium chloride ($Cd\ Cl_2\ .\ H_2O$), lead as lead nitrate ($Pb\ (NO_3)_2$) and copper as copper sulphate ($Cu\ SO_4\ .\ 5H_2O$).

Standard solutions were stored in clean bottles and diluted to desired concentration just before the beginning of the experiment.

Sublethal concentrations used were 8.4 ppm for cadmium, 2.3 ppm for Pb & 0.2 ppm for Cu (Abdel Barr 1997).

R.B.Cs count:

R.B.Cs counts were done according to the method of Hesse (1960) & Wintrob (1967) using improved Neubar haemocytometer.

Hemoglobin content was determined according to the calorimetric method of Sahli (Hesser 1960).

Results:

Normal red blood cells:

The erythrocytes of normal fish are ellipsoidal in shape (the short axis nearly two thirds of the long axis). They are nucleated with a centrally located nucleus, which is ellipsoidal as well. The measurement of the cells varies between 11 x 5 μm and 7 x 6 μm with a mean of 8.8 ± 1.4 x 5.7 ± 0.9 μm for long and short axis. The nuclei are dark purple in color and measure between 4 x 3.5 μm and 5 x 3 μm with a mean of 4.2 ± 0.4 μm x 3.3 ± 0.5 μm or the long and short axis respectively. R.B.Cs count in normal fish ranged from

420000 to 720000 cell / mm^3 with a mean value 445000 ± 250000 cell /mm^3).

Hemoglobin content:

Hemoglobin content was found to vary between 1.38 & 1.54/ 100 ml blood for normal fish of *S. Rivulatus.*

(I) Morphological variations in red blood cells:

(1) Effect of sublethal concentration of Lead (2.3 ppm):

Lysis of red blood cells is quite clear upon examination of the blood films after 4 days of exposure to 2.3 ppm Pb (cell wall of erythrocyte disintegrated and the nuclear material were clumped) (plate 1a). On the other hand diameter of the red blood cells showed a nonsignificant decrease (8.1 ± 1.2 μm in treated fish and 8.8 ±1.4 μm in control, t = -2.0 & P £ 0.5). After 8 days of exposure fragmentation of some red blood cells was clear (plate 1b), with appearance of tear drop like cells and acanthocytes. After 12 and 16 days of exposure the red blood cells lose their normal oval shape so that they become nearly rounded. Crenated cells acanthocytes and tear drop like cells were frequent (plate 1c). Beside lysis of some red cells, some cells appear to be stacked together.

(2) Effect of sublethal concentration of Cadmium (8.4 ppm) :

In the blood film of fish exposed to sublethal concentration of Cd (8.4 ppm) for 4 days it appears that most of the red blood cells lose their normal oval shape (plate 2a). Acanthocytes and crenated cells were apparent beside lysis of some red blood cells. Sticking of the red blood cells and fragmentation of red blood cells were also observed. Upon further exposure (8 days) the phenomena of sticking of the cells is further apparent (plate 2b). Acanthocytes as well as Crenated cells become common. Tear drop cells and sickle cells were apparent. After 12 days of exposure sticking of the cells become more apparent. The stacked cells lose their original shape with appearance of sickle shaped

cells (plate 2b & c). The nuclei of the red blood cells show some variation in their shape. By 16 days of exposure cells acquired different shapes with obvious disintegration of cell wall of some cells and acanthocytes were frequent (plate 3a).

(3) Effect of exposure to sublethal concentration of copper (0.2 ppm) :

The first observed change in the red blood cell morphology after treatment with Cu was the rouleaux appearance that appeared in the first 4 days of treatment (plate 3b). Also cells lose their normal shape acquiring different shapes or become rounded (plate 3c). After 8 days of exposure the red blood cells started to be stacked with each other with nuclei losing some amount of central dye showing a sign of karyolysis (plate 4a) and significantly increase in size ($t = 3.5$ & $P < 0.5$). Sickle cells were apparent and most of erythrocyte cells lose their normal shape. Upon 12 days of exposure most of the red blood cells lose their normal oval shape and become polygonal or irregular in shape (plate 4b). Sickle cells were apparent. Also spherocyte and tear drop like cells were observed (plate 4c).

(II) Variations in R.B.Cs count and haemoglobin content:

(1) Lead :

R.B.Cs count significantly decreased than that of control fish after 4, 8 and 12 days of exposure to sublethal concentration of lead (2.33 ppm) (Pb = -5.8 (4 d), -25.7 (8 d) & -13.4(12 d) $P \leq 0.05$). A significant decrease in haemoglobin content as compared to control fish also occurred (t =-9.25 (4 d), -10.66 (8 d) and -16.48 (12 d) $p \leq 0.05$).

(2) Cadmium :

Significant decrease in RBCs count was noticed (t = -3.9 (4 d), -14 (8 d) & -3.6 (12 d) $P \leq 0.05$). A significant decrease in hemoglobin content hemoglobin content of fish also occurred (t = -14.9 (4 d), -23.1 (8 d) and -16.5 (12 d) $P \leq 0.05$).

(3) Copper :

A slight increase in RBCs of the fish that exposed to 0.2 ppm Cu after 4, 8 & 12 day was observed ($t = -4.07$ (4 d), -4.2 (8 d) & -3.05 (12 d) $P \leq 0.05$). Significant increase in hemoglobin content was also noticed ($t = 8.2$ $P \leq 0.05$) after 4 days of exposure. However this rise did not continue, either after 8 days or 12 days.

Discussion*:*

The effect of pollution on the blood picture of fishes made the subject of study of various workers (Osman et al 1993, Zia & McDonald 1994 & Adak, 1995). Comparison of hematological characteristics of S.rivulatus, with those of other fishes are given according to the present study exposure of *Siganus rivulatus* to sublethal concentrations of heavy metals Pb (2.3 ppm), Cd (8.4 ppm) and Cu (0.2 ppm) resulted in some changes in the morphology of the R.B.Cs which tended to lose its ellipsoidal shape acquiring different shapes. Among the abnormal cells were the tear drop like cells, acanthocytes, crenated cells and sickle cells. Another effects of metals observed in the present study, are the sticking of the cells together, decrease (in case of Pb) or increase (in case of Cu) in size and appearance of spherocytes. In case of lead and cadmium cell wall of the erythrocytes may disintegrate leaving the nuclei free in the blood film, as well as cell fragments. Few studies are concerned with study of the morphological changes in red blood cells of fishes upon exposure to heavy metals or pollutants as a whole according to available literature. Smith (1968) recorded poikilocytosis and anisocytosis of red blood cells of coho salmon *Onchorhynchus kistuch* fed on folic acid deficient diet. He claimed that these results are characteristic feature of megaloblastic, normoblastic and macroblastic anaemia. Sarivastava and Mishra (1978) found that exposure of *Colisa fasciatus* to sublethal concentration of lead resulted in lysis of the erythrocytes. Shabana (1983) found by microscopical examination of R.B.Cs of poisoned *Clarias lazera* with lead that cells were deteriorated, as clumping of chromatin material and lysis of their

cellular and nuclear membrane were noticed. Ahmed and Munshi (1992) found that exposure of Indian carp *Calta calt*a to copper resulted in shrinkage and crenation of the configuration of the red blood cells with slight anisocytosis and with a tendency to overlap.

Poikilocytosis of red blood cells resulted in association with various types of anaemia e.g. megaloblastic anaemia, iron deficiency anaemia, thalassemia, myelosclerosis or from damage to circulating red blood cells as in microangiopathic hemolytic anaemia (Dacie & Lewis, 1975). This suggests that fish exposed to heavy metals suffer from anaemia.

Acanthocytosis is seen in the liver diseases (Cooper, 1980) or in association with abnormal phospholipid metabolism (Dacie and Lewis, 1975). This suggests that the fishes exposed to heavy metals (at sublethal doses) suffer from liver disease. Tear drop cells may result from the Pitting action of the spleen which removes autophagocytic vacuoles or inclusions. They are also seen in beta thalassemia, Heinz body anaemia, megaloblastic processes and disorders or leukemia and are caused by the removal of precipitated haemoglobin inclusions by the spleen (Bike, 1993). Sickle cells are present in homozygous Hb-S disease in films of freshly withdrawn blood and subjected to anoxia (Dacie and Lewis, 1975).

Exposure of *Siganus rivulatus* to sublethal concentrations of Pb and Cd resulted in significant decrease ($p \leq 0.05$) in erythrocyte count and haemoglobin content. This case was parallel to the observation of Smith et al. (1971) on chinook salmon *Onchorhynchus tshawytsha* subsequent to treatment with phenylehydrazin; in coho salmon *Onchorhynchus kistuch* following exposure to residual chlorine (Buckly et al., 1976) and generally in teleosts exposed to pulp mill effluent (Warner, 1967 and McLeay, 1975) and to cadmium (Larsson et al., 1976). The same observation was also recorded on *Colisa fasciatus* following exposure to lead (Sarivastava and Mishra, 1979), in *Clarias lazera* after acute exposure to lead (Shabana, 1983) on *Anguilla vulgaris* after exposure to mercury (Hilmy et al., 1987), on *Clarias lazera* exposed to hexavelant chromium (Hilmy

et al., 1988) on *Oreochromis niloticus* exposed to cadmium (AL-Akel et al., 1988), on *Crassius carassius* exposed to lead (Fantin et al., 1989), on *Clarius lazera* exposed to mercury and gallant (Abd-Allah et al., 1991) on European silver eel exposed to lead (Amin, 1992) and on *Oreochromis mossambicus* exposed to arsenic and zinc (Adak, 1995).

Abdel-Maguid (1997) found that starvation resulted in decrease of both R.B.Cs count and haemoglobin content of *Gobius niger*. On the other hand upon exposure of *Siganus rivulatus* to sublethal concentration of copper significant increase in erythrocyte count and haemoglobin content was observed. Ghazaly and Said (1995) found similar results in *Tilapia nilotica* after exposure to sublethal concentration of copper. Some other metals were found to induce the same effect of copper on fish blood. Thus Frovola (1960) recorded an increase in erythrocyte count and haemoglobin content in carp *Cyprinus carpio* after exposure to cobalt. Also the same results were found in rainbow trout *Salmo gairdneri* (Schiffman and Forman, 1959) and in *Colisa fasciatus* (Srivastava et al., 1979) following exposure to chromium.

SUMMARY

Red blood cells of *Siganus rivulatus* are of ellipsoidal shape with ellipsoid nucleus that stained dark purple. They varied in size between 7 x 6 ىm and 11 x 5ى m with nucleus varying in size between 4 x 3.5ى m and 5 x 3ى m for short and long axis respectively. Their number ranged from 420000 to 470000 cell / mm^3 blood.

Hemoglobin content of the bloodof *Siganus rivulatus* was found to be about 1.38/100 ml blood.

Exposure of *Siganus rivulatus* to sublethal concentrations of Pb and Cd resulted in significant decrease ($p < 0.05$) in erythrocyte count and haemoglobin content, while there were slight increase in case of copper.

The microscopical observations showed that exposure of *Siganus rivulatus* to sublethal concentrations of heavy metals Pb (2.3 ppm), Cd (8.4 ppm) and Cu (0.2 ppm)

resulted in some changes in the morphology of the R.B.Cs which tended to lose its ellipsoidal shape acquiring different shapes (poikilocytosis or anisocytosis). Among the abnormal cells were the tear drop like cells, acanthocytes, crenated cells and sickle cells. Another effects of metals observed in the present study, are the sticking of the cells together, decrease (in case of Pb) or increase (in case of Cu) in size and appearance of spherocytes. In case of lead and cadmium cell wall of the erythrocytes may disintegrate leaving the nuclei free in the blood film, and appearance of cell fragments.

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