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Sublethal effects of cadmium and copper on the blood characteristics of catfish *Clarias batrachus* (Linn.)

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Abstract

The present study was conducted to determine the effects of sublethal concentrations of $CdCl_2$ and $CuSO_4$ as a pollutant on some haematological parameters of *Clarias batrachus* were exposed to three sublethal levels. Sublethal concentrations used were 7.5 ppm for cadmium and 0.9 ppm for Cu. The results obtained revealed that there was significant difference in hematocrit, Exposure of *Clarias batrachus* to sublethal concentrations of Cd resulted in significant decrease ($p < 0.05$) in erythrocyte count and haemoglobin content, while there was a slight increase in case of copper. The microscopical observations showed that exposure of *Clarias batrachus* to sublethal concentrations of heavy metals 7.5 ppm for cadmium and 0.9 ppm for Cu 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).

Keywords: Cadmium, Copper, Hematological parameters, *Clarias batrachus*.

Introduction

Blood alterations in fish or damage of hemopoietic tissue organs may be associated either with changes in environmental conditions or with water borne pollutants (McCay, 1929; Dawson, 1935 and Gradner and Yevich 1969b). Most of the earlier studies on the effect of intoxication with pollutants on blood characteristics were based on differential counts of W.B.C. and R.B.C., as well as the relative concentration of haemoglobin content and hematocrit values (Khadre, 1990; Amin, 1992; Houston *et al.*, 1993; Adak, 1995 and Abdel Magid, 1997). In the present study morphological characteristics of red blood cells were studied beside the other haematological parameters on *C. batrachus* exposed to sublethal concentrations of some heavy metals (Cu and Cd).

Materials and Methods

Adult and live fish *C. batrachus* were collected from the fish farm Patra and Bhadbhada Bhopal M.P.) and were brought to the laboratory, cleaned by using 0.1% $KMnO_4$ to avoid dermal infection. Only healthy fishes (Length: 12-15cm, Weight: 50-60g) were taken for

experiment. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24h. They were 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.

Experimental glass aquaria were cleaned carefully and washed with water and acetone then with running tap water several times and finally.

Pollutants:

1000 ppm stock solutions of the three metals were prepared. Cadmium as cadmium chloride ($CdCl_2$. H_2O), 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 Cd (7.5 ppm) and Cu (0.9 ppm) (Abdel Barr 1997).

R.B.Cs count:

R.B.Cs counts were done according to the method of Hesser (1960) and Wintrob (1967) using improved Neubar haemocytometer. Haemoglobin 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 µgm and 7 x 6 µgm with a mean of 8.8 ± 1.4 x 5.7 ± 0.9 µgm for long and short axis. The nuclei are dark purple in colour and measure between 4 x 3.5 µgm and 5 x 3 µgm with a mean of 4.2 ± 0.4 µgm x 3.3 ± 0.5 µgm 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).

Haemoglobin content:

Haemoglobin content was found to vary between 1.38 and 1.54/ 100 ml blood for normal fish of *C. batrachus*.

(I) Morphological variations in red blood cells:

(1) Effect of sublethal concentration of Cadmium (7.5 ppm):

In the blood film of fish exposed to sublethal concentration of Cd (7.5 ppm) for 4 days it appears that most of the red blood cells lose their normal oval shape (Fig. 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 (Fig. 2b). Acanthocytes, Crenated cells, 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 (Fig. 2c). The nuclei of the red blood cells shows 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 (Fig. 3a).

(2) Effect of exposure to sublethal concentration of copper (0.9 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 Fig. 3b). Also cells lose their normal shape acquiring different shapes or become rounded (Fig. 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 (Fig. 4a) and significantly increase in size ($t = 3.5$ and $P < 0.5$). 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 (Fig. 4b). Sickle cells were apparent. Also spherocyte and tear drop like cells were observed (Fig. 4c).

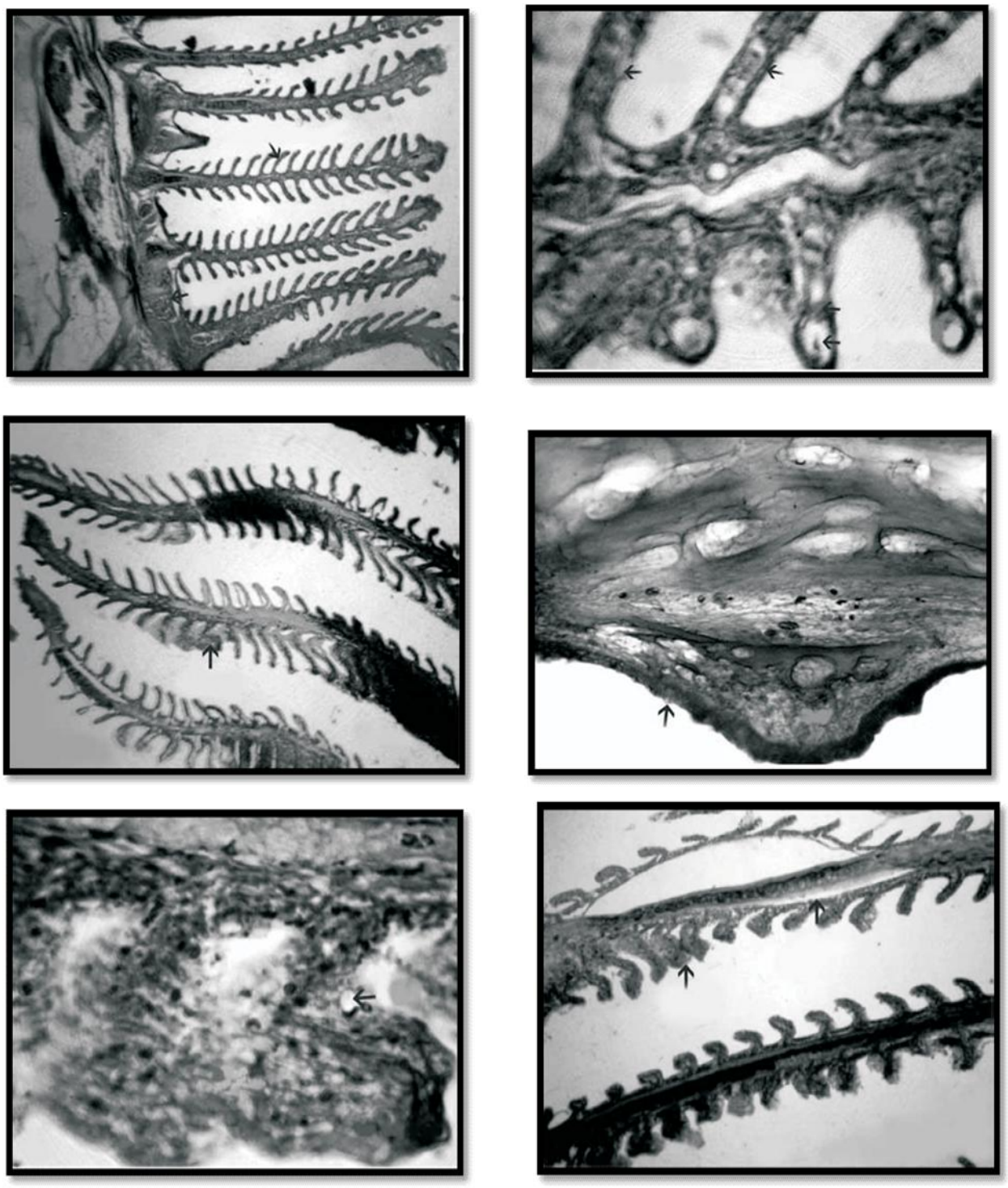

Figure shows the effect of Sublethal concentration of Cd and Cu on normal blood cells.

(2) Cadmium:

Significant decrease in RBCs count was noticed (t = -4.9 (4 d), -16 (8 d) and -4.6 (12 d) $P < 0.05$). A significant decrease in hemoglobin content hemoglobin content of fish also occurred (t = -15.9 (4 d), -24.1 (8 d) and -17.5 (12 d) $P < 0.05$).

(3) Copper:

A slight increase in RBCs of the fish that exposed to 0.9 ppm Cu after 4, 8 and 12 day was observed (t = -5.07 (4 d), -5.2 (8 d) and -4.05 (12 d) $P < 0.05$). Significant increase in haemoglobin content was also noticed (t = 9.2 $P < 0.05$) 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 profile of fishes made the significant studies of various workers (Osman *et al.*, 1993, Zia and McDonald 1994 and Adak, 1995). Comparison of haematological characteristics of *C. batrachus* with those of other fishes are given according to the present study exposure of *C. batrachus* to sublethal concentrations of heavy metals Cd (7.5 ppm) and Cu (0.9 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 Cd) or increase (in case of Cu) in size and appearance of spherocytes. In case of 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. Ahmed and Munshi (1992) have found that exposure of Indian carp *Calta calta* 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 and 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 *C. batrachus* to sublethal concentrations of Cd and Cu resulted in significant decrease ($p<0.05$) 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 *Clarias 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) have 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 *C. batrachus* to sublethal concentration of copper significant increase in erythrocyte count and haemoglobin content was observed. Ghazaly and Said (1995) have 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) had recorded that an increase in erythrocyte count and haemoglobin content in carp *Cyprinus carpio* after exposure to cobalt. Similar type of results were found in rainbow trout *Salmo gairdneri* (Schiffman and Forman, 1959) and in *Colisa fasciatus* (Srivastava *et al.*, 1979) following exposure to chromium.

Conclusion

Exposure of *C. batrachus* to sublethal concentrations of Cd resulted in significant decrease ($p<0.05$) in erythrocyte count and haemoglobin content, while

there was slight increase in case of copper. The microscopical observations showed that exposure of *C. batrachus* to sublethal concentrations of heavy metals Cd (7.5 ppm) and Cu (0.9 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 Cd) or increase (in case of Cu) in size and appearance of spherocytes. In case of copper 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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