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The Alterations in the Hematological Parameters of Brown Trout *Salmo trutta fario*, Exposed to Cobalt Chloride

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Abstract: *Salmo trutta fario* were exposed to a concentration of cobalt chloride (180 μg L^{-1} of) at 12 h intervals, for 28 days in order to determine the effects of this chemical compound on the hematological parameters of this fish. The blood samples were obtained from the all control and treatment fish at the end of the exposure period. Increases were observed in Red Blood Cell (RBC), thrombocyte count, Erythrocyte-Sedimentation Rate (ESR) and hematocrit. On the other hand White Blood Cell (WBC), hemoglobin, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) values were decreased because of the cobalt chloride exposure. Statistical analyses showed that the differences in RBC, MCV and MCH were very important ($p<0.01$) in ESR; important ($p>0.05$) but the others were not.

Key words: Fish, water pollution, toxicity, blood parameters, cobalt, Turkey

INTRODUCTION

Cobalt is an essential element for humans being necessary for the formation of vitamin B12 (hydroxycobalamin). The human body contains about 1-2 mg of cobalt; cobalt deficiency causes anemia and increases the risk of developmental abnormalities and growth failure in infants. However, excessive levels of cobalt can be detrimental to the organism (Bjorklund, 2008).

Cobalt, a natural element present in certain ores of the earth's crust is essential to life in trace amounts but excess dietary cobalt causes toxic effects in mammals (Domingo, 1989). Everyone is exposed to low levels of cobalt in air, water and food. Earlier research has indicated that exposure to cobalt might cause adverse effects on the male reproductive system (Pedigo *et al*., 1988). Co is found in the ores cobaltite, smaltite and erythrite and is usually obtained as a byproduct of copper, iron, lead, nickel and silver extractions.

Although uncontaminated natural waters generally contain no more than a few micrograms per liter of Co it can occur at elevated concentrations in surface waters affected by runoff from mines containing Co-bearing ores (Marr *et al*., 1998). Cobalt chloride is the chemical compound with the formula $CoCl_2$. Oral administration of cobalt chloride to pregnant rats at 12, 24 and 48 mg kg^{-1} body weight/day from day 14 of gestation through day 21 of lactation significantly affected the postnatal survival and development of the pups (Domingo *et al*., 1985). Besides; there are reports about the effects of $CoCl_2$ exposure on the blood biochemistry of *Salmo trutta fario* (Atamanalp *et al*., 2009a, d) and *Oncorhynchus mykiss* (Atamanalp *et al*., 2009b, c).

The route of exposure is frequently dermal or via inhalation that occurs mostly in industrial refining. Cobalt toxicity includes cardiomyopathy, adverse pulmonary effects and carcinogenicity. This heavy metal is also suspected to cause nerotoxic effects and lead to memory deficit (Bjorklund, 2008).

Hematological parameters have been used to describe the health of fish (Blaxhall, 1972), monitor stress response (Soivio and Oikari, 1976; Kocabatmaz and Ekingen, 1984) and predict systematic relationships and physiological adaptations of animals. They more quickly reflect the poor condition of fish than other commonly measured parameters (Alkinson and Judd, 1978). Hematological parameters are increasingly used as indicators of the physiological stress response to endogenous or exogenous changes in fish (Lermena *et al*., 2004). Hematological changes in some fish exposed to various toxicants have been studied in *Tilapia mossambica* (Aziz *et al*., 1993), *Ctenopharyngodon idella* (Shakoori *et al*., 1996), *Heteropneustes fossilis* (Kumar *et al*., 1999) and *Oncorhynchus mykiss* (Atamanalp *et al*., 2002; Atamanalp and Yanik, 2003). Although, Co seldom will be present as the only

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major metal toxicant in mining-related aqueous systems, we are not aware of any previous studies of the toxicity and negative effects of Co and Co compounds. For example there are no data available about the effects of cobalt chloride on the hematological parameters of *Salmo trutta fario*. Thus, we performed the present study to determine if sublethal concentration of cobalt chloride changes hematological parameters in *Salmo trutta fario*.

MATERIALS AND METHODS

Fish maintenance, treatment unit, water and toxicant: A group of 24 *Salmo trutta fario* fish (with an average weight of 125±15 g) was obtained from Ataturk University Agriculture Faculty Aquaculture Engineering Department's farm. The treatment was put into practice in the Toxicology Research Unit of Aquaculture Engineering Department. Fish were acclimated to conditions of research unit for 4 weeks.

The research platforms were 780, l fiberglass circular tanks (100 cm diameter, 100 cm depth) with a constant and fresh water flow (1.5, l min^{-1}) with no recirculation and under natural light conditions. The water temperature was 9.0±0.5°C during the acclimation and treatment period.

The dissolved oxygen and pH levels and total water hardness were 9 ppm, 7.6 and 105 mg in $CaCO_3$, respectively. The tanks were aerated with air pumps. About 24 fish were placed into 3 tanks, 2 tanks for testing the cobalt chloride (7 fish per tank) and the other one for the control group with 10 fish. The chemical compound was obtained from Sigma in its commercial package. Treatment group fish were exposed to a dose of 180 μg L^{-1} of cobalt chloride at 12 h intervals for 28 days.

Blood sampling and analyses: At the end of 4 weeks exposure, fish were taken out and their blood was subjected to hematological analysis (Aziz *et al*., 1993; Shakoori *et al*., 1996; Santhakumar *et al*., 1999; Atamanalp *et al*., 2002). Approximately, 2 cc venous blood was drawn from each group using heparin as an anticoagulant and for the estimation of the Red Blood Cell (RBC) count (Blaxhall and Daisley, 1973; Atamanalp *et al*., 2002), the total White Blood Cell (WBC) and thrombocyte count (Blaxhall and Daisley, 1973; Atamanalp *et al*., 2002) at computer which connected to microscope (Fig. 1), the Hemoglobin (Hb) concentration (Soivio and Oikari, 1976) and the Packed Cell Volume (PCV) (Schalm *et al*., 1975), and Erythrocyte-Sedimentation Rate (ESR) whereas the Mean Corpuscular Volume (MCV), the Mean Corpuscular Hemoglobin (MCH) and the Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated according to Reddy and Bashamohideen (1989).

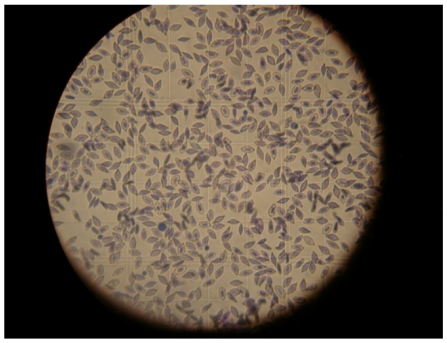

Fig. 1: The view of screen of counting process the blood cells at computer connected with microscope

RESULTS AND DISCUSSION

The red blood cell, thrombocyte, hematocrit and erythrocyte-sedimentation rate values were higher in treated fish group than in control group (Table 1). On the other hand white blood cell, hemoglobin, MCV, MCH and MCHC values were lower in treated fish blood samples. The difference in RBC, MCV and MCH parameters was found as very important ($p<0.01$), the difference in ESR was important but the others were evaluated as not significant after the statistical analyses.

Cobalt chlorite exposure increased RBC value of *Salmo trutta fario* from 1.77±0.43 10^6 mm^{-3} (in control group fish) to 4.46±0.58 10^6 mm^{-3} (in treatment group fish). The difference was evaluated very important ($p<0.01$) in the statistical analyses.

There is no report about the chemical exposure in *Salmo trutta fario* but Atamanalp *et al*. (2002) observed a significant increase in the erythrocyte count in *O. mykiss* exposed to cypermethrin (a synthetic pyrethroit). In another report mancozeb caused to light increase in the same fish RBC value (Atamanalp and Yanik, 2003). Similar findings were also observed in *Ctenopharyngodon idella* exposure to fenvalarate (Shakoori *et al*., 1991) and

Table 1: Changes in the hematological parameters of *Salmo trutta fario* exposed to cobalt chloride. Each value is the mean±SD of all observations

Parameters	Control (n = 10)	Treatment (n = 14)	Statistical analyses
RBC (10^6 mm^{-3})	1.77±0.430	4.46±0.580	**
WBC (10^4 mm^{-3})	8.33±0.702	6.20±1.772	NS
Thrombocytes (10^4 mm^{-3})	10.33±1.604	12.36±1.734	NS
Hemoglobin (g dL^{-1})	8.73±1.000	8.46±1.420	NS
ESR (mm h^{-1})	1.83±1.610	2.50±1.710	*
Hematocrit (%)	32.00±10.54	33.20±2.390	NS
MCV (μm^3)	181.56±48.31	75.56±11.57	**
MCH (pg)	50.34±6.500	19.18±3.460	**
MCHC (%)	29.44±10.79	25.72±5.810	NS

NS: Not Significant, *$p<0.05$, significant, **$p<0.01$ very significant

in *Heteropneuestes fossilis* after exposure to sumithion and sevin (Koundinya and Ramamurthi, 1979) in *Cyprinus carpio* after 48 h exposure to cypermethrin (Reddy and Bashamohideen, 1989) and in *Anabas testudineus* exposed to azodrin. Red blood cell increasing may explain with kidney deformations.

White blood cell count was observed a slight decrease in the treatment group comparing with control group. This finding was against the knowledge that infections and intoxications stimulate WBC in fish but this reason was parallel with Atamanalp *et al.* (2002) and Atamanalp and Yanik (2003) which found that Cypermethrin and Mancozeb decreased WBC in rainbow trout. In the same fish species different findings may explain with the specifications of different toxic compounds. On the contrary of these reports, Shakoori *et al.* (1991, 1996), Aziz *et al.* (1993), Kumar *et al.* (1999) and Santhakumar *et al.* (1999) reported increases in the WBC count of different fish species to different fish species.

There was an increment in thrombocyte count but not statistically significant (10.33±1.604 10^4 mm^{-3} in control and 12.36±1.734 10^4 mm^{-3} in treatment group). Kocabatmaz and Ekingen (1984) reported that thrombocyte count may be affected from stress quickly. Therefore the increased thrombocyte in the present study can be explained by the stress causing role of cobalt chloride. This finding was similar with Atamanalp *et al.* (2002) which observed increase in *O. mykiss* Plt count exposed to cypermethrine.

The decrease in hemoglobin concentration may be attributed to the fact that the oxygen carrying capacity of the fish was affected by the cobalt chloride. This chemical compound appears to interfere with the ability to bind hemoglobin to oxygen during respiration. Due to an insufficient supply of oxygen, respiration was not maintained efficiently. As a result, the demand for hemoglobin content increased. Hb content increasing may depend on the deformed osmoregulation balance of fish. The difference in this value was not statistically important in the present study. Atamanalp and Yanik (2003) observed a significant decrease in Hb content of *O. mykiss* and reported this situation with Hb destroying or to a decrease in the rate of Hb synthesis. Similarly in freshwater catfish (*Heteropneustes fossilis*) the Hb (%) decreased after 30 days exposure to deltamethrin (Kumar *et al.*, 1999). The against findings with these researches may be explain with different fish species and different chemicals effects.

The Erythrocyte Sedimentation Rate (ESR) increased from 1.83±1.61-2.50±1.71 mm h^{-1} with the exposure of cobalt chloride. This was statistically significant ($p>0.05$). The increase in ESR shows that fish were intoxicated by this toxic chemical compound. This finding was similar with Atamanalp *et al.* (2002) and Kumar *et al.* (1999). In the first report the researcher observed that cypermethrin exposure to *O. mykiss* and in the 2nd one deltamethrin exposure to *H. fossilis* increased ESR values of fish.

The alteration in the hematocrit value was slightly in positive route and not statistically important. This value was measured in control as 32.00, 2.50±1.71 and 33.20±2.39% in treatment fish group. Against to the findings, exposure to danitol and fenvalarate caused a significant reduction in the hematocrit (PCV) value in *Ctenopharyngodon idella* (Ahmad *et al.*, 1995; Shakoori *et al.*, 1996) in *O. mykiss* Cypermethrin (Atamanalp *et al.*, 2002) and Mancozeb (Atamanalp and Yanik, 2003). Aziz *et al.* (1993) observed an increase in *Tilapia mossambica* exposed to mercury chloride and supported the present study findings.

Cobalt chloride exposure decreased MCV, MCH and MCHC, the 1st and 2nd parameters too significantly but not the last one ($p<0.01$, Table 1). The decreased MCV is indicative of hypochronic microcytic anemia. MCV reduction shows that cobalt chloride may interfere with the normal physiology of RBC.

The physiological and chemical properties of fish blood are very sensitive to environmental changes (Hughes and Nemcsok, 1988) and pollutants (Yanik and Atamanalp, 2001).

CONCLUSION

In this study the present study reveals that cobalt chloride has profound effects on the hematological parameters of *Salmo trutta fario*. Further research are needed since different fish species and at different growing stages of fish may respond at different levels to pollution factors.

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