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RESEARCH ARTICLE

The Alterations in the Hematological Parameters of Rainbow Trout, *Oncorhynchus mykiss,* Exposed to Cobalt Chloride

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Summary

Rainbow trout, *Oncorhynchus mykiss* were exposed to a concentration of cobalt chloride (180 µg/l of) at 8 hours 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), white blood cell (WBC), thrombocyte count, hemoglobin, erythrocyte-sedimentation rate (ESR) and mean corpuscular hemoglobin concentration (MCHC). On the other hand, hematocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were decreased because of the cobalt chloride exposure. Statistical analyses showed that the differences in WBC, hemoglobin, MCV and MCH were very important ($P<0.01$), in MCHC was important ($P<0.05$) but the others were not important.

Keywords: *Fish, Water pollution, Toxicity, Blood parameters*

Kobalt Klorite Maruz Kalan Gökkuşağı *(Oncorhynchus mykiss)*'nın Kan Parametrelerindeki Değişimler

Özet

Gökkuşağı alabalıkları *(Oncorhynchus mykiss)* kobalt kloritin 180 µg/l'lik dozuna 8 saat aralıklı uygulamalarla 28 gün maruz bırakılarak hematolojik parametreler üzerine etkilerinin belirlenmesi amaçlandı. Deneme periyodunun sonunda kontrol ve muamele gruplarındaki balıkların hepsinden kan numuneleri alındı. Eritrosit sayısı (RBC), lökosit sayısı (WBC), trombosit sayısı, hemoglobin düzeyi, eritrosit sedimentasyon oranı (ESR) ve eritrosit başına düşen ortalama hemoglobin konsantrasyonu (MCHC) değerlerinde artışlar gözlendi. Buna karşılık ortalama eritrosit hacmi (MCV) ve eritrosit başına düşen ortalama hemoglobin miktarı değerlerinde düşüşler belirlendi. İstatistiksel analizler sonucunda WBC, hemoglobin düzeyi, MCV ve MCH değerlerindeki fark çok önemli ($P<0.01$), MCHC değerinde önemli ($P<0.05$), diğerlerinde ise önemsiz olarak değerlendirilmiştir.

Anahtar sözcükler: *Balık, Su kirliliği, Toksisite, Kan parametreleri*

INTRODUCTION

Cobalt (Co) is much less abundant in nature than is copper (Cu) and many other metals, in both igneous and sedimentary rock. Co is found in the ores cobaltite, smaltite, and erythrite, and is usually obtained as a by product 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 run off from mines containing Co-bearing ores [1]. Cobalt chloride is a chemical compound with the formula $CoCl_2$.

Hematological parameters have been used to describe the health of fish [2], monitor stress response [3,4] and predict systematic relationships and physiological adaptations of

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animals. They more quickly reflect the poor condition of fish than other commonly measured parameters [5]. Hematological parameters are increasingly used as indicators of the physiological stress response to endogenous or exogenous changes in fish [6]. Hematological changes in some fish exposed to various toxicants have been studied in *Tilapia mossambica* [7], *Ctenopharyngodon idella* [8], *Heteropneustes fossilis* [9] and *Oncorhynchus mykiss* [10,11].

Although Co seldom will be present as the only 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 is no data available about the effects of cobalt chloride on the hematological parameters of rainbow trout. Thus, we performed the present study to determine if sublethal concentration of cobalt chloride changes hematological parameters in rainbow trout *(Oncorhynchus mykiss)*.

MATERIAL and METHODS

Fish Maintenance, Treatment Unit, Water and Toxicant

A group of 48 rainbow trout fish (with an average weight of 145±12 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 three 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 9.5±0.5°C during the acclimation and treatment period.

The dissolved oxygen, pH levels and total water hardness were 8-9 mg/l, 7.8 and 102 mg/l as $CaCO_3$, respectively. The tanks were aerated with an air pump. Twelve fish were placed into each of tanks, two tanks for testing the cobalt chloride and two tanks as the control. The chemical compound was obtained from Sigma in its commercial package. Treatment group fish were exposed to a dose of 180 µg/l of cobalt chloride at 8 h intervals for 28 days. Treatment group fish were exposed to a dose of 180 µg/l of cobalt chloride at 8 h intervals for 28 days according the rules of renewal environment tests [12].

Blood Sampling and Analyses

At the end of four weeks exposure, all fish were taken out and their blood was subjected to hematological analysis [7,8,10,13]. All fish of treatment groups (24 fish) but 10 fish of control group were sampled. 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 [10,14], the total white blood cell (WBC) and thrombocyte count [2,10] at computer which connected to microscope *(Fig. 1)*, the hemoglobin (Hb) concentration [3] and the packed cell volume (PCV) [15], 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 [16].

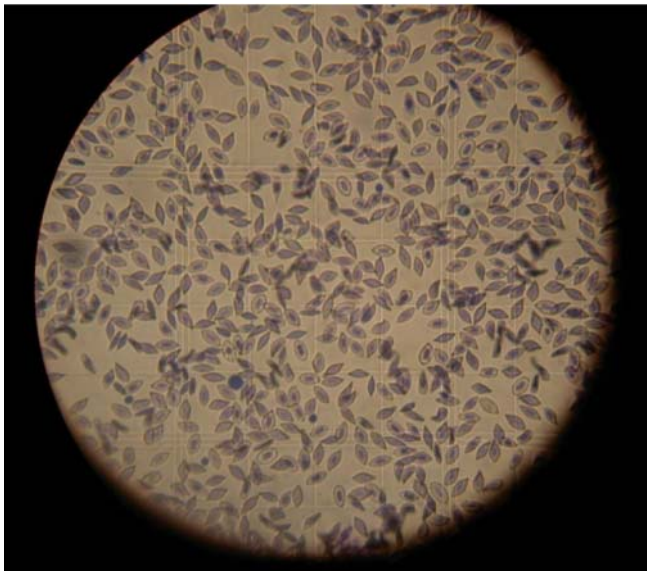


Fig 1. The view of screen of counting process the blood cells at computer connected with microscope

Şekil 1. Bilgisayar bağlantılı mikroskop ile kan hücrelerinin sayım işlemi

RESULTS

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

DISCUSSION

Although no significant differences were observed in RBC, it increased slightly during the exposure period. Atamanalp et al.[10] 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 [11]. Similar findings were also observed in *Ctenopharyngodon idella* exposure to fenvalarate [8] and in *Heteropneuestes fossilis* after exposure to sumithion and sevin [17], in *Cyprinus carpio* after 48 h exposure to cypermethrin [16], and in *Anabas*

Table 1. *Changes in the hematological parameters of Oncorhynchus mykiss exposed to cobalt chloride. Each value is the mean ± SD of all observations*

Tablo 1. *Kobalt klorite maruz kalan gökkuşağı alabalığı (Oncorhynchus mykiss)'nın kan parametrelerindeki değişimler. Her değer tüm değerlerin serbestlik derecesinin ortalamasıdır*

Parameters	Control (n=10)	Treatment (n=24)	Statistical Analyses
RBC (10^6/mm^3)	0.655±0.046	0.695±0.020	N.S.
WBC (10^4/mm^3)	6.972±0.382	22.333± 5.131	**
Thrombocytes (10^4/mm^3)	9.000±2.000	10.933±4.406	N.S.
Hemoglobin (g/dl)	6.783±0.189	9.233±1.001	**
ESR (mm/h)	0.577±0.368	0.900±0.400	N.S.
Hematocrit (%)	40.38±7.63	36.67±5.68	N.S.
MCV (µm^3)	621.207±148.004	193.021±21.312	**
MCH (pg)	104.459±10.721	48.735±4.719	**
MCHC (%)	17.346±3.387	25.644±5.470	*

NS: *Not significant, * P<0.05, important, ** P<0.01 very important*

testudineus exposed to azodrin. Red blood cell increasing may explain with kidney deformations.

White blood cell count was observed very high increase (P<0.01) in the treatment group comparing with control group. This was an inevitable result because infections and intoxications stimulate WBC in fish. This reason was opposite with Atamanalp et al.[10] and Atamanalp and Yanik [11] 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, [7-9,13,18] reported increases in the WBC count between different fish species. So our finding is supported by these ones.

There was a not statistically significant increase in thrombocyte count, (9.000 10^4/mm^3 in control and 10.933 10^4/mm^3 in treatment group). Kocabatmaz and Ekingen [4] reported that thrombocyte count may be affected from stress quickly. Therefore, increased thrombocyte in the present study can be explained by the stress causing role of cobalt chloride.

The increase 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 of hemoglobin to bind 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 the deformed osmoregulation balance of fish. Atamanalp and Yanik [11] 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 [9].

The erythrocyte sedimentation rate (ESR) increased slightly, as the exposure of cobalt chloride. The increase in ESR shows that fish were intoxicated by this toxic chemical compound. Previous two studies [9,10].

Similar to ESR parameter; the alteration in the hematocrit value was slightly and not statistically important. This value was measured in control as 40.38% and 36.67% in treatment fish group. Similar our findings, exposured to danitol and fenvalarate caused a significant reduction in the hematocrit (PCV) value in *Ctenopharyngodon idella* [8,19], in *O. mykiss* cypermethrin [10] and mancozeb [11]. However, an increase was observed in *Tilapia mossambica* exposed to mercury chloride [7]. In spite of an increase in the RBC count, the PCV reduction shows the magnitude of the shrinking cell size due to this chemical compound intoxication [19]. The decrease in PCV shows that cobalt chloride may interfere with the normal physiology of RBC.

Cobalt chloride exposure decreased MCV and MCH too significantly (P<0.01) and increased MCHC significantly (P<0.05). The decreased MCV and increased MCHC are indicative of hipochronic microcytic anemia. The increased Hb content may also be attributed to increased erythropoiesis and hemoglobin synthesis which, in turn, explains in increased MCHC. MCV reduction shows that cobalt chloride may interfere with the normal physiology of RBC *(Table 1)*.

The physiological and chemical properties of fish blood are very sensitive to environmental changes [20] and pollutants [21]. The present study reveals that cobalt chloride has profound effects on the hematological parameters of rainbow trout. Further works 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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