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Alterations in Hematological Parameters of Rainbow Trout (*Oncorhynchus mykiss*) Exposed to DDVP

Muhammed Atamanalp*, Simay Angis, Pinar Oguzhan and Ercument Aksakal

Fisheries Department, Agricultural Faculty, Ataturk University, 25240 Erzurum, Turkey

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Abstract

The aim of this study was to assess the effects of a sublethal dose (1.6 mg/l) of DDVP (dichlorvos) on hematological parameters of rainbow trout (*Oncorhynchus mykiss*) after 28 days of exposure. The DDVP caused increases in the red and white blood cell counts, hemoglobin, erythrocyte sedimentation rate, mean corpuscular volume, and mean corpuscular hemoglobin concentration (MCHC). On the other hand, it decreased thrombocyte (Plt), hematocrit (packed cell volume), and mean corpuscular volume. The only statistically significant differences between exposed and unexposed fish were in white blood cell count and MCHC.

Introduction

Dichlorvos (DDVP; 2,2-dichlorovinyl dimethyl phosphate) is an organophosphate pesticide commonly used to eradicate crustacean ectoparasites and treat sea lice (*Lepophtheirus salmonis* and *Caligus elogatus*) on commercial fish farms (Murison et al., 1997; Varo et al., 2003).

Dichlorvos is volatile and highly toxic by inhalation, dermal absorption, and ingestion. As with all organophosphates, dichlorvos is readily absorbed through the skin. Compared to poisoning by other organophosphates, dichlorvos causes a more rapid onset of symptoms and is often followed by a similarly rapid recovery (EXTOXNET, 1996). The toxicity of DDVP has been studied in various aquatic invertebrates and fish. Also, the dispersion of the chemical after release from the cages has been simulated and monitored (Murison et al., 1997).

Hematological parameters respond quickly to changes in environmental conditions and have been studied in a wide range of marine to freshwater fish. Hematological parameters are used to describe fish health, monitor stress response, and predict systematic relationships and physiological adaptations of animals. Hematological parameters more quickly reflect the poor condition of fish than other commonly measured parameters.

The goal of this research was to determine changes in rainbow trout blood parameters following exposure to DDVP.

Materials and Methods

Fish and maintenance. Rainbow trout of both sexes (145±20 g) were obtained from the Trout Breeding and Research Center of the Faculty of Agriculture at Ataturk University. Seven fish were allocated to the treated group

* Corresponding author. E-mail: mataman@atauni.edu.tr

and five to the control. Fish were acclimated to conditions in the research unit for four weeks in 600-l fiberglass tanks with a fresh water flow of 0.5 l/min/kg fish, a waste water discharge outlet, and a water temperature of 11.0±0.5°C. Fish were fed commercial trout pellets throughout acclimation and the experiment.

Toxicant. A commercial package of DDVP was obtained from IMPA Tarim (Turkey). All tanks were filled with 600 l water. Treated tanks received DDVP at a sublethal concentration of 1.6 mg/l. The chemical was added to the tanks once a day for 28 days.

Blood collection and blood analyses. Approximately 2 cc blood was collected from the caudal vein of each fish, anticoagulant heparin was added, and the blood was placed in Vacutainer tubes. Red and white blood cells and thrombocytes (Plt) were counted according to Blaxhall and Daisley (1973). The hemoglobin (Hb) concentration was determined as in Hoffmann and Lomel (1984), the erythrocyte sedimentation rate as in Blaxhall and Daisley (1973), and the hematocrit (PCV) as in Schalm et al. (1975). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Heath (1987) and as in Reddy and Bashamoiden (1989).

Statistical analysis. Differences among groups were tested with variance analyses and group averages were analyzed using Duncan's test.

Results

The red and white blood cell counts, Hb, erythrocyte sedimentation rate, MCH, and MCHC were higher in treated fish than in control fish (Table 1). On the other hand, thrombocyte (Plt), hematocrit (PCV), and MCV were lower in treated fish. The only statistically significant differences were in white blood cell count and MCHC.

Discussion

The red blood cell count in the control group was within the range of Kocabatmaz and Ekingen (1984; 0.538-1.185 x 10^6/mm^3) and similar to that found by Atamanalp et al. (2002; 0.603 x10^6/mm^3). After DDVP exposure, the red blood cell count was higher in the treated group but did not significantly differ from the control. Likewise, this parameter increased in *Tilapia mossambica* treated with cadmium (Aziz et al., 1993), *Ctenopharyngodon idella*

Table 1. Hematological parameters of rainbow trout treated with dichlorvos for 28 days, compared to untreated control fish (means±SD).

Parameter	*Control (n = 5)*	*Treated group (n = 7)*
Red blood cells (10^6/mm^3)	0.64±0.19	0.65±0.17
White blood cells (10^4/mm^3)	6.9±1.84	11.79±1.76**
Thrombocytes (10^4/mm^3)	8.80±2.65	8.57±2.30
Hemoglobin (g/100 ml)	6.90±2.46	7.70±2.21
ESR (mm/h)	0.40±1.74	1.42±1.33
Hematocrit (%)	43.00±12.40	41.00±11.16
MCV (µm^3)	661.54±212.84	655.75±200.84
MCH (pg)	106.15±51.18	124.58±48.83*
MCHC (%)	16.05±2.99	18.75±2.07

ESR **=** erythrocyte sedimentation rate
MCV = mean corpuscular volume
MCH = mean corpuscular hemoglobin
MCHC = mean corpuscular hemoglobin concentration
* = p<0.05; ** = p<0.01

treated with fenvalerate (Shakoori et al. (1996), *Heteropneustes fossilis* treated with deltamethrin (Kumar et al., 1999), and *Oncorhynchus mykiss* treated with cypermethrin (Atamanalp et al., 2002). On the other hand, some researchers reported a decrease in red blood cell count after exposure, for example, in *Cyprinus carpio* treated with fenvalerate (Reddy and Bashamohideen, 1989), *Ctenopharyngodon idella* treated with mercury chloride (Shakoori et al., 1991), and *Anabas testudineus* treated with monocrotophos (Santhakumar et al., 1999).

The white blood cell count also increased after exposure to DDVP, similar to results of Shakoori et al. (1991), Aziz et al. (1993), Kumar et al. (1999), and Santhakumar et al. (1999). In contrast, Shakoori et al. (1996) and Atamanalp et al. (2002) reported that pesticides caused a reduction in white blood cells in their studies.

The number of thrombocytes (Plt) in the control was much higher than obtained by Kocabatmaz and Ekingen (1984) and Atamanalp et al. (2002) but did not statistically differ from the value in the treated fish. Kocabatmaz and Ekingen (1984) reported 2.1 x $10^4/mm^3$ thrombocytes in rainbow trout before stress and 4.3 x $10^4/mm^3$ after. These differences can be explained by differences in stress level.

Hemoglobin (Hb) was greater in treated fish, perhaps because the oxygen carrying capacity of the fish was affected by the DDVP. Dichlorvos appears to interfere with the ability to bind hemoglobin to oxygen during respiration. Due to a possibly insufficient supply of oxygen, respiration may have been affected. As a result, the demand for hemoglobin increased. The increased hemoglobin may also be attributed to increased erythropoiesis and hemoglobin synthesis which, in turn, explains the increased MCHC. Sublethal doses of mercury chloride increased the hemoglobin of *C. idella* (Shakoori et al., 1991) as did cadmium in *T. mossambica* (Aziz et al., 1993), fenvalerate in *C. idella* (Shakoori et al., 1996), and cypermethrin in *O. mykiss* (Atamanalp et al., 2002). In contrast, hemoglobin decreased in *C. carpio* treated with fenvalerate and cypermethrin (Reddy and Bashamohideen, 1989), *C. idella* treated with fenpropathrin (Ahmad et al., 1995), and *Heteropneustes fossilis* treated with deltamethrin (Kumar et al., 1999).

DDVP caused a insignificant reduction in hematocrit concentrate (PCV), as it did in *C. carpio* exposed to fenvalerate (Reddy and Bashamohideen, 1989), *C. idella* exposed to fenpropathrin (Ahmad et al., 1995), *C. idella* exposed to fenvalerate (Shakoori et al., 1996), and *O. mykiss* exposed to cypermethrin (Atamanalp et al., 2002). The decrease in hematocrit concentrate suggests that DDVP may interfere with the normal physiology of red blood cells. On the other hand, exposure to mercury chloride raised the hematocrit concentrate in *C. idella* (Shakoori et al., 1991) and *T. mossambica* (Aziz et al., 1993), possibly due to specific effects of this chemical in or reactions of these fish species.

The erythrocyte sedimentation rate (ESR) of the control fish was similar to that of healthy rainbow trout found by McCarthy et al. (1975) but lower than that found by Kocabatmaz and Ekingen (1984). ESR was greater in treated fish, in agreement with Kumar et al. (1999), indicating that they were intoxicated by the toxic chemical. ESR is raised by acute infection, heavy metal toxicity, and kidney deformation (Blaxhall and Daisley, 1973).

The difference in mean corpuscular volume (MCV) between the control and treated fish was not statistically significant. The drop in treated fish agrees with Shakoori et al. (1996) and Atamanalp et al. (2002).

DDVP significantly increased the mean corpuscular hemoglobin (MCH) in the treated fish. Toxicity and stress factors have been reported to raise MCH (Reddy and Bashamohideen, 1989; Aziz et al., 1993; Pages et al. 1995) or lower it (Shakoori et al., 1996; Kumar et al., 1999). This discrepancy may originate from the chemicals or differences among fish species.

The mean corpuscular hemoglobin concentration (MCHC) rose in treated fish, similar to Shakoori et al. (1991, 1996) but in contrast to Reddy and Bashamohideen (1989), Aziz et al. (1993), and Santhakumar et al. (1999).

This research shows that dichlorvos has profound effects on the hematological parameters of rainbow trout. More such studies are needed because different life stages within this species and other species may react differently.

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