Hematological Analysis in Fish Toxicology

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Hematological analysis is commonly used to assess the physiological state of fish. It includes red blood cell parameters, white blood cell parameters, and the number of thrombocytes per blood volume unit. Hematological analysis is one of the basic tools (often accompanied by biochemical and histopathological analyses) to assess the influence of organic and inorganic substances on fish. It is, therefore, applicable in both ecotoxicology and pharmacotoxicology.

fish blood parameters head kidney toxicity

1. Introduction

Hematological analysis of peripheral blood parameters and quantitative evaluation of blood cell morphology are useful and relatively inexpensive tools often used in fish toxicology. Blood indices are sensitive and fast reacting biomarkers of various environmental impacts, including water pollution with toxic agents. Blood parameters reflect a wide range of physiological alterations, both adaptive and disruptive. They provide extensive information about various physiological functions as reliable biomarkers of an organism's performance. Blood sampling is less invasive compared to the collection of other tissues from live organisms and is possible under both laboratory and field conditions. Basic hematological parameters: hematocrit (Ht), hemoglobin concentration (Hb), erythrocyte count (RBC), leukocyte count (WBC), and blood smear can be obtained using a small amount of blood (about 200 µL is sufficient). Additional derived red blood parameters can also be calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) using Ht, Hb, and RBC values and appropriate formulas. Stained blood smears may be used for quantitative evaluation of erythrocyte and leukocyte populations to calculate the percentage of immature erythrocytes (erythroblasts), erythrocyte cellular and nuclear anomalies, differential leukocyte count, and thrombocyte count. These parameters are useful to evaluate erythropoietic activity, cytotoxic and genotoxic effects, and the status of the immune system. Hematological analysis is laborious, and to obtain reliable results requires skilled and experienced personnel since most measurements are performed using manual methods—all cells in fish blood are nucleated, and thus standard automatic analyzers applied in mammalian hematology cannot be used. This may be overcome by using modern veterinary analyzers that can be adjusted to work with fish blood [1][2][3].

Toxic agents, e.g., metal ions, pesticides, or other anthropogenic aquatic pollutants, as well as pharmaceuticals such as immunomodulators, antimicrobial and antiparasitic therapeutics, or anesthetics, were proven to cause hematological changes in fish [4][5][6][7][8][9][10]. To observe toxicity-induced alterations, it is necessary to provide reference values. Unfortunately, as in all poikilothermic vertebrates, the internal environments of fish are variable

and considerably affected by external conditions, and it is very difficult to establish reliable hematological reference values for a species since their ranges are very wide [11][12][13]. Witeska et al. [14] summarized the data obtained over eight years from 146 clinically healthy juvenile individuals of *Cyprinus carpio* used as controls in various studies carried out under similar environmental conditions, and the results showed different levels of variability in various hematological parameters; some of them were stable (e.g., frequency of lymphocytes), most were moderately variable (e.g., hemoglobin concentration and red blood cell count), while others turned out to be highly variable (e.g., thrombocyte count). Therefore, some parameters have better biomarker potential than others. According to Ahmed et al. [15], variability of hematological parameters in fish results from the variable internal environment of the fish and the changes of environmental factors. It should be emphasized that reference (normal) values should be obtained in each particular experiment or field study as values for a control group of fish not exposed to the pollutants, kept in a tank with clean water, or sampled from a non-polluted site at the same time as the exposed individuals.

Hematological alterations in fish subjected to xenobiotics may be different and depend on the toxic agent, its concentration and time of exposure, environmental conditions, and intrinsic factors such as fish species, age, and size [1][15][16]. The observed hematological changes may indicate adaptive response of organisms to toxicity, show damage, or both; therefore, they are sometimes difficult to interpret. Hematological responses of fish to toxic exposures may also differ among the species and life stages that differ in their sensitivity to environmental impacts. Most toxic agents induce general and nonspecific stress and oxidative stress response, as well as cytotoxic effects and compensatory reactions.

2. Changes in Red Blood Cell Parameters Induced by Chemicals

Red blood cell parameters include hematocrit (Ht), hemoglobin concentration (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Additional information about the red blood cell system may be obtained by microscope analysis of blood smears: percentage of immature erythrocytes (erythroblasts) as an indicator of erythropoietic activity and percentage of various erythrocyte anomalies including cellular and nuclear deformities as indicator of cytotoxicity and genotoxicity. The observed toxicity-induced changes may be different and show an increase or decrease in the values of all or some red blood cell parameters, indicating the changes in oxygen transport capacity. An increase in Ht, Hb, RBC, or MCV may occur as a compensatory response, facilitating oxygen transport when a toxic agent causes a general stress, impairs gas exchange by affecting gill epithelium, or activates fish metabolism, e.g., increasing detoxification pathways. According to Carvalho and Fernandes [17], copper caused an increase in Ht, Hb, and RBC values in *Prochilodus scrofa*, and the authors concluded that these changes indicated ionoregulatory or respiratory disturbances that caused an increase in energetic metabolism to restore the impaired functions. Dias de Moraes et al. [18] reported an increase in Ht, Hb, and RBC values of *Brycon amazonicus* following acute exposure to pyrethroid insecticide cypermethrin and concluded that it was an adaptive response to hypoxia due to gill morphological changes that probably impaired the oxygen uptake and red blood cells were

probably released from the spleen or hematopoietic tissue. Azithromycin induced a dose-dependent increase in Ht and Hb in *Oreochromis niloticus* [19]. An increase in Ht and MCV was observed by Rożyński et al. [20] in *Perca fluviatilis* after anesthesia with etomidate. Guimaraes et al. [21] reported a concentration-related increase in Ht, Hb, and RBC in *Oreochromis niloticus* fed diets supplemented with various levels of vitamin A.

A decrease is observed if a toxic agent causes damage to circulating erythrocytes; direct hemolysis in circulation or shortened erythrocyte life span, or/and impairs erythropoiesis. Such changes are described as an anemic response. However, usually it is difficult to identify an exact cause of anemia. Ligina et al. [22] reported a decrease in Ht, RBC, and Hb, and an MCV increase in *Anabas testudineus* treated with acrylamide. Yonar et al. [23] found a decrease in RBC, Hb, Ht, MCV, MCH, and MCHC in *Cyprinus carpio* subjected to organophosphate insecticide chlorpyrifos and explained anemia with possible impairment of erythropoietic activity, osmoregulatory disturbances, or accelerated eryptosis in the hematopoietic tissue. According to Jayaprakash and Shettu [24], pyrethroid insecticide deltamethrin caused a significant decrease in Ht, Hb, RBC, MCV, and MCHC of *Channa punctatus*. The authors explained the anemic response with impaired iron absorption and/or inhibition of enzymes involved in hemoglobin synthesis. Haider and Rauf [25] reported a significant decrease in RBC, Hb, HCT, MCV, and MCH compared to the control in *Cirrhinus mrigala* after chronic exposure to organophosphate insecticide diazinon. According to the authors, the anemic response might have been attributed to the failure or suppression of the hematopoietic system of the fish. Javed et al. [26] observed macrocytic hypochromic anemia (considerable decrease in Ht, Hb, RBC, and MCHC accompanied by increase in MCV and MCH), causing a strong impairment of oxygen carrying capacity in *Channa punctatus* subjected to industrial effluents containing metal ion mixture Co, Cr, Cu, Fe, Mn, Ni, and Zn, and interpreted anemia with the inhibition of erythropoiesis. Ko et al. [27] reported a concentration-related decrease in Ht, Hb, and RBC of *Platichthys stellatus* intoxicated with hexavalent chromium and attributed these changes to hemophilia, osmoregulatory disturbances, or a direct adverse effect of Cr on hematopoietic stem cells. According to Bishkoul et al. [28], anesthesia with MS-222 caused a decrease in Hb, Ht, and RBC in *Acipenser ruthenus*. According to Dawood et al. [29], deltamethrin caused a decrease in Hb and RBC in *Oreochromis niloticus*. A decrease in Ht, Hb, and RBC in the same fish species after oxytetracycline exposure was also reported by Omoregie and Oyebani [30].

Microscope analysis of the red blood cell population is another useful tool to evaluate the effects of toxic agents on fish. In Pappenheim stained smears, erythrocytes are well visible as regular elliptical cells with uniform acidophilic cytoplasm and centrally located elliptical basophilic nucleus. Thus, any changes in the cell or nucleus shape and staining properties may be considered anomalies. On the other hand, erythroblasts may be also observed, usually as smaller, rounder, and polychromatophilic (purplish) cells, with a larger and less condensed nucleus compared to the mature erythrocytes. A percentage of erythroblasts can be calculated as a good indicator of erythropoietic activity. Pala and Dey [31] reported an increase in the frequency of abnormal erythrocytes (crenated, ruptured, and contracted cells, echinocytes, spherocytes, lobopodial projections, and membrane internalization) in *Channa gachua* exposed to municipal wastewater. Kaur and Kaur [32] reported various nuclear and cellular abnormalities detected using light and scanning electron microscopy in *Labeo rohita* subjected to acute and subchronic exposure to Basic violet-1 dye. The authors observed erythrocyte anomalies before the appearance of other toxicity symptoms such as behavioral anomalies or mortality. A high frequency of micronuclei and lobed nuclei were

observed in *Channa punctatus* exposed to thermal power plant effluent containing a mixture of heavy metals [26]. According to Farag and Alagawany [33], nucleated erythrocytes of fish can be used to evaluate genotoxicity of xenobiotics using various assays: comet, DNA fragmentation, or micronucleus test. Erythrocytes may be also useful for evaluation of toxicity-induced apoptosis, oxidative stress, and other cellular damage measures [34][35][36].

3. Changes in White Blood Cell Parameters Induced by Chemicals

Leukocyte count (WBC) and leukogram also called as differential leukocyte count (DLC—percentage of various types of leukocytes) are the most commonly used indicators of fish immune potential. Toxic agents often affect leukocyte count and, similar to the case of red blood parameters, an increase or decrease may be observed. An increase in WBC (leukocytosis) is usually interpreted as activation of the immune response due to tissue damage by a toxic agent and often neutrophilia or/and monocytosis is observed, indicating an inflammatory response. On the other hand, leukopenia (decrease in WBC) is attributed to a toxicity-induced general stress response (causing particularly lymphopenia and an increase of neutrophil to lymphocyte ratio) or specific toxic action affecting circulating leukocytes or leukopoiesis resulting in immunosuppression. Leukocytosis (increase in numbers of all types of leukocytes) was reported by Ligina et al. [22] in *Anabas testudineus* during intoxication with acrylamide. According to Zahran et al. [37], exposure to insecticide chlorpyrifos caused leukocytosis in *Oreochromis niloticus*. The numbers of both main leukocyte populations increased: neutrophils and lymphocytes but the increase in neutrophils was more pronounced (neutrophilia). The authors interpreted these changes as compensation action to the potential compromised immune functions. Bujjamma and Padmavathi [38] reported a concentration-dependent increase in WBC in *Heteropneustes fossilis* subjected to cadmium exposure. According to the authors, this might have resulted from immunomodulation caused by cadmium-induced tissue damage. According to Javed et al. [26], who observed increased WBC in *Channa punctatus* subjected to power plant effluent containing mixture of metal ions, leukocytosis was related to the magnitude of damage and stress induced by heavy metals which might have resulted in the stimulation of immunological defense. Leukocytosis caused by azithromycin was reported by Shiogiri et al. [19] in *Oreochromis niloticus*. According to Mahboub et al. [39], *Oreochromis niloticus* exposed to Hg showed leukocytosis, lymphopenia, and neutrophilia accompanied by impaired immune functions. Oluah et al. [40] observed leukocytosis, lymphocytosis, neutropenia, and monocytopenia in *Clarias gariepinus* exposed to the herbicide Ronstar.

Leukopenia was reported in *Sebastes schlegelii* exposed to ammonia [41] and, according to the authors, it was induced by stress. Tavares-Dias et al. [42] observed a decrease in WBC (both lymphocyte and neutrophil count) in *Colossoma macropomum* exposed to copper. *Oreochromis niloticus* exposed to nonylphenol showed leukopenia related to lymphopenia, and monocytopenia which indicates immunosuppression [43]. Lymphopenia and granulocytosis resulted from exposure of *Coregonus lavaretus* to anesthetic propofol [44] which indicates stress. Leukopenia was reported by Dawood et al. [29] in *Oreochromis niloticus* exposed to deltamethrin and after oxytetracycline treatment by Omoregie and Oyebani [30]. Maklakova et al. [45] observed monocytosis and neutropenia in *Oncorhynchus mykiss* subjected to benzylpenicillin or oxytetracycline treatments.

4. Changes in Thrombocyte Count Induced by Chemicals

Thrombocyte count (TC), being an estimator of blood coagulation and also a marker of nonspecific immune functions, is rarely measured. It is usually obtained indirectly by counting thrombocytes in a smear and then estimating TC from their proportion to leukocytes or erythrocytes and WBC or RBC, respectively. Thrombocyte count in fish is very variable [14] and they are sometimes included in leukocyte population as they are involved in both blood coagulation and defense mechanisms [46][47]. Thrombocyte count may show different alterations due to intoxication but due to high individual variability of this parameter, the results are often inconclusive.

According to Witeska and Kościuk [48], *Cyprinus carpio* subjected to acute exposure to Zn showed stress-related thrombocytosis. Lemly [49] observed thrombocytosis in *Lepomis cyanellus* from Belews Lake contaminated with selenium. Corredor-Santamaria et al. [50] reported thrombocytosis in *Astyanax bimaculatus* and *Aequidens metae* from Ocoa River polluted with domestic and industrial wastewater. The authors explain it with a pollution-related increase in the defense response. Fredianelli et al. [51] reported thrombocytopenia in *Rhamdia quelen* sublethally intoxicated with pesticide fipronil and explained it with stress-related cortisol secretion and its action reducing the quantity and quality of thrombocytes. Khan et al. [52] reported different reactions of thrombocytes to glyphosate and atrazine; the first herbicide induced an increase in TC, while the latter, a decline. Thrombocytopenia was also observed by Omoregie and Oyebani [30] in *Oreochromis niloticus* after oxytetracycline treatment.

Hematological analysis is one of the tools commonly used to evaluate the health and welfare of fish, both under aquaculture conditions and in scientific studies, to assess the influence of environmental factors on fish [1][16]. The study conducted by Bojarski et al. [53] demonstrated that hematological indices were the most sensitive and reliable biomarkers of exposure of *Cyprinus carpio* to the herbicide Roundup. Far fewer changes in comparison to hematological ones were observed in the blood biochemical parameters, while microstructure of the analyzed organs (gills, liver, trunk kidney) was unchanged. Thus, the authors concluded that hematological analysis is a basic and necessary tool in evaluation of the effects of Roundup exposure (in the case of *Cyprinus carpio*). Undoubtedly, hematological parameters are sensitive and early indicators of physiological alterations, and they provide valuable and extensive information about the effects of various chemicals in fish. Hematological indices inform us about oxygen transport capacity, immune status, stress response, cytotoxicity, and genotoxicity. Blood sampling is relatively noninvasive and easy even under field conditions. Evaluation of basic hematological parameters is inexpensive and does not require sophisticated laboratory equipment but to obtain reliable results must be performed and interpreted by highly skilled personnel. The observed changes are usually nonspecific and thus do not help to identify a cause of intoxication in case of exposure to unknown pollutants. To evaluate hematotoxicity of a particular compound or polluted environment, it is necessary to compare the results with the values obtained at the same time in the same fish species living under control conditions since unambiguous reference hematological values for the vast majority of fish do not exist. Nevertheless, taking into consideration the advantages of hematological analysis, the researchers recommend this method for evaluation of toxicity in fish.

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