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Hematological Alternation In Common Carp Fish (*Cyprinus Carpio L.* 1758) After Exposing to Dursban

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Abstract

Hematological parameters were used as a biomarker of sub chronic effect of dursban in *Cyprinus carpio L*. The common carp was exposed sub chronically to the 36, 53,113 µg /l concentrations of dursban for six weeks. During the experimental period the results showed a decrease in the red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), and packed cell volume (PCV) in the last two weeks of exposure, while the Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were significantly increased. The present study indicates that sub chronic exposures of *C.carpio* to dursban alter the hematological parameters.

Keywords: *Cyprinus carpio L*, Dursban, hematological parameters, pollution

التغيرات الدمويه في أسماك الكارب الشائع (Cyprinus carpio L. 1758) بعد تعرضه للدورسبان

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الخلاصه

تم استخدام المعلمات الدموية كمؤشرحيوي للتأثيرالشبه المزمنة للدورسبان في *Cyprinus carpio L*. تم تعريض الكارب الشائع بشكل مزمن لتركيز (36,53,113مايكروغرام/لتر) من الدورسبان لسته اسابيع. خلال التجربة النتائج أظهرت النتائج انخفاض في خلايا الدم الحمراء (RBC) وخلايا الدم البيضاء (WBC) والهيموغلوبين (Hb) ومتوسط حجم الخلية (MCV)و حجم الخلية المرصوصة (PCV) في اخراسبوعين من التعرض ، في حين سجلت زياده متوسط خليه الهيموغلوبين (MCH) و تركيز جزيئه الهيموغلوبين (MCHC) بشكل ملحوظ. تشير الدراسة الحالية إلى أن التعرضات الشبه المزمنة لأسماك الكارب الشائعة للدورسبان تؤدي الى تغير في معلمات الدم.

Introduction

Water is the most natural resource that exists on our planet and is essential for survival and the development of modern technology [1]. All water pollution affects organisms that live in the water bodies and in almost all cases the effect is damaging not only to the individual species and populations but also to the natural biological communities [2]. Pesticides in aquatic ecosystems to affect fish diet and subsequent growth are high, because toxicity to zooplankton and macro-invertebrates is often greater than for fish [3]. Also the pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them[4].The measurement of hematological parameters its helpful in diagnosis many infection disease[5].These parameters may

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vary depending on age, gender, race, environmental and genetic background[6]. Hematological parameters such as red blood cells (RBC),white blood cell(WBC),hemoglobin (Hb) concentration, Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH) and mean Cell Hemoglobin Concentration (MCHC). Values outside normal ranges are diagnostic for disorders, including cancer, immune diseases, and cardiovascular disease[7].Hematological parameters, can also be considered biomarkers of wide use and with great potential applications in terrestric and aquatic ecotoxicological studies[8,9]. Fishes exposed to metals, pesticides and effluents exhibit hematological changes, not only after laboratory exposure, but also when the exposure occurs in the field [10]. Hematological changes in fish may be used for assessing the effects of contaminants, because blood parameters respond to low doses of pollutants [11, 12]. Fish blood parameters have been increasingly employed in environmental monitoring programs to indicate physiological changes due to toxicants [11, 13]. The study on the hematology of fish has contributed significantly to the understanding of comparative physiology, phylogenetic relationship, mode of animal life, food selection and other significant ecological parameters [14]. Common carp (*Cyprinus carpio Linnaeus*, 1758.) have been largely employed in ecotoxicology assessment, as they can serve as bioindicators of environmental contamination [15]. Common carp is one of the most important freshwater fish species in aquaculture. A number of unique features have contributed for its attraction as a prime model in our study, such as rapid growth, easy maintenance in the aquarium. Also, carp can tolerate four weeks exposure to pollutants with minimal stress handling [16].The aims of this study identify the effect of the pesticide in hematological parameter of common carp.

Material an methods

Experiment fish

The fish used in this study were a common carp (*Cyprinus carpio L.* 1785), which obtained from local fish hatchery incubators and then measured and weighted by using a sensitive balance. The standard length and average body weight of the experimental fish were 15 ± 1.3 cm and 70 ± 8.2 g, respectively. These fish were subjected to the acclimation process to the laboratory conditions for five days before starting the experiment. The healthy fish were selected, while the weak and sick fish were rejected [17].

Prepare the concentrations of the studied insecticide

The concentrations of dursban (Chlorpyrifos480 gm, US) that used in the experiment were prepared by dissolved 2ml in 1 liter of deionized water. The concentrations that used in the experiment were calculated depending on the following equation:

In (µg/l) unit $\boldsymbol{C1V1 = C2V2}$

Sub chronic toxicity test for dursban

The fish were exposed to three concentrations of Dursban which were 113µg/l, 53µg/l and 36µg/l respectively. Three groups of fishes were used in each aquarium subjected to113, 53 and 36 µg/l from the selected concentrations .Water was refreshed every 48 hr to remove any wastes to protection of depletion oxygen. Sampling was done each two weeks of pesticide exposure . After the stipulated exposure periods, 12 fishes were removed from each pesticide-treated group.

Determination of Hematological biomarkers

Blood was sampled from four fish of each control and exposed groups during each exposure period of 2 ,4 and 6 weeks. It was collected by heart puncture by using sterile disposable insulin syringe which that previously washed with heparin to prevent blood clotting of fish because fish blood clots extremely quickly, and samples almost always require anticoagulant treatment[18] then quickly placed in EDTA tubes to determine hematological test and eppendorf tubes to determine other biomarker. The blood parameters such as hemoglobin , Red blood cell , White blood cell, Packed cell volume , Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were tested by using complete blood count test (CBC).

Statistical analysis

The data in this study were analyzed by analysis of variance (ANOVA), F-test, t-test and least significant difference (LSD) was used to explain the difference between mean at ($p< 0.05$).

Result and discussion

Hematological parameters

Hematological techniques have been utilized to determine sublethal impacts of pollutants during the clinic diagnosis of fish physiology [19]. Blood parameters are considered good physiological indicators of the whole body conditions and therefore can be used in diagnosing the structural and functional status of fish exposed to toxicants [20].

Changes in hematological profiles of fish common carp exposed to selected concentrations of chlorpyrifos are given in Table-1.

The highest mean RBC value (1.52 $x10^3$/mm ± 0.032) mm was recorded in blood sample exposed to 36 µg/l after two weeks, while the lowest mean value (0.09 $x10^3$/mm ±0.01) was recorded in blood sample exposed to 36 µg/l after six week and control blood sample was recorded (0.09 $x10^3$/mm ± 0.04). The analysis of variance results shows significant decrease of dursban pesticide especially after period of six weeks in 36 µg/l. The decrease in erythropoietic activity cause anaemic state to fish that exposed to pesticides. The reduction in erythrocyte count might be caused either by the inhibition of erythropoiesis or by the destruction of red blood cells. The highest mean WBC value (214.74 $x10^3$/mm ± 2.77) mm was recorded in blood sample exposed to 36 µg/l after four weeks, while the lowest mean value (56.12 $x10^3$/mm ±4.05) was recorded in blood sample exposed to same concentration after six week and control blood sample was recorded (146.81 $x10^3$/mm ±3.233). The analysis of variance results shows significant decrease in pesticide after period of six weeks in36µg/l. The changes in leucocytes counts after exposure to pollutants may be associated to a decrease in nonspecific immunity of the fish . The highest mean Hb value (11.30 g/dL ±0.024) was recorded in blood sample exposed to 53µg/l after two weeks, while lowest mean value (5.50 g/dL ±0.27) was found in blood sample subjected to 53µg/l after four weeks. Control blood sample had a mean of(8.00g/dL ±0.231). The result of statistical analysis showed significant decrease in Hb during the last four weeks of exposure. For packed cell volume (PCV), mean values were varied from(30.60%±0.380)in blood sample of a fish treated with 36µg/l after two weeks of exposure to (1.20%±0.11) of that treated with 36µg/l and six weeks of exposure, while control (1.20%±0.035). The result showed significant decrease in PCV after six weeks of exposure in compared to control. Drop in PCV could be attributed to low RBC count or haemodilution[21]. The lowest mean value of MCV (89.80 fl/cell ±1.62) was found in blood sample subjected to 53µg/l after six weeks while, the highest mean MCV value (230.40 fl/cell ±5.784) was recorded in blood sample exposed to 113 µg/l after two weeks, Control blood sample gave a mean of (133.30 fl/cell ±3.848).The values of MCV are significantly decreased in the last six weeks of exposure when compared to control.

Regarding MCH test, the present study was found that the highest mean value (788.90 Pg/cell ± 38.01) of fish sample treated with 36µg/l after six weeks of exposure, while the lowest mean (52.40 Pg/cell ±1.315) was recorded in 113µg/l after two weeks of exposure, while the control sample had a mean of (888.90 Pg/cell ±25.660). We note that the values are significantly increased in the last weeks of exposure compared to control. The highest mean MCHC value (591.70%±25.76) was recorded in blood sample exposed to 36µg/l after six weeks, while the lowest mean value (35.30%± 0.698) was found in blood sample subjected to 36µg/l after two weeks. Control blood sample gave a mean of (666.70%±19.246) .The result shows that the values of MCHC are significantly increased in the last weeks of exposure compared to control. These results express a probable condition of anemia as it is known that many chemical pollutants, including agrochemicals, can induce anemia in fish.

Table 1-Mean ± standard deviation of several blood contents of *C. carpio* subjected to different concentrations of dursban for two, four and six weeks.

	2			**4**				**6**			
	36	53	113	Control	36	53	113	36	53	113	Control
RBC (Cell $x10^3$/mm)	1.52a ± 0.032	0.085c ± 0.023	0.98b ± 0.025	0.09d ± 0.004	0.22c ± 0.02	0.35b ± 0.04	1.19a ± 0.06	0.09b ± 0.01	0.59a ± 0.03	0.13b ± 0.01	0.09b ± 0.02
WBC (Cell $x10^3$/mm)	214.00a ± 5.094	108.12 c± 2.869	98.18c ± 2.465	146.81b ± 3.233	214.74a± 2.77	109.0 0c± 0.40	93.6 6d± 2.46	56.12d ± 4.05	109.61 b ± 6.33	87.70c ± 5.06	146.81a ± 2.23

Hb ((g/dl)	10.80a± 0.024	11.30a ± 0.311	11.10a ± 0.279	8.00 b ± 0.231	10.50a± 0.76	5.50a ± 0.27	11.20a ± 0.54	7.10c ± 0.04	7.70ab ± 0.02	7.40bc ± 0.36	8.00a ± 0.07
PCV (%)	30.60a ± 0.380	22.70b ± 0.624	22.10b ± 0.555	1.20c ± 0.035	4.20b ± 0.30	4.90b ± 1.02	26.20a ± 1.26	1.20c ± 0.11	5.30a ± 0.05	3.90b ± 0.10	1.20c ± 0.03
MCV (fl/cell)	115.30d ± 2.151	160.00 b± 4.399	230.40 ±a 5.784	133.30c ± 3.848	190.90 ±b 13.78	140.00c ± 3.86	220.20 ±a 10.59	133.30 a± 2.41	89.80b ± 1.62	125.80 a ± 2.27	133.30 a ± 3.50
MCH (Pg/cell)	455.50b ± 13.375	177.50 c ± 4.880	52.40d ± 1.315	888.90a ± 25.660	477.30b ± 34.45	157.10c ± 3.03	94.10c ± 4.53	788.90 a ± 38.01	130.50 c ± 15.62	238.70 b ± 22.97	888.90a ± 23.33
MCHC (%)	35.30b ± 0.698	49.80b ± 1.369	40.60b ± 1.019	66.70 a ± 19.246	250.0b ± 18.04	112.20c ± 3.34	42.70 d ± 2.05	591.70 b ± 25.76	145.50 c ± 6.38	189.70 c ± 9.13	660.70a ± 17.50

Different superscript letters (a, b, d and c) in a row show significant differences ($p \leq 0.05$)

Conclusion

The current study gives further evidence that dursban can adversely affect the non-target organisms like fish organism. Fish have proved to be useful experimental models for assessing aquatic ecosystems that are expose to environmental pollution and associated hematological changes. Dursban induces decrease in RBC, WBC, Hb , MCV and PCV,and increases in MCHC and MCH. The hematological parameters are significant to show the hazardous nature of the insecticide.

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References

1. Zeitoun, M. M. and Mehana, E. **2014**. "Impact of water pollution with heavy metals on fish health: overview and updates." *Global Veterinaria*, **12**(2): 219-231 .
2. Agrawal, A., Pandey, R .and Sharma, B. **2010**. "Water Pollution with Special Reference to Pesticide Contamination in India," .*Journal of Water Resource and Protection*, **2**(5): 432-448. doi: 10.4236/jwarp.2010.25050.
3. Johnson, W.L.V. and Finley, M.T. **1980**. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. *US Dept Int Fish Wildl Serv Res Publ*, **137:** 1-98.
4. Miller, G.T. **2004**. *Sustaining the Earth*. 6th Ed. Thompson Learning, Inc. Pacific Grove, California. Chapter **9**: 211-216.
5. Kone, B., Maiga, M., Baya, B., Sarro, Y.D.S., Coulibaly, N., Kone, A., Diarra, B., Sanogo, M., Togo, A.C.G., Goita, D.,Dembele, M,; Polis, M.A.,Warfield ,J., Belson, M., Dao ,S., Orsega, S., Murphy, R.L., Diallo, S. and Siddiqui, S. **2017**. Establishing Reference Ranges of Hematological Parameters from Malian Healthy Adults. *J Blood Lymph*. **7**:1. DOI: 10.4172/2165-7831.1000154.
6. Lugada, E.S., Mermin, J., Kaharuza, F., Ulvestad, E. and Were, W. **2004**. Population-based hematologic and immunologic reference values for a healthy Ugandan population. *Clin Diagn Lab Immunol*.**11**: 29-34.
7. Kelada ,S. N. P., Aylor, D. L., Peck ,B. C. E., Ryan ,J. F., Tavarez,U., Buus ,R. J., Miller ,D.R., Chesler, E.J., Threadgill ,D.W., Churchill ,G. A.,de Villena F.P., and Collins, F.S.**2012**.Genetic Analysis of Hematological Parameters in Incipient Lines of the Collaborative Cross. *Mouse Genetic Resources*, (2).
8. Fairbrother, A., Landis, W. G., Dominguez, S., Shiroyama, T., Buchhloz,P., Roze, M. J., and Matthews, G. B. **1998**. A novel nonmetric multivariate approach to the evaluation of biomarkers in terrestrial "elds studies.*Ecotoxicology*, **7**: 1-10.

9. Sancho, E., Ceron,J. J. and Ferrando , M. D.**2000**. Cholinesterase Activity and Hematological Parameters as Biomarkers of Sublethal Molinate Exposure in Anguilla Anguilla. *Ecotoxicology and Environmental Safety*.**46**: 81 -86.

10. Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C.T. and Ayyappan, S. **2004**. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost; *Labeo rohita* (Hamilton). *Ecotoxicol. Environ. Safety*, **58**: 220–226.

11. Seriani, R., Dias, D.C., Silva, M.R.R., Villares, E.O., Abessa, D.M.S., Moreira, L.B., Ranzani-Paiva, M.J.T. and Rivero, D.H.R.F.**2009**. Hematological parameters of *Oreochromis niloticus* from a polluted site. *Annals of World Aquaculture Society*. Vera Cruz, México.

12. Seriani, R., Moreira, L. B., Abessa, D. M. S., Maranho, L. A., Abujamara, L. D., Carvalho, N. S. B., Kirschbaum, A. A. and Ranzani-Paiva, M. J. T.**2010**.Haematological analysis in *Micropogonias furnieri* from two estuaries at Baixada Santista, São Paulo, Brazil. *Braz. J. Ocean.*, **58**: 87-92.

13. França, M.B., Panek, A.D. and Eleutherio, E.C. **2007**. Oxidative stress and its effects during dehydration. *Comp Biochem Physiol A Mol Integr Physiol*. **146** (4): 621-31.

14. Deshmukh, D. R. and Sonawane ,S. R. **2007**. Effect of Long-Term Exposure to Endosulfan on Blood Cell Count in *Channa Gachua* . *J Indian Fish. Assoc*. **34**: 83- 85.

15. Alinnor, I.J. **2005**. Assessment of elemental contaminants in water and fish samples from Aba River. *Environ. Monit .Asses.***102**: 15-25.

16. Oikari, A. **2006**. Caging techniques for field exposures of fish to chemical contaminants. *Aquatic Toxicology*, **78**: 370–381.

17. Environmental Protection Agency (EPA).**1996**. Ecological effects test guidelines OPPTS 850. 1075 fish acute toxicity test, freshwater and marine. Office of prevention, pesticides and toxic substances (7101). U.S. EPA. 712-C-96-118.

18. Witeska, M. and Wargocka, W. **2011**. Disodium EDTA used as anticoagulant causes hemolysis in common carp blood. *Turkish Journal of Veterinary and Animal Sciences*, **35**: 99-104. doi:10.3906/vet-0908-51.

19. Bhagwant S, Bhikajee M. **2000**.Induction of hypochromic macrocytic anaemia in Oreochromic hybrid (cichlidae) exposed to 100µg/l (sublethal dose) of aluminium. *Sci. Techn*. **5**: 9-20.

20. Seriani, R., Abessa, D.M.S., Kirschbaum, A.A., Pereira, C.D.S., Romano, P., Ranzani-Paiva, M.J. T**. 2011**. Relationship Between Water Toxicity And Hematological Changes In *Oreochromis Niloticus* Braz. *J. Aquat.Sci.Technol*. **15**(2): 47-53.

21. David, M., Sangeetha, J., Shrinivas, J., Harish, E.R., Naik, V.R. **2015** .Effects Of Deltamethrin On Haematological Indices Of Indian Major Carp, *Cirrhinus Mrigala*(Hamilton). *Int. J. Pure Appl. Zool*, **3**(1): 37-43.