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Effect of Short Term Exposure of Ethoxyquin on Hematological Parameters in Fresh Water Fish, *Oreochromis mossambicus.*

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Ethoxyquin; Lethal Concentration; *Oreochromis mossambicus*; Haematological parameters; RBC and WBC count

ABSTRACT:
Ethoxyquin (EQ, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) is a synthetic antioxidant that is included in fish feeds in order to protect it against lipid peroxidation. Many unfavourable side-effects have been observed in animals fed with EQ-containing feeds. Studies on the harmful effects of EQ on vertebrates are growing, but the effects of EQ in aquatic systems have rarely been described. In the present study LC_{50} 96 hr value of EQ to the freshwater fish *Oreochromis mossambicus* (Thilapia) was determined through Probit analysis.The bioassay found that the median lethal dosage in fish after 96 hours (LC50-96h) was 11.37 mg/L. The fish were reared in sublethal concentration of the chemical for 24hr, 48hr, 72hr and 96hr. Blood was taken from the treated and control fish and haematological parameters were analyzed. It was found in the present study that RBC count and hemoglobin values in fish decreased significantly as a result of EQ application. Such a situation can be an indicator for haemolytic anemia. An increase in white blood cells was noticed, which probably represent an adaptive response to help the organism to counteract the toxic effects of EQ. A significant increase in neutrophil count was noticed suggesting that the fish is trying to overcome the toxic effect of chemicals by leukocytosis mechanism.

INTRODUCTION

Ethoxyquin (EQ, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) is used in different food products and in animal feeds, because of its strong antioxidant activity. In recent times, many unfavourable side-effects have been observed in animals fed with EQ-containing feeds [1]. Adverse effects were also observed in people who are exposed to this compound at work [2]. The use of EQ in any food for human consumption (except spices, e.g., chili) is not allowed, but it can pass from feed to farmed fish, poultry, and eggs, so human beings can be exposed to this antioxidant. All the same, some harmful effects in animals and people occupationally exposed to EQ were noticed.

Ethoxyquin as fish meal in aquaculture is much more likely launched directly to the wider aquatic surroundings around an aquaculture facility as waste feed or may be taken up by using fish after which excreted once more into the environment. As an aquatic vertebrate fish is in direct touch with the aquatic environment and whatever added to its surroundings might also exert some effect on its hematological traits [3]. Haematological parameters appear to be the utmost dependable indicator of alteration inside the body structure of fishes [4]. That is because blood participates without delay or circuitously in almost all biochemical tactics within the body of fish, for instance homeostasis as well as disease approaches. Various toxicants make alteration in haematological and blood chemical indices. Thus haematological parameters are dependable indicator of systemic response of fish to external stimuli [5].

Haematological indices such as RBC and WBC counts and haemoglobin concentrations undergo changes in stressful conditions such as disease, adverse environmental conditions and contaminants [6]. Alteration in blood parameters can be used as an

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indicator of variation in biochemical processes due to xenobiotic treatments. It is believed that changes of blood constituents could adversely affect the performance of the fish. Haematological parameters are important biomarkers used to identify the adverse effects of toxicants in an organism and are broadly useful to weigh up environmental risk of any pollutants [7]. Hence, the present study was designed to evaluate the effect of ethoxyquin exposure on haematological indices in fresh water fish, *Oreochromis mossambicus*.

MATERIALS AND METHODS

Experimental fish

Oreochromis mossambicus, a kind of freshwater fish, were collected from fish farm at Kottakal in the Malappuram District, Kerala. The fishes were kept in the lab for a week prior to the experiment, where they were provided with a constant supply of water and a well-lit environment. Fish were fed the recommended quantity of commercial fish pellets three times a day and housed in thirty-litre glass aquariums with plenty of oxygen throughout the acclimation period. Preliminary screening and standardisation of physicochemical properties of tap water were carried out in accordance with the criteria established by the American Public Health Association [8].

Chemicals

The chemicals used were of analytical purity. They were used without being refined further. Ethoxyquin was obtained from Sigma Aldrich in Germany.

Determination of median lethal concentration (LC_{50}-96h)

In order to estimate the median lethal concentration (semi-static; 96 h-LC_{50}) over a period of 96 hours, ethoxyquin concentration 5-25mg/L is applied to five different groups with 15 fishes each group. The mortality is observed for 96 hrs. Triplicates were kept in perfect condition. Probit Analysis was performed for determining LC_{50} values. Median lethal concentration (96 h-LC_{50}) is the concentration at which 50% of the fish population dies; this value was verified using the Probit tool of regression analysis with a 5% confidence limit [9].

Selection of sublethal concentrations

Sublethal doses, comprising one-tenth of the 96 h-LC_{50}, were selected for further toxicological evaluation based on the median lethal concentration for 96 h duration.

Experimental design

For toxicity induction studies the experimental design was done as follows; Fishes were divided into five groups, with nine specimens in each group. Group I was negative control group (without toxicant). In group II to V sub lethal concentration of 96h -LC_{50} (ie, $1/10^{th}$ of medial lethal concentration) was added and exposed to various time intervals like 24hr, 48hr,72hr,96hr. Blood was collected from each group after the time of exposure and used for various hematological tests. Collection of blood was done by caudal puncture of the fish. The whole blood was used for the estimation of erythrocyte, leucocyte counts and haemoglobin in both control and experimental groups [10,11]. Differential Count was performed by preparing a smear and Leishman staining.

Statistical analysis

The experiments were repeated on three different occasions in triplicate and the data were analysed by Student's *t*-test. Statistical comparisons were done between control and exposure data from the same species. Standard deviation, probability level below 0.05 was taken as statistically significant. SPSS 22 Version.

RESULT AND DISCUSSION

The LC_{50} was found to be 11.37mg/l. Lethal Concentration 50 (LC_{50}) is a standard toxicity dose measurement. This is the concentration of a chemical that kills 50% of a test population within a set period of time, usually 24 to 96 hours. Not all chemical poisoning result in the immediate death of an animal.

Haematological Parameters

Hemoglobin –Drabkin's Method

Haemoglobin values are one of the haematological parameters mostly used for evaluating fish health. Haemoglobin profile is indicative of oxygen carrying capacity of the blood and anaemia is indicated by low haemoglobin [12]. In the present study, the level of Hb was found to be decreased, which is summarized as follows;

Table 1: Hb level in fish on EQ toxicity induction

TIME OF EXPOSURE	Hb level (g%)
CONTROL	8.53 ± 0.768
24 hour	8.29± 0.702
48 hour	7.75 ± 0.685
72 hour	7.45 ± 0.657
96 hour	7.17 ± 0.614

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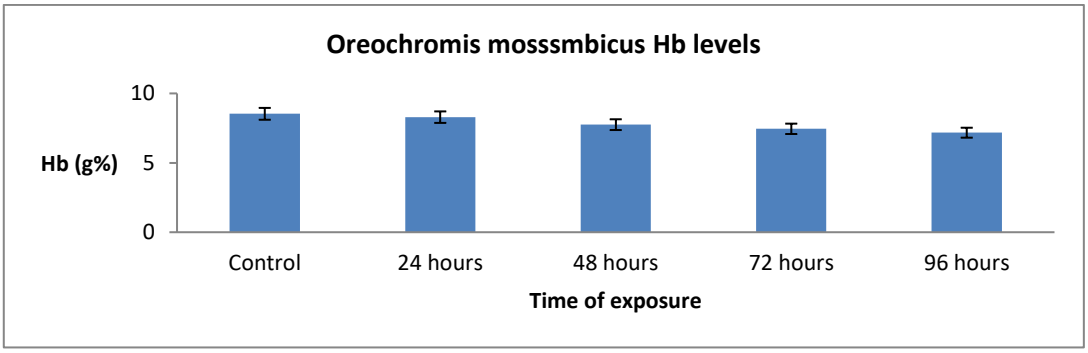


Figure 1: Bar graph showing Hb level in fish on EQ toxicity induction

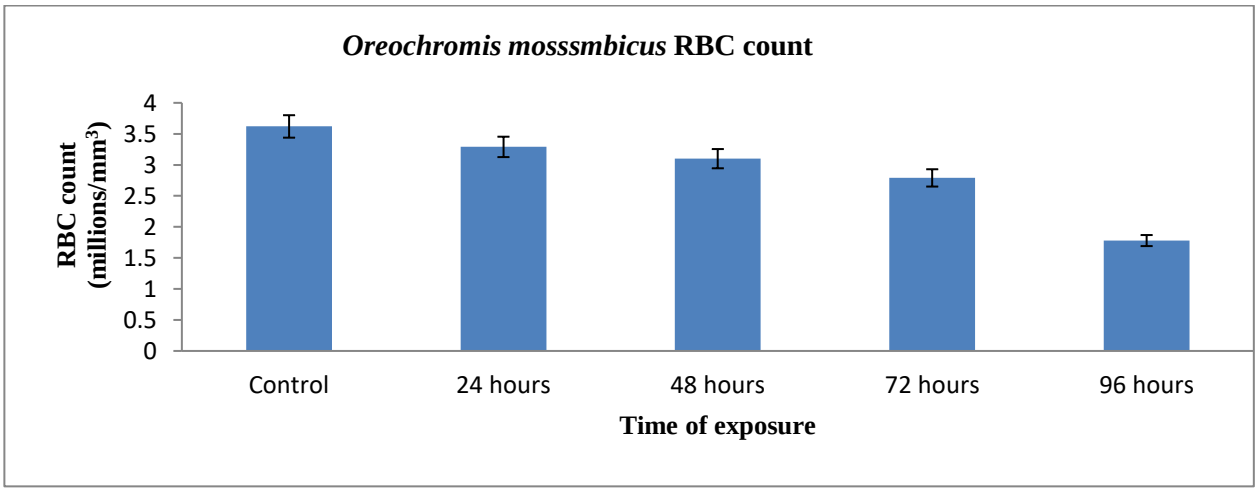
Haemoglobin levels were found to be decreased in many fishes on induction of heavy metal toxicity. The decrease may be due to the reduction in RBC count, which represents hemolysis, hemorrhage and decreased erythropoiesis [13].

Determination of RBC & WBC Count

The results obtained for RBC and WBC count on EQ exposure at different time intervals were as follows;

Table 2: RBC & WBC count in fish on EQ toxicity induction

TIME OF EXPOSURE	RBC COUNT(millions/mm^3)	WBC COUNT(Cells/mm^3)
CONTROL	3.62 ± 0.275	10.78 ± 0.848
24 hour	3.29 ± 0.262	30.98 ± 1.765
48 hour	3.1 ± 0.22	34.21 ± 1.941
72 hour	2.79 ± 0.981	38.66 ± 2.426
96 hour	1.78 ± 0.84	44.86 ± 3.75



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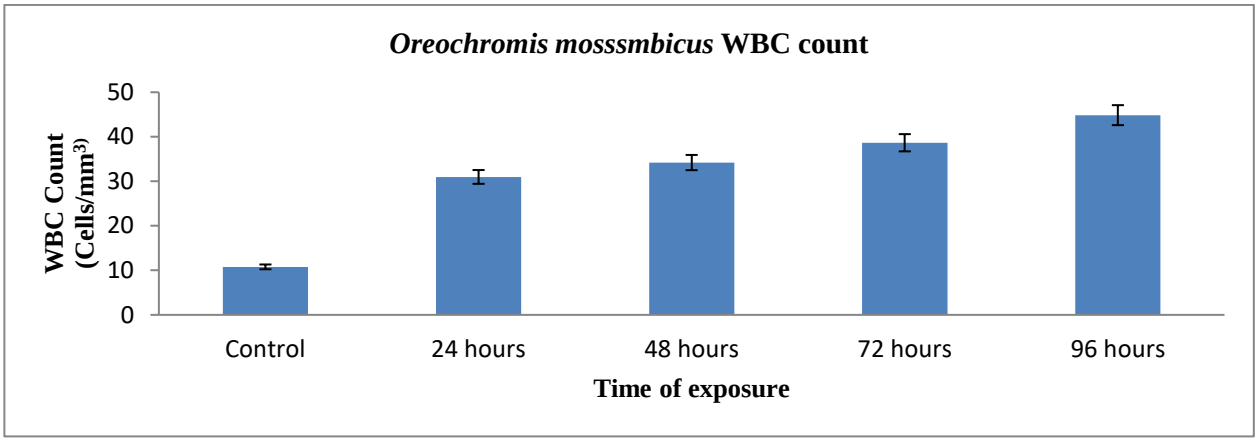

Figure 2&3: Bar graphs showing RBC & WBC in fish on EQ toxicity induction

In the present study the decrease in RBC count during the treatment of ethoxyquin might have resulted from severe anaemic state or haemolysing capacity of ethoxyquin. It was found that the WBC Count was found to be increased. This may be explained by a reaction of the defense mechanism of the fish by leucocytosis under pathological conditions and against foreign bodies [14]. White blood cell (leucocytes) count in fish blood varies according to season, age and sex [15]. Leucopoenia or leucocytosis is pathological conditions associated with abnormal leucocyte count indicating the possibility of alteration in immune function of the individual [16]. A response of the cellular immune system to infection or treatments manifests as an increase in WBC count [17].

Differential Count

The results for differential count were as follows;

Table 3: Differential Count in fish on EQ toxicity induction

	TIME OF EXPOSURE				
Differential Count	Control	24hr	48 hr	72 hr	96 hr
Neutrophil(%)	19.67 ± 0.142	21.25± 0.185	22.43± 0.198	23.67± 0.202	27.22 ± 0.216
Eosinophil(%)	4	6	8	10	13
Basophil(%)	7	9	12	14	16
Monocyte(%)	6.3 ± 0.042	7.4 ± 0.056	7.4 ± 0.059	8.3 ± 0.064	9.4 ± 0.057
Lymphocyte (%)	53.21 ± 0.365	53.34 ± 0.387	55.56 ± 0.357	56.67 ± 0.398	57.91 ± 0.407

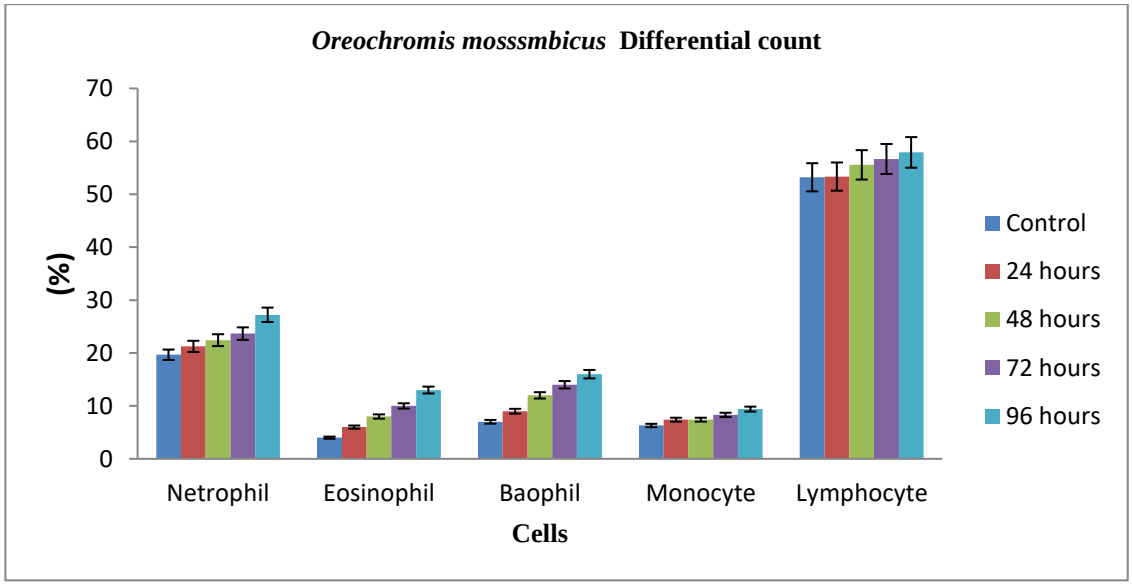

Figure 3: Bar graph showing differential count in fish on EQ toxicity induction

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It was found that there was a transient increase in neutrophil count on EQ exposure suggesting that extravasation of neutrophils is taking place as a result of toxic exposure. There was no significant change in other cells [18].

CONCLUSION

The present study focuses on changes in haematological parameters on EQ exposure. Haematological parameters in fish can significantly change in response towards chemical stressors; however, these alterations are non-specific to a wide range of substances. Some of these changes may be the result of the activation of protective mechanisms. The results obtained in this work allow us to conclude that exposure to EQ promotes alterations in hematologic parameters in *Oreochromis mossambicus*. It was found in the present study that RBC count and Hb values in fish decreased significantly as a result of EQ application. Such a situation can be an indicator for haemolytic anaemia. An increase in white blood cells was noticed, which probably represent an adaptive response to help the organism to counteract the toxic effects of EQ. It is also important to point out that the effects of environmental toxicants on hematological characteristics of fish vary according to the target species. A significant increase in neutrophils suggests that the fish is trying to overcome the toxic effect of chemicals by leukocytosis mechanism.

REFERENCES

1. Little, A.D., 1990. Chemical Committee Draft Report, Ethoxyquin, CAS Number, Submitted to National Toxicology Program, Executive Summary of Safety and Toxicity Information. 91-53-2.
2. Drewhurst.,I., 1998. Ethoxyquin. JMPR Evaluations http://www.inchem.org/documents/jmpr/jmpmono/v098pr09.htm
3. Gabriel, U.U., Amakiri, EU and Ezeri, G.N.O., 2007. Haematology and gill pathology of *Clariasgariepinus* exposed to refined petroleum oil under laboratory conditions. J. Ani. Vet. Adv. 6(3): 461–465.
4. Akinrotimi, O., Agokei, E., & Aranyo, A. (2012). Changes in blood parameters of *Tilapia guineensis* Exposed to Different Salinity Levels. *Journal of Env Engineer Technol, 1*(2), 4-12.
5. Tavares-Dias M., & Moraes F. R.2007. Haematological and biochemical reference intervals for farmed channel catfish. *J Fish Biol, 71*, 383-388. https://doi.org/10.1111/j.1095-8649.2007.01494.x
6. Mali, R. P., & Chavan, P. N. 2014. Temperature Dependent Haematological Alterations of Freshwater Cultivable Fish, *Oreochromis Mossambicus* from Nanded District (Ms) India. *Int J Innovative Res Sci,Engineer Technol, 3*(11), 17178-17180. https://doi.org/10.15680/IJIRSET.2014.0311013.
7. Van der Oost R., Beyer, J and Vermeulen ,N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13(2): 57–149.
8. APHA.,1998,Standard methods for the examination of water and waste water, 20th Edition, Washington, DC.
9. Finney D.J.1971. Probit analysis, 3rd (Ed.), Cambridge University Press, London, 333 pp.
10. Russia. U. and Sood. SK. 1992. Routine haematological tests. In: Medical laboratory technology. (KL Mukerjee, Ed). Tata McGraw Hill Publishing Co. Ltd, 252-258.
11. Drabkin, D.L. 1946. Spectrophotometric studies XIV- The crystallographic and optical properties of the haemoglobin of man in comparison with those of other species. *J.Biol.Chem*.164:703-723.
12. Zhang, X., Xie, P., Li, D., & Shi, Z., 2007 .,Hematological and plasma biochemical responses of crucian carp (*Carassius auratus*) to intraperitoneal injection of extracted microcystins with the possible mechanisms of anemia. *Toxicon, 49*(8), 1150-1157. https://doi.org/10.1016/j.toxicon.2007.02.009
13. Sharma, R,., Bakera , P.P. and Patil., BN.1995. Haematological and biochemical changes in Ring dove, Streptopella dicaocto after repeated exposure to thiodan 35EC. J.Ecobiol.7(2):131-138.
14. John, P,J. 2007. Alteration of certain blood parameters of freshwater teleost *Mystusvittatus* after chronic exposure to Metasystox and Sevin. Fish Physiol. Biochem. 33: 15-20.
15. Charoo, S. Q., Chalkoo, S. R., & Qureshi, T. A. 2013. Sexual differentiation in blood biochemistry of rainbow trout (*Oncorhynchus mykiss*). *Int J Adv Fish Aquatic Sci, 1*(1), 32-38.
16. Elahee, K.B., Bhagwant, S., 2007. Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the west coast of Mauritius. Ecotoxicol. Environ. Saf. 68, 361–371
17. Rapatsa M. M., & Moyo N. A. 2013. Haematological, histological and growth characteristics of *Oreochromis mossambicus* exposed to effective microorganisms in organically manured aquadams. *Asian J Ani Vet Adv, 8*(7), 852-862.
18. Cazenave, J., Wunderlin, D.A., Hued, A.C., Bistoni, M.A., 2005. Haematological parameters in a neotropical fish, Corydoras paleatus (Jenyns,

Journal of Chemical Health Risks

www.jchr.org

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1842) (Pisces,Callichthyidae), captured from pristine and polluted water. Hydrobiologia 537, 25–33