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RESEARCH ARTICLE

EFFECT OF NONYLPHENOL LC-50 ON HEMATOLOGICAL PROFILE OF *OPHIOCEPHALUS PUNCTATUS* (BLOCH, 1793)

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ABSTRACT

Xenoestrogen nonylphenol at lethal concentration 50 causes dramatic change in the haematological profile of *Ophiocephalus punctatus*. On short term exposure to NPanaemic conditions were found to induce in fish, which result in decrease in erythrocytes, Hb, PCV. However MCV and MCH increased. These changes can be attributed to inhibition of erythrogenesis or destruction of erythrocytes. Total leucocyte and lymphocytes count decreased indicate the potential decreased in immunity of exposed fish. It is concluded that the NP is hemotoxic in *O. punctatus*.

INTRODUCTION

Aquatic toxicology is emerging as a challenging area in the field of biological research due to its various facets in the field of environment, agriculture, veterinary pharmaceutical and medical sciences. The phenolic wastes are considered to be the common water pollutants generated from a variety of industrial processes used in oil refineries, gas operation, coke ovens, coal gasification and also by natural processes such as the decomposition of plant matter (Buikema *et al*., 1979). However, relatively high concentrations of phenol are found in rivers near the outlets of channels where industrial waste-water is discharged (Buikema *et al*., 1979; Loh *et al*., 2000). The term Nonylphenol (NP) used to refer to a group of isomeric phenolic compounds each consisting of a nine carbon alkyl chain attached to a phenol ring, with the chemical formula $C_{15}H_{24}O$. There are various isomers that are differing in the degree of alkyl chain branching and the position of the phenol ring. Most NP produced commercially is in the form of 4-Nonylphenol or p-Nonylphenol (i.e. alkyl chain attached at para position) with varied alkyl chain branching (CCME, 2002).Fishes belonging to different taxonomic groups are adopted variously depending on different prevailing ecological conditions (Benarjee *et al*., 2010). The fluctuations in the blood constituents in fishes are subjected to change in temperature, ecological conditions, food habits, chemical and environmental stress. The fish hematological parameters such as RBC, WBC, Hb and PCV values etc., are thus shown to be influenced by many factors include environmental factors (Pandey, 1977), seasonal conditions (Joshi and Tondon, 1977; Khan, 1977) different period of reproductive cycle and chemical stress (Khan, and Siddiqui, 1970). In the present study attempt has been made to found out the changes in hematological profil on exposure to noxious chemical nonylphenol in *O. punctatus*.

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MATERIALS AND METHODS

All the experimental fingerlings of *O. punctatus* selected for this present study were purchased from fisherman of Mulchera, District-Gachchiroli (M.S.) India. Fish were brought to the laboratory and bathed in 0.01% potassium permanganate solution for fifteen minutes for two subsequent days to kill the external infectious pathogenic microorganisms if any to avoid the possible mortality of fish due to microbial infections. After the treatment with disinfectant fingerlings were placed in a large glass aquarium for acclimatized for fifteen days. During these period of acclimatization fish were fed alternate days with boiled egg albumen and dried minced prawn powder. Fingerlings were separated according their size and weight. Fish selected for the experiment had an average length 25±4 cm and weight 80±5 gm. Health of aquarium was monitored time to time for physiochemical parameters like pH, temperature, dissolved oxygen, conductivity; free carbon dioxide and total alkalinity. Lethal concentration 50 for nonylphenol was evaluated (Finney, 1971) and found to be 15.51 ppm for in case of *O. punctatus* (Khandale *et al*., 2015). After the determination of LC-50, this concentration was employed on the fish *O. punctatus.* Six small glass aquarium having 30 liter of water holding capacity were set in the laboratory. One out of six was used as a control and remaining five for experimental. All the five tanks having 20 liters of dechlorinated water were diluted with working solution of NP until reaching lethal concentration 50, water mix properly after the addition of working solution of NP. Later fish were placed into the aquarium. Precautions were taken, if any fish found dead it was quickly removed from the aquarium. Fish during experiment were fed with albumen of boiled eggs and dried minced prawn powder in alternate days. This short term exposure of *O. punctatus* to NP LC-50 ran for four days. The blood samples were collected every day at the interval of twenty-four hours and similarly water samples were used for physico-chemical monitoring. Water of both the experimental and a controlled tank were changed every day to remove the dirt. All the statistical calculations were carried out by one-way ANOVA method by using trial statistical software Prism Graph pad and Microsoft Excel- 2008.

RESULTS

Influence of NP LC-50 on some hematological parameters of fish, *O. punctatus* was studied in detail. RBCs, Hb, PCV, MCV, MCH, MCHC, WBCs, Neutrophil, Lymphocytes, Monocytes, Eosinophils and Basophils were the main objects of the present work. However, all these objects showed changes on exposure to NP LC-50 have been explained as follows:

Hemoglobin Percentage (Hb %)

In the laboratory acclimatized fish *O. punctatus*hemoglobin percentage was recorded to be 12.590 ± 1.453 gms%. In NP LC-50 exposed fish, hemoglobin percentage got insignificantly reduced to 12.110 ± 0.557 gms% at 24-hours of exposure. This reduction was only 3.81% as compared to control one. However, from 48 hours to 96-h of exposure successive decreased in the hemoglobin level was significantly observed. The maximum reduction (10.68 ± 0.178 gms %) in hemoglobin percentage was apparently recorded on last day of exposure. This reduction was 15.17% compared to normal fish (Table 1).

Packed Cell Volume (PCV)

In normal fish PCV was recorded to be 35.43 ± 0.291%. As compared to normal fish, percentage of PCV went on decreased right from very first day of exposure and it was recorded to be 33.707 ± 0.402% at 24 hrs (Table-1). However significantly lowest PCV percentage was recorded at fourth days i.e. on 96 hrs of exposure and it was 31.233 ± 0.201%. The percent decrease in PCV percentage on first day was 4.86 and on the last day it was 11.85.

Mean Cell Volume (MCV)

Mean cell volume in normal *O. punctatus* was calculated to be 35.43 ± 0.291 fl. On first day insignificant increase in MCV was observed (Table 1). Compared to normal fish MCV increased by 7%. However, MCV consistently increased on each day of exposure up to 96 hrs. The maximum increased was observed on fourth days of exposure and as far as percent increase was concerned it got shoot up by 129.81%.

Table 1. Effect of NP LC-50on hematological parameters

Sr. No.	Hematological parameters	Control	24-hrs	48-hrs	72-hrs	96-hrs
1	RBCs mill/mm3	2.97±0.14	2.64±0.29	2.12±0.16*	1.87±0.09*	1.14±0.13*
2	Hb %	12.51±1.73	12.110±0.35	11.88±0.20	11.48±0.58	10.68±0.18
3	PCV %	35.43±3.38	33.71±0.40	33.07±0.482	32.71±0.14	31.23±0.20
4	MCV %	12.15±13.61	13.5±13.76	155097±14.65**	174.6±8.04*	273.82±32.46*
5	MCH pg	42.06±5.25	45.81±4.16	55.79±3.50**	61.25±5.29**	93.63±9.27*
6	MCHC%	35.3±2.01	35.93±0.78	35.77±1.13	35.08±1.66*	34.19±0.68
7	WBC tho./mm.3	16.6±0.34	16.48±0.09	15.68±0.23*	15.12±0.14*	14.86±0.11*
8	Neutrophil %	20.67±0.36	22.98±0.46*	24.39±0.19*	26.46±0.45*	28.36±1.22*
9	Lymphocyte %	60.43±0.49	52.33±0.81*	47.67±0.67*	43.74±1.52*	38.86±0.38*
10	Monocyte %	11.08±0.14	8.897±0.23*	8.14±0.19*	7.61±0.37*	7.19±0.76*
11	Eosinophils%	5.23±0.14	14.86±0.57*	19.23±0.68*	21.93±1.08*	25.25±0.87*
12	Basophils %	2.563±0.357	1.63±0.84*	0.93±0.01*	0.57±0.37*	0.46±0.21*

Note: *indicates p<0.01, ** indicates p<0.05 and without signed are statistically non-significant

Total Red Blood Corpuscles Counts (RBCs)

In controlled fish *O. punctatus,* the total RBCs or total Erythrocyte counts (TEC) were estimated to be 2.974 ± 0.142 million/mm^3 (10^6/mm^3). On 24 hrs of exposure to NP LC-50, total RBCs count decreased (2.643 ± 0.296 million/mm^3) insignificantly ($p > 0.05$) as compared to normal fish by 11.13%. From day first (24-hour) onward significant ($p < 0.001$) decrease in total RBCs was observed up to days fourth of exposure (96-hours). Significantly lowest total RBCs count was recorded to be 1.141 ± 0.132 million/mm^3 on the fourth days of exposure and as far as percent decrease as compared to normal count was concerned, it is reduced by 61.63% on fourth days of exposure (Table 1).

On first day of exposure to NP LC-50, increase in MCV was insignificant ($P>0.05$) but subsequently on third day onwards it was significantly increased (Table-1).

Mean Cell Hemoglobin (MCH)

In control fish, MCH measured was 42.058 ± 5.246 pg. In an experimental fish, on the 1st day of exposure to NP LC-50 it got insignificantly increased to 45.808 ± 4.162 pg. Continuous significant increase in MCH was observed from 2nd to 4th days of exposure (Table-1). Maximum increased however was on the last day of exposure and it was 93.629 ± 9.270 pg. MCH decreased as compared to normal fish on 1st day of exposure by approximately 8.92% and on forth day by 122.62%.

Mean Cell Hemoglobin Concentration (MCHC)

MCHC in normal fish *O. punctatus* was calculated to be 35.535 ± 2.509 gm%. On first and second days of short term exposure to NP LC-50, insignificant increased in MCHC as compared to controlled one was observed (Table-1). However, on the 3rd and 4th days of exposure, comparison to normal fish, consistently insignificant decrease in MCHC percentage was observed (Table-1). But maximum increase in MCHC was recorded on the 1st day of exposure 35.932 ± 0.545 gm%. And as far as percentage increase was concerned, it was 1.12 gms%. Lowest MCHC was observed on fourth day of exposure i.e. 34.196 ± 0.678 gm% and percentagewise decreased was calculated to be 3.77 gms% as comparison to normal fish.

Total Leucocyte count (TLC)

Total White Blood Cells (WBCs) count is the TLC. In normal fish *O. punctatus,* TLC accounted was 16.61 ± 0.236 thousands/mm^3 or 10^3/mm^3. On the first day of exposure to NP LC-50, TLC insignificantly increased to 16.477 ± 0.0.096 thousands/mm^3 and approximately by 0.8 thousands/mm^3 percent increase was observed as compared to in normal fish. From days 2nd consistent significantly increase in TLC was observed till the 4th days of exposure (Table-1). However, the lowest TLC was on 4th days of exposure 14.863 ± 0.112 thousands/mm^3. As far as percent decreased as compared to control fish was concerned TLC got decreased by 10.52 thousands/mm^3 percent.

Neutrophil (%)

The percentage of Neutrophil in the normal or control fish was estimated to be 20.67 ± 0.359. In an experimental fish, the neutrophil percentage increased from the very first day to last day of short term exposure to NP LC-50 (Table-1). On day first, significant ($P<0.01$) increase was noted to be 22.987 ± 0.464%. The rate of increase as compared to control was 11.21%. However, obvious significant ($P<0.001$) increased through all days of exposure till the days fourth was observed (Table-1). Maximum increase, of course was on fourth days i.e. 28.36 ± 1.224% and rate of percentage increase as compared to control was 37.20.

Lymphocytes (%)

Percentage of Lymphocytes in the blood of control fish was observed to be 60.43 ± 0.489. On short term exposure to NP LC-50, the lymphocytes percentage went on decrease consistently from 24 hrs to 96 hrs of exposure (Table-1). Maximum significant decreased was noted on fourth day of exposure and it was 38.863 ± 0.379%. However minimum significant decrease was seen on first day 52.33 ± 0.813%. Percent decrease in lymphocytes on first day of exposure compared to control fish was calculated to be 13.40 and on days fourth it was approximately 35.69.

Monocytes (%)

On short term exposure to NP LC-50 percentages of monocytes gradually decreased. In the normal fish *O. punctatus*, monocytes counted were 11.08%. The monocytes significantly decreased on first day of exposure to 8.897%. The minimum percent of monocytes during entire period of exposure was recorded on fourth days (Table-1 and Fig.1). However, the percentage decrease as compared to control fish at 24 hrs of exposure was 19.70 and on fourth days it was 35.08.

Eosinophil (%)

The normal fish *O. punctatus* were shown to have 5.233% of eosinophil. The percentage of eosinophil on exposure to NP LC-50 for short period was found to increase significantly (Table-1) for all days of exposure. Nonetheless, on the first day of exposure eosinophil increased to 14.853% and percentile decrease was calculated to be 183.83%. Maximum increased in the eosinophil were noted on 4th day of exposure and it was 25.253%, the percent decrease as compared to normal fish was 282.57.

Basophils (%)

Insignificant decrease in basophil percentage was noted on the first day of exposure to NP LC-50 and it was 1.633%. In the normal fish, basophils percentage was calculated to be 2.563 (Table-1). The percent decrease on first day as compare to normal fish was 36.29 and on fourth days it was quite fell down to 0.463. However, decrease in the basophil level as compared to control fish was 99.82%.

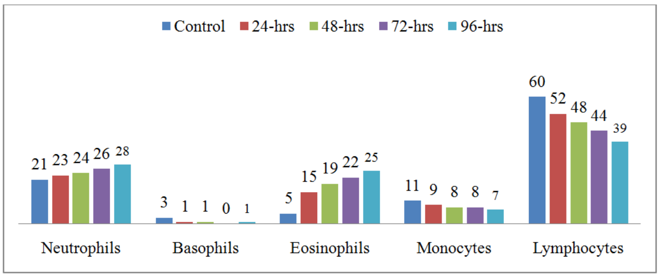

Figure 1. Percentage of differential Leucocytes count during short term exposure to NP LC-50

Percentages of Differential Leucocytes Count

The white blood cells (WBCs) or leucocytes are differentiated into eosinophil, basophils, neutrophils, lymphocytes and monocytes. In the normal fish *O. punctatus* the percentage of eosinophil's accounted to be 5%, basophils 3%, neutrophils 21%, lymphocytes 60% and monocytes 11% (Fig.1).From first to fourth days of exposure to NP LC-50 leucocytes percentage in *O. punctatus* variably got changed (Fig.1).

DISCUSSION

The chemical poisons that enter in the water body variably damages the cells and tissues of the aquatic animals thereby causing the disturbance in general metabolism. The blood cells in the fishes are produced in the region of head kidney where hematopoietic tissues are present. There are varieties of blood cells that include Red blood cells, White blood cells and blood platelets. Besides these White blood cells classified into eosinophil, basophil, neutrophil, monocyte and lymphocyte. There are many reports that the blood cells quantitatively show the variation on exposure to domestic wastes, industrial effluents pesticides and also some other chemicals. It is well known for the couple of decades that the exposure of fish to different chemical agents induce alteration in many hematological variables (Heath, 1995; Modesto and Martinez, 2010) and it helps to evaluate the health status of fish (Martinez and Souza, 2002). Blood cell responses are one of the most important markers for changes in the internal and external environment of animals (Sharma and Chadha, 2015). Blood cell count is a stable index and normally fish tries to maintain it between certain limits (Sharma and Chadha, 2015). But presence of toxicant in water may lead to change in water quality which may ultimately change one or more hematological aspects (VanVuren, 1986). Increase or decrease in hematological parameters can be found in fish exposed to chemical pollutants. However, change in blood parameter may also depend on fish species, age, the stage of reproductive cycle and disease (Golovina, 1996; Lusora, 1997).Evaluation of hematological parameters in fish is used for the detection of physiopathological changes in the stress conditions (Nussey *et al*., 1995). Hematological aspects such as hematocrit, hemoglobin, total erythrocytes and leucocytes are the indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Sancho *et al*., 2000; Barcellos *et al*., 2003). Beside these it is also an indicator of water balance, nutritional status and overall health condition of fish (Chang and Hur, 1999; Denson *et al*., 2003).

It is reported that the effects of environmental toxicants on hematological parameters of fish differ according to the target species (Elahee and Bhagwant, 2007; Glusczak *et al*., 2006; Modesto and Martinez, 2010). In the present study when *O. punctatus* was treated with NP LC-50 for short term duration, total erythrocytes or total red blood corpuscles (RBCs) were found decreased significantly till the end of exposure as compared to control. But the hemoglobin percentage decreased non-significantly upon exposure to all sort of concentration both for short and long time exposure. The decreased in RBC and Hb was also reported in *Salmotruttacaspius* (Mousavi and Yousefian, 2012), *Cichlasomadimerus* (Vazquez and Lo Nostro, 2014).There are some reports of getting affected the number of white blood cells by variety of physiological and environmental factors and such responses generally found when fish were subjected to an array of toxicant (Witeska, 2005; Jayprakash and Shettu, 2013; Sayed and Moneeb, 2015). It was observed that during short term exposure, the TEC decreased insignificantly on first day (24 hrs) and later consistently decreased significantly along with continuous non-significant decrease in hemoglobin concentration in *O. punctatus*. However, on exposure to sub-lethal concentrations of NP LC-50 TEC decreased significantly but Hb decreased insignificantly over entire long period of exposure. Similarly, Ramesh and Saravana (2008), and Yonar *et al*. (2012) reported significant decrease in RBC counts and Hb level in chlorpyrifos induced fish *Cyprinuscarpio.* The decrease in RBC count and Hb level may be due to the inhibition of erythorpoiesis, haemosysnthesis, or osmoregularotory dysfunction or due to an increased rate of erythrocyte destruction in the hematopoietic organ (Vani *et al*., 2011).

The PCV showed insignificant decreased during entire short term of exposure. MCV and MCH exhibited both insignificant increased on first day (24 hrs) and later it significantly increased till four days (96 hrs) of exposure. However, MCHC was found increased insignificantly during 24 hrs and 48 hrs of exposure but later it decreased insignificantly for the next two days. In long term exposed *O. punctatus*, PCV and MCHC decreased but MCV and MCH increased consistently for both the sub-lethal concentration of NP LC-50 during. Besides decrease in total RBC counts WBC counts were found decreased during entire period of exposure to NP LC-50 and also significant decreased were noticed during the long term exposure to sub-lethal concentrations of NP LC-50. The total number of neutrophils and eosinophils increased with decreased in numbers of lymphocytes, monocytes and basophils during short term exposure to NP LC-50 and also during long term exposure to NP LC-50. As far as differential counts were concerned for all the four days (short term duration) and twenty-one days (long term exposure to both sub-lethal concentrations of NP LC-50) leucocytes showed dramatic changes.Leucocytes play an important role in the nonspecific or innate immunity, and the leucocyte count/activity can indicate the health status of a fish (Secombes, 1996). The significantly maximum increase in WBCs counts in *C. carpio* exposed to chlorpyrifos (Okechukwu *et al*., 2007; Ramesh and Saravana, 2008; Yonar *et al*., 2012). Such alternation could be the result of the activation of immune system in the presence of contaminant, which in turn is an adaptive response of the organism, resulting in more effective immune defense response (Modesto and Martines, 2010).

Conclusion

Decreased in hemoglobin, PCV, MCHC and TEC/RBCs counts indicate the anemic condition of fish, may be due to inhibition of erythrogenesis or destruction of erythrocytes on exposure to both the lethal and sub-lethal concentration of NP. But on short term exposure to NP LC-50 MCHC showed no consistency and the cause could not be understood. Total leucocyte and lymphocytes count decreased on exposure to lethal and sub-lethal concentrations indicated potential decreased in the immunity. It is concluded that the NP is hemotoxic in *O. punctatus*.

Conflict of interest- Authors stated that no conflict of interest.

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