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Immune response in *Channa punctatus* after sub chronic 4-nonylphenol treatment and recovery

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Abstract

The effect of 4-nonylphenol (Surfactant) on blood parameter of fish *Channa punctatus* were evaluated after sub chronic exposure of 90 days and post treatment recovery was also ascertained by keeping fish in normal water for 30 days. The fish were exposed to different sub-lethal concentrations which were decided after calculating safe application rate for 90 days. Blood samples were collected after 30, 60 and 90 days respectively. Dose and time dependent effect of 4-NP was seen on differential leucocyte count (monocyte, lymphocyte and neutrophils). WBC differential count revealed a significant increase in the number of monocyte and neutrophils and decrease in the number of lymphocytes after 90 days of treatment. This can be considered as a clear evidence of altered immune system caused by 4-NP. On the other hand recovery indicates a considerable compensatory potential of *C. punctatus* immune system. This may be suggested parameter considered as useful biomarker for monitoring effect of toxicants in fish.

Keywords: Nonylphenol, monocytes, lymphocytes, neutrophils

Introduction

Aquatic systems are exposed to a large number of pollutants which are mostly released in effluents from industries. Globally, industrial waste water represents the major source of water pollution. Nonylphenol is a degradation product of nonylphenol ethoxylates (NPEO) which is highly persistent and toxic, posing a serious threat to humans and other organisms [1]. Nonylphenol is by far the most important non-ionic surfactant used in detergents, pesticides and cleaning agents [2]. Since its discovery, the production of nonylphenol has increased exponentially between 100 to 500 million pounds every year and meets the definition of high production volume chemical [3]. In India NP has been detected at extremely high level in few studies conducted on the different river system [4, 5]. The range of concentrations commonly found in rivers between 0.2 - 12 µg/L that is already causing problem to a number of species [6]. It may cause ecological effects due to its high risk quotient, according to the studies conducted on effluent of various textile industries from different countries. Concentration of NP and NPEO was found to be high which shows the current problematic condition of Asian countries. Studies indicate the presence of these chemicals in waste water treatment plants, surface water and ground water [7]. A number of studies on its behavioural, physiological, genotoxic effect is present in the literature [8, 9].

Toxicant alters the immune response by immune suppressive or immune stimulatory response [10]. Severe or chronic stress is often associated with poor performance and has long been associated with immune-suppression in fish [11]. Immune mechanisms are of significance to fish as they face several environmental challenges. Adverse ecological conditions may have acute or chronic influence on the health status of fish, by modulating immune responses [12].

A wide variety of organisms have been used to monitor areas with different pollution levels. Among these fishes represent an excellent experimental model for toxicological studies as they inhabit all zones of aquatic habitat, bio accumulate the environmental pollutants, respond to mutagens at low concentration and provide early warning for pollution induced environmental changes [13]. So the present study has been designed to assess the immunotoxic effect of 4-nonylphenol on fish *C. punctatus* after sub chronic exposure of 90 days and recovery was ascertained after 30 days post treatment to observe the potential of the immune system of fish to overcome the stress.

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Materials and Methods

Experimental fish specimen and chemicals

Freshwater air-breathing fish *C. punctatus* was procured from local sources. The specimens had an average weight and length of 16.50 ± 2.14 g and 11.40 ± 2.01 cm, respectively. Fish specimens were subjected to a prophylactic treatment by bathing twice in 0.05% potassium permanganate (KMnO₄) for 2 minutes to avoid any dermal infections. The specimens were then acclimatized for two weeks under laboratory conditions in semi-static systems. They were fed with boiled eggs. The fecal matter and other waste materials were siphoned off daily to reduce the ammonia content in water. 4-nonylphenol used in the present study was obtained from Himedia (India). A stock solution was prepared by dissolving NP in ethanol as a carrier solvent.

Experimental fish specimens and chemicals

To determine the 96-hour LC₅₀ value of 4-nonylphenol, acute toxicity bioassay was conducted in a static system in the laboratory the test solution was changed every day to ensure that the chemical level stayed the same throughout the test period. The 96-hours LC₅₀ value of 4-nonylphenol was determined as 1.27 mg/l for *C. punctatus* [14], following the probit analysis method as described by Finney (1971). Safe application rate (SAR) was calculated using the formula given by Basak and Konar (1977). After calculating the SAR three sublethal concentrations were decided, which were 1/10th (0.15mg/l), 1/15th (0.10mg/l) and 1/20th (0.07 mg/l) of the SAR.

$$\text{SAR} = \frac{\text{LC}_0 \times \text{LC}_{100}}{\text{LC}_{50}}$$

In-vivo exposure experiment

The fish specimens were exposed to the three aforementioned test concentrations of 4-nonylphenol and in tap water and ethanol in a static renewal system with the change of test water every day to maintain concentration consistently. In order to eliminate the leaching potential of 4-nonylphenol, plastic material was avoided and glass aquaria of 200 liters capacity were used for the experiment. The exposure was continued up to 90 days, and blood sample were taken at the intervals of 30, 60, and 90 days at the rate of five different fish per interval. The specimens maintained in tap water were considered as negative control and those in ethanol as a positive control. After 90 days of exposure fish were kept in water without 4-nonylphenol for 30 days and at the end of 30 days recovery was ascertained.

Differential leucocyte count

For differential leukocyte count blood smear was made immediately after taking blood. Dried at room temperature and then fixed in ethanol for 10 minutes. Differential leukocytes (lymphocytes, neutrophils and monocytes) were counted in blood smear after staining the slides with Wright stain for 30 minutes, dried and observed under microscope. 100 WBC's were counted with blood cell counter [17].

Statistical Analysis

The results are expressed as mean \pm S.E. The Tukey-HSD test was considered for multiple comparisons and designed to study significance of difference in the different leukocytes among treated and control groups at different time intervals. One way analysis of variance was applied to assess the effect

of concentration and time duration.

Results and Discussion

A significant change in the number of differential leucocyte was observed after subchronic exposure of 4-NP to fish *C. punctatus*. The lymphocyte number decreased significantly, while monocyte and neutrophil number increase significantly ($p \leq 0.05$) with time duration as well as concentration when compared with control. The differential leucocyte count has been estimated in the study. The change in the differential WBCs count can be used an indicator of stress reaction and for immunity decrease after exposure to toxic substances^{18, 19}. Despite some anatomical and physiological differences, the immune system of fish is similar to other vertebrates, and consists of various types of cells and chemical mediators. In fish, cellular and humoral, non-specific and specific immune mechanisms are present. The fish immune response may serve as an alternate or additional model for predicting the immunotoxicity of environmental contaminants as shown by many workers^[20, 21].

Change in leucocyte number indicate the response of fish organism to stress reaction. The study shows that 4-nonylphenol caused immunological impairment in fish which weaken its immune system and may lead to severe physiological problems, ultimately leading to death of fish. The results also showed an increase in neutrophils and decrease in lymphocytes number and these results are in agreement with results of Davis *et al.* (2008). Dhanekar *et al.* (1985) reported the elevation in the number of lymphocytes, monocytes, neutrophils and eosinophils in *H. fossilis*. Differential leukocyte count showed a significant increase in the population of lymphocytes whereas neutrophils and monocytes were found to be decreased in *C. punctatus* after exposure to copper^[24]. Similar increase in lymphocytes and decrease in neutrophils were found by Shahi *et al.* (2013) in fish *C. punctatus* after exposure to synthetic and plant origin pesticides and by Sampath *et al.* (1993) when they exposed Nile tilapia (*O. niloticus*) to a toxic environment. Decrease in monocyte number is observed by Venkatramana *et al.* (2013) in *C. carpio* in response to pyrethroid based pesticide. In contrast to this, Queensly *et al.* (2015) reported a significant decrease in lymphocytes, neutrophils and monocytes in *C. carpio* when exposed to cypermethrin. Decreased value of lymphocytes along with other haematological parameters was seen in *L. rohita* after exposure with butachlor^[29]. Ajima *et al.* (2015) reported that when fish *C. gariepinus* were exposed with NPK fertilizer for 56 days, the number of neutrophils and monocytes get increased while lymphocyte number decreased.

The rate of recovery of *C. punctatus* exposed to 4-NP was examined by assessing responses of differential leucocyte count in treatment and post treatment tests. After 30 days recovery period a significant recovery was seen in all the parameters tested. The differential leucocyte count return to a normal level, if exposure to contaminants is discontinuous. The fish *C. gariepinus* exposed to Parquat dichloride recovered spontaneously and the aberrated parameters in all the treatments normalized after 12 days in insecticide free aquarium^[31]. The fish species are able to overcome rapidly the toxic stress induced by insecticide flucycloxuron^[32]. Kroupova *et al.* (2002) found that in common carp the values of haematocrit, erythrocyte count and Hb concentration after recovery were markedly lower as compared to after nitrite poisoning.

Table 1: Differential leukocyte count in fish *C. punctatus* after treatment with different concentrations of 4-NP for 30, 60 and 90 days.

		30 Days	60 Days	90 Days
Treatments				
Lymphocytes	Control	84.00±0.57 ^{a,p}	84.00±0.57 ^{a,p}	83.00±0.57 ^{a,p}
	Ethanol	82.33±0.33 ^{a,p}	83.33±1.2 ^{a,p}	84.00±0.57 ^{a,p}
	0.07 mg/l	78.00±0.57 ^{a,q}	75.00±0.57 ^{b,q}	72.00±0.33 ^{c,q}
	0.10 mg/l	76.00±0.57 ^{a,q}	74.00±0.57 ^{a,q,r}	71.00±0.57 ^{b,q}
Neutrophils	Control	11.00±0.50 ^{a,p}	11.66±0.30 ^{a,p}	11.33±0.30 ^{a,p}
	Ethanol	11.66±0.30 ^{a,p}	11±0.50 ^{a,p}	11.60±0.30 ^{a,p}
	0.07 mg/l	12.66±0.30 ^{a,q,r}	13.66±0.30 ^{ab,q}	14.66±0.30 ^{b,q}
	0.10 mg/l	14.33±0.30 ^{a,q}	14.66±0.30 ^{a,q}	15.66±0.30 ^{a,q}
Monocytes	Control	5.33±0.3 ^{a,p}	5.33±0.6 ^{a,p}	4.6±0.3 ^{a,p}
	Ethanol	6±0.001 ^{a,p}	5.33±1.25 ^{a,p}	4.33±0.8 ^{a,p}
	0.07 mg/l	6±0.001 ^{a,p}	5.33±1.45 ^{b,p}	4.33±0.8 ^{a,p}
	0.10 mg/l	9.33±0.3 ^{a,q}	11.6±0.8 ^{ab,q}	13.33±0.6 ^{b,q}
	0.15 mg/l	11.33±0.8 ^{a,q}	10.33±0.3 ^{a,q}	11±0.001 ^{a,q}

*-($p \leq 0.05$)**-($p \leq 0.01$)

The values given as mean \pm standard error. Different letters (a, b, c) between the columns are significantly different (Tukey's test, $p \leq 0.01$) and signify the effect of duration of exposure at each concentration. Similarly, different letters (p, q, r, s)

within the columns are significantly different (Tukey's test, $p \leq 0.01$) and signify the effect of different concentrations of 4-nonylphenol at the same time interval.

Table 2: Values of different blood parameters after 90 days exposure and 30 days recovery.

		0.07 mg/l	0.10 mg/l	0.15 mg/l
Lymphocytes	Exposed gp	72.00±0.33 ^a	71.00±0.57 ^a	70.66±0.33 ^a
	Recovery gp	81.33±0.3 ^b	82.33±0.3 ^b	81.00±0.5 ^b
Neutrophils	Exposed gp	14.66±0.3 ^a	15.66±0.3 ^a	18.33±0.3 ^a
	Recovery gp	10.33±0.3 ^b	10.66±0.6 ^b	11.33±0.3 ^b
Monocytes	Exposed gp	4.33±0.8 ^a	13.33±0.6 ^a	11.00±0.001 ^a
	Recovery gp	4.66±0.3 ^b	4.66±0.8 ^b	6.00±0.57 ^b

**-($p \leq 0.01$)

The values given as mean \pm standard error. Different letters (a, b) are significantly different (Tukey's test, $p \leq 0.01$) and signify the difference between exposed and recovery group.

Conclusion

4-nonylphenol has immunosuppressive effect on fish *C. punctatus* after sub chronic exposure. These alterations may suppress normal growth, reproduction, immunity and even survival of fish in natural environment. But the values of all the parameters returns to normal level after recovery period that showed great potential of fish *C. punctatus* to overcome stress condition.

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