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Acute Toxicity of 4-nonylphenol on Haemotological profile of Fresh water Fish *Channa punctatus*

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Abstract

Haematological parameters have been recognized as valuable tool for monitoring fish health. In the present study the haematological profile of fresh water fish Channa punctatus was studied. Fish were exposed to sub lethal concentration of 4-nonylphenol decided after LC_{50} determination for a period of 24, 48, 72 and 96 hrs. In haematological profile, effect is seen on RBC count, WBC count, Hb, and PCV as well as on Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). 4-nonylphenol significantly decreases the value of RBCs, Hb, PCV, MCH, MCV, MCHCand WBC count increases with 4-nonylphenol treatment and at different time of exposure. It was concluded that the 4-nonylphenol caused haemotoxicity in fish.

Keywords: Haematology, *Channa punctatus,* 4-nonylphenol, RBC, WBC.

Introduction

Marine and aquatic environments are a home to a vast variety of organisms ranging from prokaryotes to higher vertebrates. Unfortunately, these environments also act as sinks for a great variety of anthropogenic organic pollutants many of which are toxic at elevated concentrations due to their low biodegradability. These organic compounds are mainly derived from domestic agricultural and industrial waste water resources. These include polycyclic aromatic compounds, pesticides, azo dyes, aromatic amines and various surfactants used in textile industry.

Nonylphenol ethoxylate (NPE) is a non ionic surfactant which is used worldwide and found in aquatic environment. This compound is cost effective and hence used in the formation of detergents, paints, lubricants, stabilizer polymers, antioxidants, polystyrene tubes, insecticides and herbicides. They are used in paper, textile and many other industries as well as in shampoos, deodorants, detergents used in household activities. Due to extensive use it reaches in to sewage where it is broken down to 4-nonylphenol by microorganisms, which is known to be more stable and persistent[1]. As it is persistence in the environment so it get bio accumulate in aquatic organisms, the concerns regarding the adverse effects of 4-nonylphenol has been increased recently[2].

Blood cell responses are one of most important marker for changes in the internal and external environment of animals. Blood cell count is a stable index and normally fish tries to maintain it between certain limits. But presence of toxicants in water may lead to change in water quality which may ultimately change one or more haematological parameters[3]. Increase or decrease in haematological parameters can be found if fish is exposed to chemical pollutants. Change in blood parameter may also depend on fish species, age, the cycle of the sexual maturity of spawners and diseases[4,5]. So keeping all these things in view 4-nonyl phenol is selected as testing chemical to assess its haematological effects on fish *C. punctatus*. Ecotoxicological characteristics of air breathing fresh water fish *C. punctatus* such as its wide distribution and availability throughout the year, easy maintenance in aquarium and commercial importance make this species an excellent model for toxicity studies[6].

Material and Method

Experimental fish and chemical: Fresh water teleost fish *C. puctatus* (Bloch; family; Channidae, Order; perciformes) were procured from local fish market having weight 17 ± 2 g and measured 12 ± 2 cm. Fish was treated with 0.02% $KMnO_4$ for 2 minutes to avoid any dermal infection. The fishes were then acclimatized under laboratory conditions in static conditions for 15 days and kept in rectangular glass aquaria of capacity 200 litres. They were fed with boiled egg. The faecal matter and other waste material were siphoned off daily to reduce ammonia content in water. 4-Nonylphenol was obtained from himedia (India). Stock solution was prepared in ethanol.

Determination of LC_{50}: LC_{50} for NP comes out to be 1.27mg/l. Based on LC_{50} value sub lethal concentration 0.158 mg/l (1/8) of LC_{50} were chosen and fish were exposed to sub lethal concentrations for 24, 48, 72 and 96 hrs to see the effect on haematological profile. Fishes in tap water was considered as negative control and in ethanol as positive control The physicochemical properties of test water, namely temperature - 23.9±0.19, pH -7.4±0.20, dissolved oxygen-3.2±0.30mg/l, total alkalinity-16.65±0.30, free CO_2.8±0. 23 mg/l, TDS-0.4±0.01 g/l,

TSS-0. 5±0.01, TS-0. 9±0.02g/l were analyzed by standard methods[7].

Haematological Parameters: Present study deals with the comparison of important blood parameters like Hb, PCV, RBC's, and WBC's count. Haematological analysis was done by taking blood from fish by heart puncture in eppendorf tubes containing EDTA anticoagulant. Blood samples from treated group and control was used for haematological analysis viz RBC counting, WBC counting, PCV and haemoglobin. All the haematological parameters were determined by using the standard technique described by Jain N.C.[8]. Red blood cells (RBC) were counted using Neubaur haemocytometer. Blood was diluted 1: 200 with Hayem's fluid. Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were counted per 10^6 mm^3. Sahli's haemoglobinometer was used to estimate haemoglobin (Hb). Haematocrit levels were determined by drawing fresh blood into microhaematocrit tubes and centrifuged in a microhaematocrit centrifuge (Micro Centrifuge, Remi, Remi Motors, Bombay, India) at 9000 g for 5 min. Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated using formulae mentioned by Dacie S.[9].

$$MCV = \frac{\text{Packed cell volume as percentage} \times 10}{\text{RBC in millions}}\ \mu m^3$$

$$MCHC = \frac{\text{Hb in grams . 100 g per 100 ml}}{\text{Packed cell volume}}$$

$$MCH = \frac{\text{Hb in grams . 10pg}}{\text{RBC in millions}}$$

Statistical analysis: The results are expressed as mean ± S.E. The Tukey-HSD test was considered for multiple comparisons and designed to study significance of difference in the RBC's, WBC's count, Hb, PCV, MCH, MCHC and MCV among treated and control groups at different time intervals. One way analysis of variance was also applied to assess the effect of interaction between concentration and time on all the haematological parameters.

Results and Discussion

In the present study, exposure of fish to concentration 0.158 mg/l ($1/8^{th}$ of LC_{50}) of 4-nonylphenol for 24, 48, 72 and 96 hr caused significant alterations in haematological parameters of fresh water fish *C. punctatus*. Hb, PCV, RBC's and WBC's are given in figures 1-4 and MCH, MCV, MCHC are given in figures 5-6. RBCs number (million/µl), Hb content (mg/dl) and packed cell volume (Haemocrite) percentage decreased significantly after exposure to 4-nonylphenol in comparison with controls ($p \leq 0.05$). There was slight change in all the parameters in positive control as compared to negative but change was not significant. Hb and PCV level decreases up to 96 hrs and the decrease is significant with treatment as well as with increase in time of exposure. Hb level decrease from 11 to 6.16 % and value of PCV decreases from 35.33 to 24.5% at the end of 96 hrs. In case of RBCs decrease is significant when compared with controls but no significant change is observed with time duration. RBCs level decrease from 5.72×10^6 to $4.52 \times 10^6/mm^3$. While WBC count increases with 4-nonylphenol treatment as well as increase was found with time duration. Highest WBC count was found at 96 hrs and increase was significant. Value of WBCs count increase from 4.38×10^3 to $6.73\times10^3/mm^3$. There is significant decrease ($p\leq 0.05$) in MCHC value. Simliar trend was found for MCH. Change is significant with treatment as well as with time duration. MCV remains almost same expect at 96 hrs there is a significant decrease in the value after treatment with 4-nonylphenol as compared to controls.

When fishes are exposed to stressors they evoke non specific response to cope with changes and to maintain homeostatic state[10]. But in case the disturbance remain for longer time it may threaten the fish health and well being. In fishes change in blood cell distribution is correlated with the change in environment. Therefore in presence of toxicant or other stressors blood parameters can be used as standared laboratory test to determine diseased conditions and metabolic disturbances. The exposure of *C. punctatus* to sub lethal concentration of 4-nonylphenol caused a significant decrease in erythrocyte count, haemoglobin and haematocrit. All mentioned alterations indicate that exposed fish suffered from anemia induced by the 4-nonylphenol. This is an indication of disruptive effects of 4-nonylphenol on erythropoietic tissues as well as cells viability[11]. It is also possible that 4-nonylphenol adversely suppressed fish osmoregulation. The disturbed osmoregulation may finally results in dilution of blood. Other reasons for anemia may be altered membrane permeability or increased mechanical fragality or may be due to defective iron metabolism or intestinal uptake of iron due to mucosal lesions[12]. NP has higher affinity for membrane phospholipids, which accounts for its lytic activity[13]. Anemia found in present study is a consequence of an interaction between NP and erythrocyte membrane. Erythrocytes and their hemoglobin contents are responsible for oxygen transportation within the body. Low number of red blood cells or insufficient amount of their hemoglobin content could influence energy balance of the body[14]. In this case fish may suffer from oxygen deficiency, which ultimately prohibits its normal growth. Moreover it seems that reduction in red blood cells is a key factor which could be responsible for productivity reduction. Similar decrease in value of erythrocyte count, haemocrit and haemoglobin was reported after exposure to benzene derivatives[15] and exposure to diazine[16].

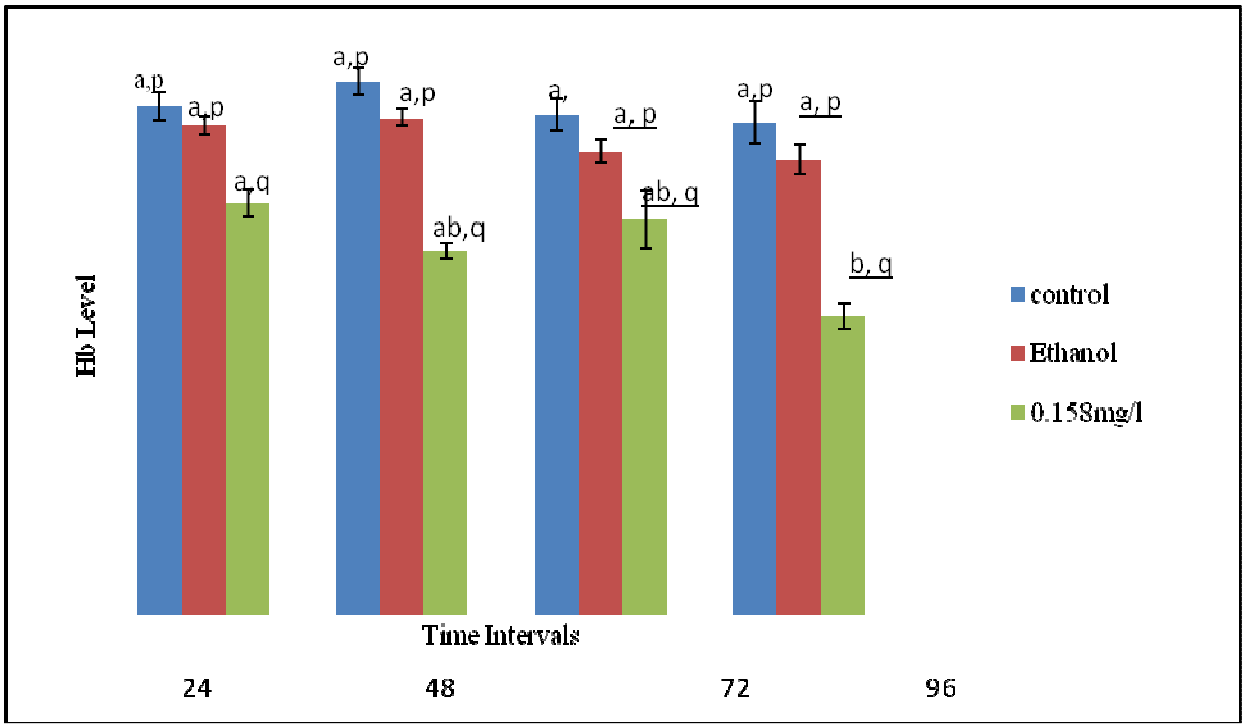

Figure-1
Showing Hb level in controls and after treatment with 4-NP (letters a, b and p, q are showing significant differences)

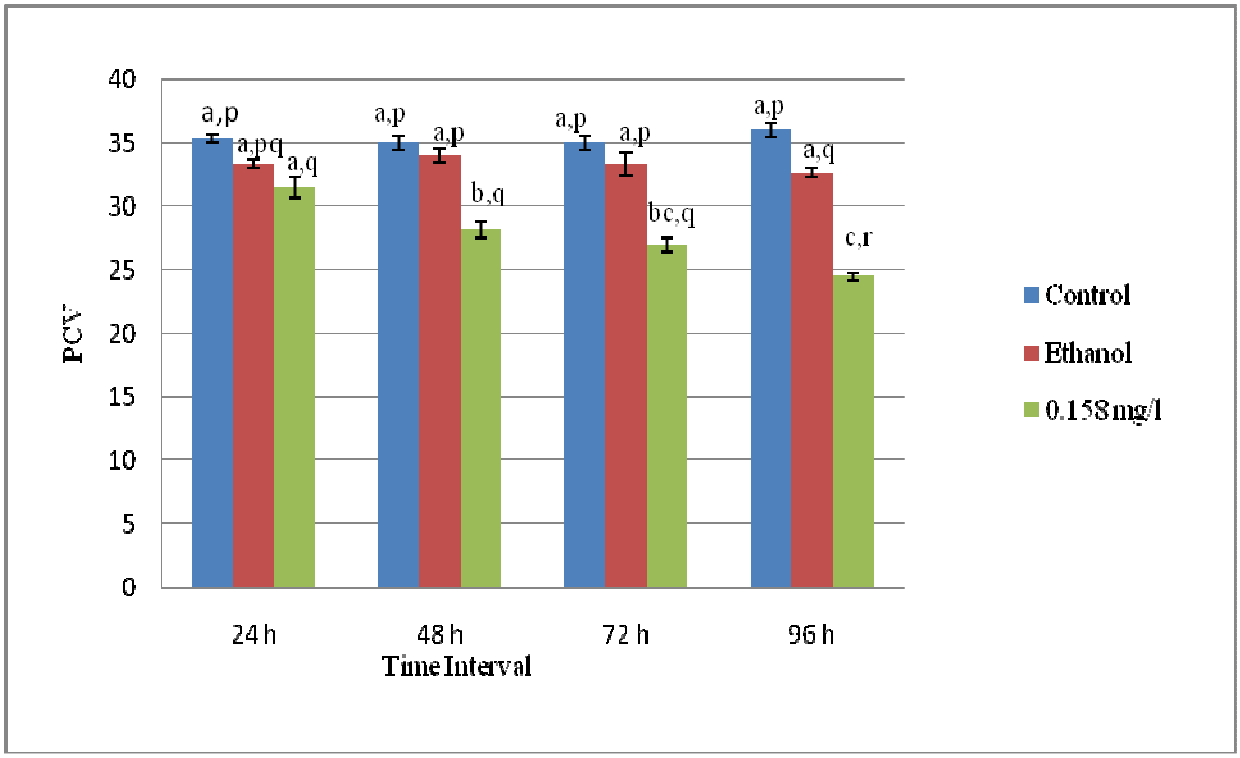

Figure-2
Showing Packed cell volume in both controls and after treatment with 4-NP (a, b, c and p, q are showing significant differences)

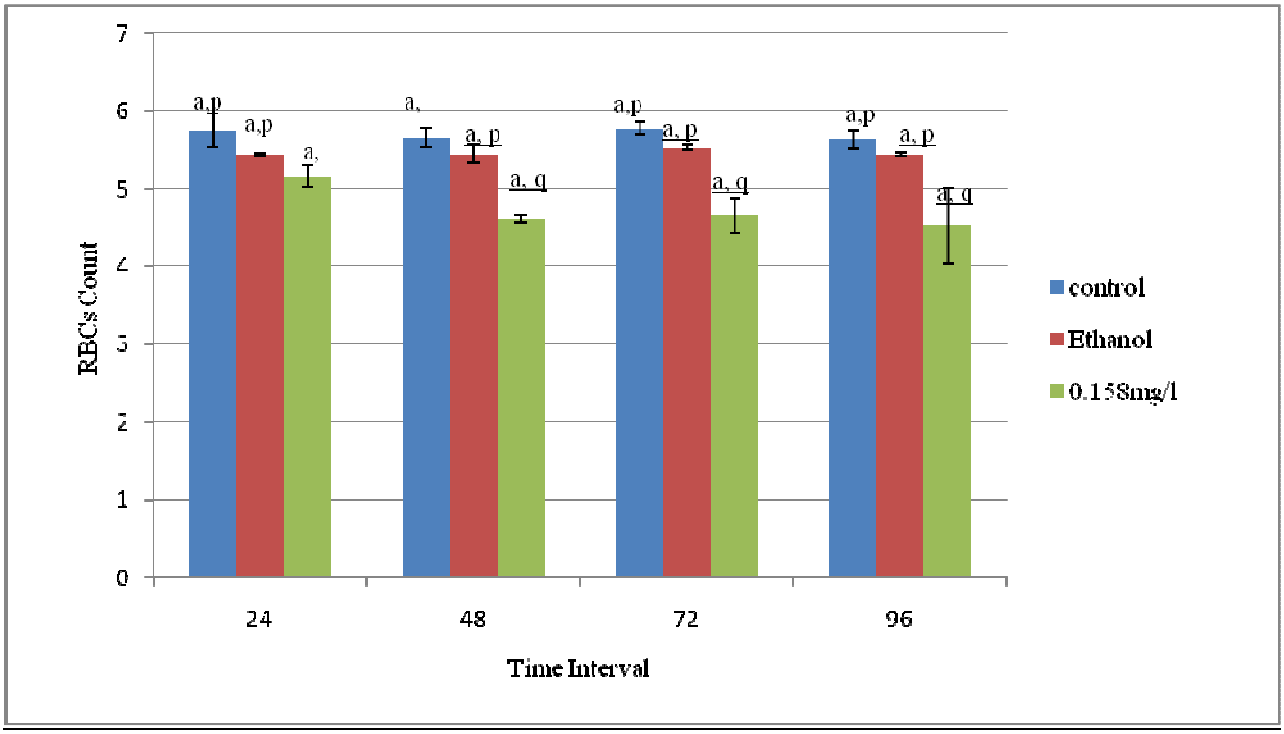

Figure 3
Showing RBCs count in controls and after treatment with 4-NP (letters a and p, q showing significant differences)

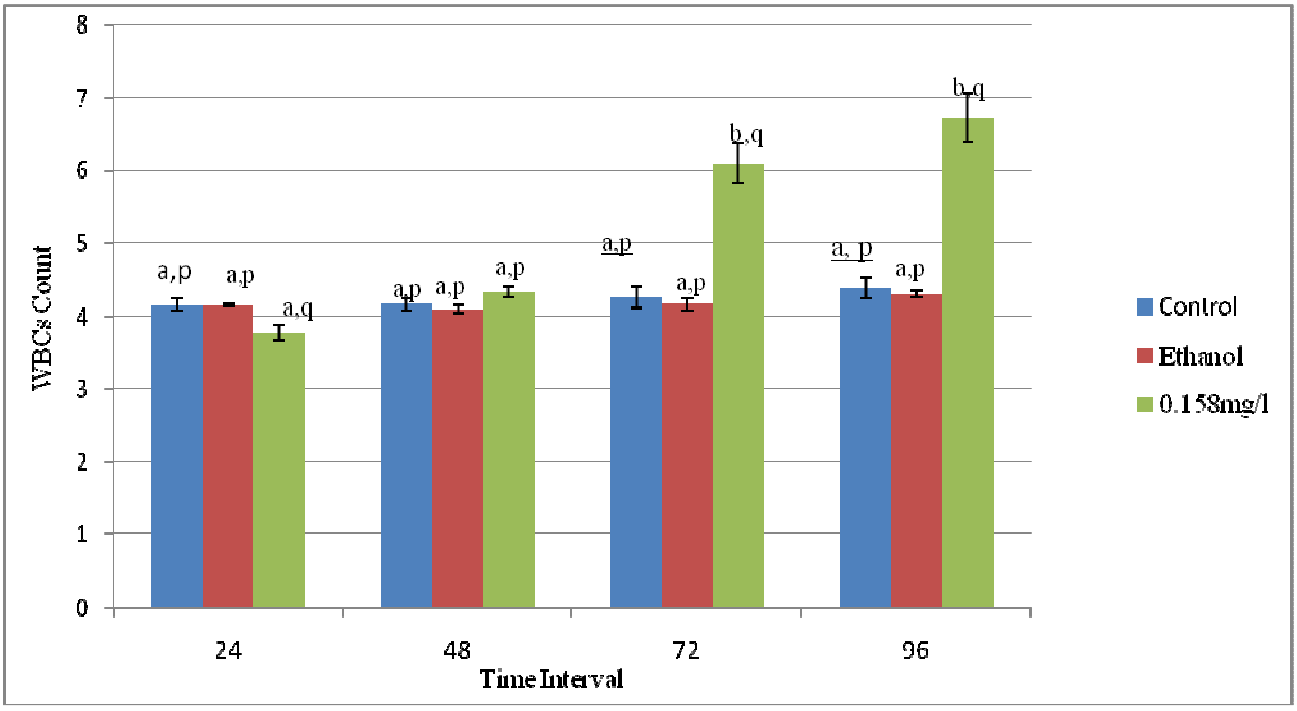

Figure-4
Showing WBCs count in controls and after treatment with 4NP (letters a, b and p, q are showing significant differences)

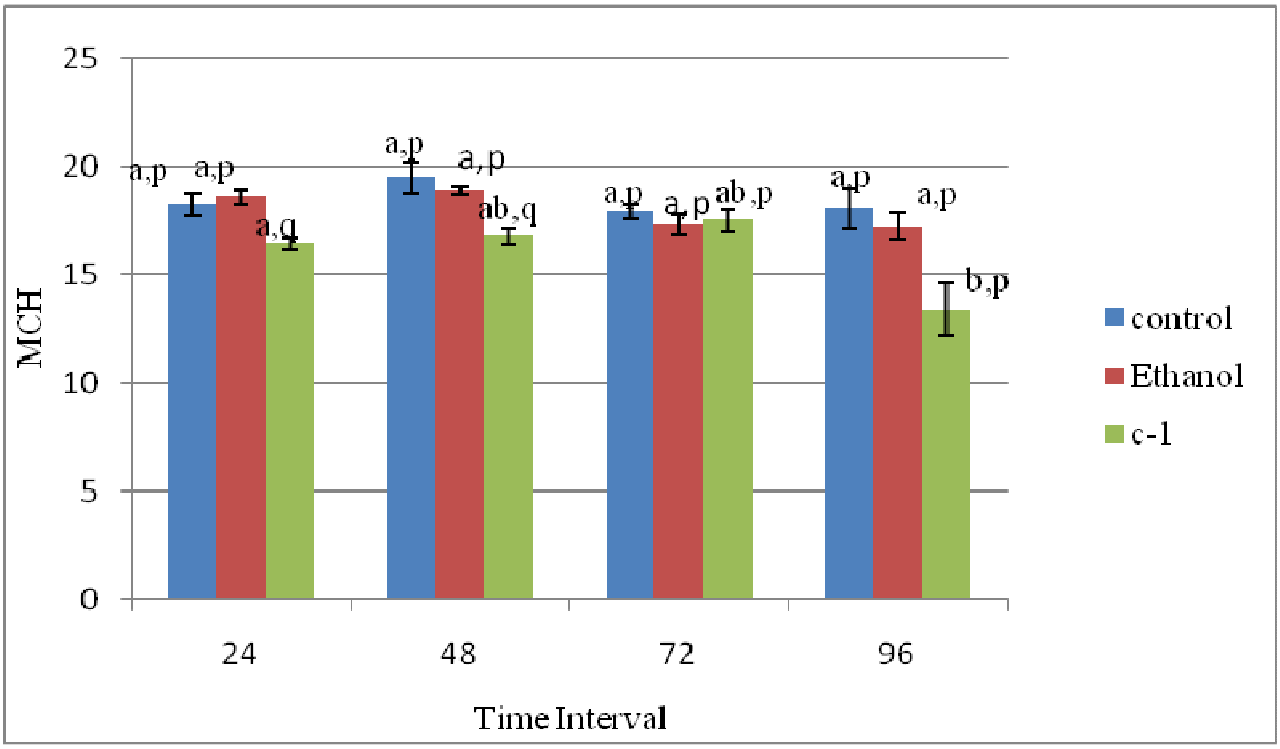

Figure 5
Showing MCH in controls and after treatment with 4NP (letters a, b and p, q are showing significant differences)

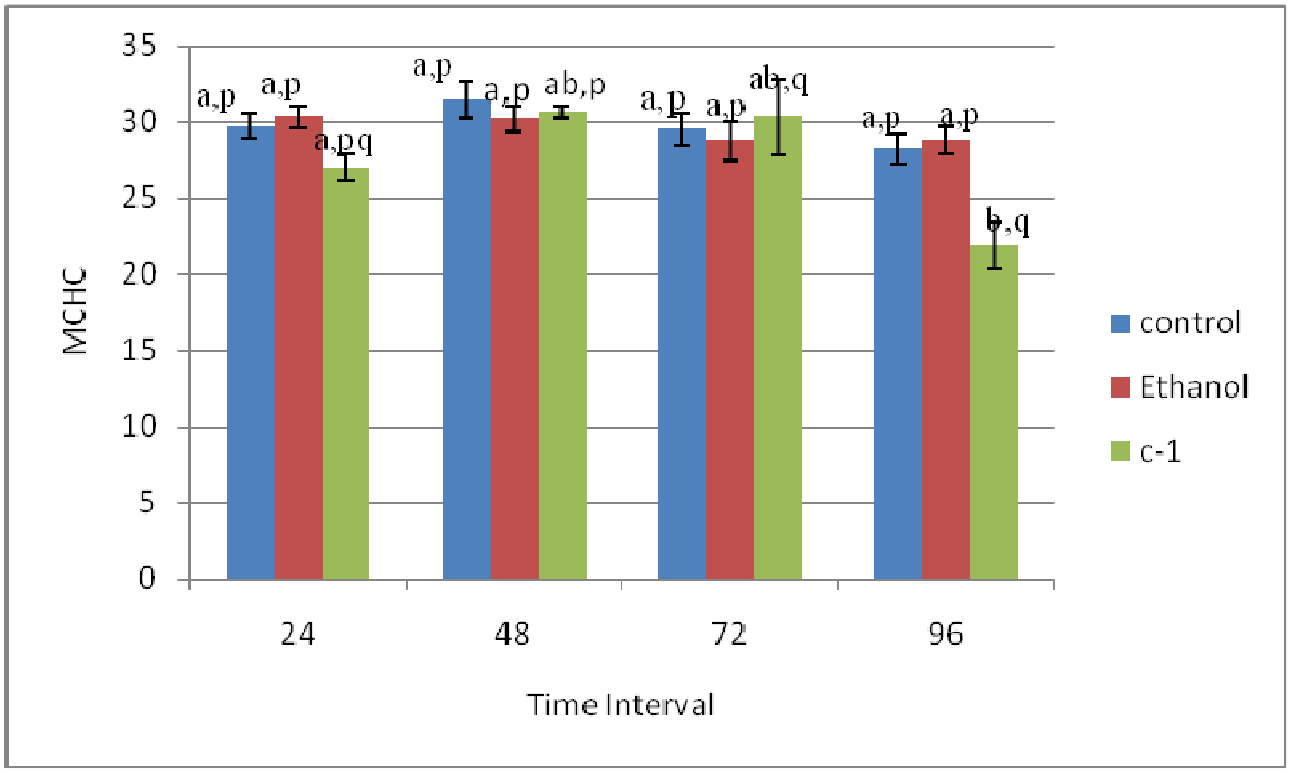

Figure-6
Showing MCHC in controls and after treatment with 4NP (letters a, b and p, q are showing significant differences)

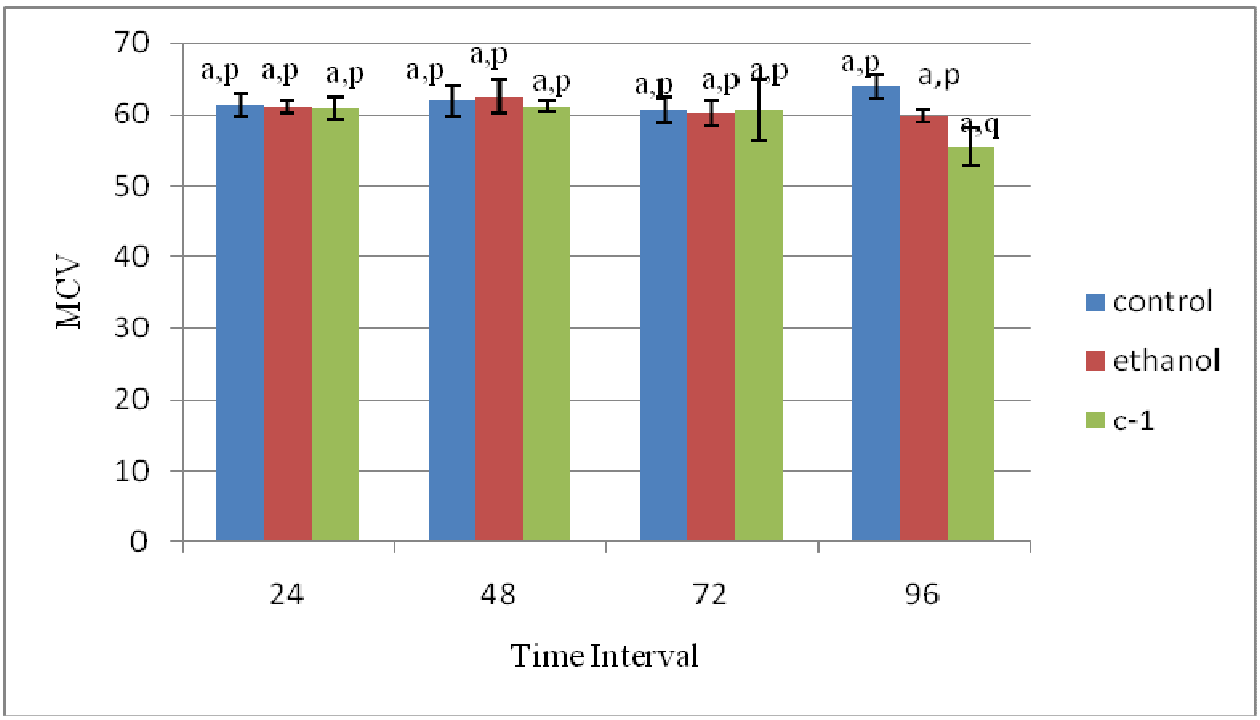

Figure-7
Showing MCV in controls and after treatment with 4NP (letters a, b and p, q are showing significant differences)

Figure 1-7 Showing mean values (n=3) of % haemoglobin, % packed cell volume, RBCs count, WBCs count, MCH, MCHC, MCV after treatment with three sub lethal concentrations of 4-nonylphenol for different time of exposure. Error bars represent standard errors (SE). Different letters (a, b, c,) show the significant different (Tukey"s test, $p \leq 0.05$) and signify the effect of 4-nonylphenol at different time intervals. Similarly, different letters (p, q) show significant different (Tukey's test, $p \leq 0.05$) and signify the effect of nonylphenol treatment and controls at the same time intervals.

The increase in WBCs count or leucocytosis in treated fish probably reflects increased demand for WBC for removal of cellular debris at a faster rate. The increase in stressor probably puts a greater demand for phagocytic cells[17]. Increase in total WBC count in the present study was a result of direct stimulation for its defense. Progressive increased levels of total WBC count have also been reported in *C. punctatus* exposed to lead[18], *Clarias batrachus* exposed to mercuric chloride[14], *Clarias gariepinus* to metal finishing company effluents[19]. With the help of the haematological parameters, the erythrocytes constants (MCV, MCH, and MCHC) for the blood of the *C. punctatus* were calculated. Their diagnostic value is very important because they help to detect the presence of some physiological lesions in the process of formation of haemoglobin and offers information on the size, shape and haemoglobin quantity in erythrocytes[20]. The increase value of MCH may indicate a condition of macrocytic anaemia. Change in MCH is due to reduction in cellular blood ions, resulting in reduced O_2 carrying capacity and eventually stimulating erythropoises. The significant decrease in MCHC for exposed group is an indicator for variations in erythrocytes shape, size and Hb content[21]. This leads to anemia in fish[22]. Decrease in the value of Hb, PCV, RBCs may results in hypochromic microcytic anemia which may be due to deficirncy of iron and its decreased utility[23].

The present study showed that 4NP is hematotoxic to *C. punctatus*. The exposure of fish to NP resulted in significant reduction in the studied haematological parameters. These alterations may negatively suppress normal growth, reproduction, immunity and even survival of fish in natural environment as well as culture conditions.

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