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Effect of diethylphthalate on the haematological parameters of the freshwater fish *Oreochromismossambicus* (Tilapia)

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ABSTRACT

Diethyl phthalate is a kind of plasticizer widely used in industries. Phthalate esters have recently attracted special attention of the scientific community, regulatory agencies and the general public as a consequence of their high production volume, widespread use and possible endocrine related effects. Hence the present study was conducted to assess the chronic and sub lethal toxicity level of diethylphthalate (DEP) on the freshwater fish Oreochromismossambicus. The 96h LC_{50} value of diethyl phthalate exposed fish Oreochromismossmbicus was estimated by probit analysis method (with 95% confidence limits). The fish was treated with different concentrations namely, 5 and 15 ppm w/v of DEP. The survivability of fish exposed to different concentrations of diethylphthalate was assessed. Hematological parameters such as red blood cell count (RBC), white blood corpuscles (WBC),hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV), were observed in the exposed fish and compared with the control group. The results revealed that sub lethal concentrations of diethyl phthalate produced changes in all the measured hematological parameters. Present findings revealed that Diethylphthalate exerts its toxic action even at sub lethal concentrations and hematological parameters and abnormal behavior may be sensitive indicators to evaluate phthalate intoxication.

Keywords: Diethylphthalate; *Oreochromismossambicus;*Chronictoxicity; Haematology

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INTRODUCTION

Anthorpogenic estrogenic compounds like phthalate esters have been used in the manufacture of plastics, pharmaceutical coatings, celluloid (Joblingetal 1995; Sonnescheinetal., 1995). In India ,DEP is also extensively used in the manufacture of license sticks,as a perfume binder (Sonde*etal*.,2000 ;Ralio etal .,1985) reported that the blood parameters of diagnostic importance are erythrocyte and leucocytes counts, haemoglobin ,haematocrit and leucocyte differential counts which would readily respond to incidental factors such as physical stress and environmental stress due to water contamination.Tilapia is a good biological model for toxicological and immunotoxicity studies (Casas-Solis et al., 2007;Giron-perez et al .,2007;Giron-perez et al ., 2008)due to diverse characteristics, namely their high growth rates, efficiency in adapting to diverse diets, great resistance to diseases and handling practices, easy reproduction in captivity at prolific rate and finally, good tolerance to a wide range of environmental conditions (Fontainhas et al .,1998).Haematological indices are very indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels (Kori-Siakpere ;Oboh, (2011).

Hematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc.(Blaxhall et al.,1972;Duthie et al ., 1985).The use of haematological technique in fish culture has made it possible for researchers to use it in environmental monitoring and fish health conditions (Mulcahy 1975). The present study aim to gain insight into the changes induced in haematological parameters of *Oreochromismossambicus* on chronic exposure to diethyl phthalate.

MATERIALS AND METHODS

Experimental design

Diethylphthalate toxicity were assessed using tilapia as a aquaculture model in this experiment and thirty mature adult Tilapia (*Oreochromismossambicus)* were obtained from local breeders and acclimatized under laboratory conditions for a month. These adult fishes were reared in aquarium tanks for a period of 30 days at standard environmental conditions (28^0C and 14 hour photoperiod), and used for further experiments.Diethylphthalate was purchased from Sigma .St.Louis,USA and was dissolved in acetone to form a stock solution (500000 mgL^-1) and stored at room temperature.10 fishes were randomly selected from the stock and exposed to different concentrations of DEP (10,20,30,40,50,60,70,80,90 and 100ppm) for 96 hours to determine the median lethal concentration (LC_{50}) of DEP with selection exposure concentration of 5 and 15 ppm for chronic sub-lethal concentration exposure studies. Water was replaced daily with fresh DEP mixed water to maintain constant level of DEP during exposure period. The LC_{50} value for DEP was 50 ppm. For sub-lethal study , $1/5^{th}$, $1/10^{th}$ and $1/20^{th}$ of the LC_{50} value were chosen.A control group was maintained simultaneously. All these experiments were performed in triplicates.

Hematological analysis

The fishes were removed and anaesthetized and blood was taken from the caudal vein and collected in heparinized capillary tubes. Blood samples were used to measure hemoglobin (HB) concentration and red blood cell count and white blood cell count (RBC and WBC) which was done immediately. The HB concentration was determined using the cianometahemoglobin method Lee et al ., (1998)and RBC count was carried out in a modified Neubauer chamber after saline (0.9% NaCl solution) dilution of the blood. The blood indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were then calculated using the blood measurements above Lee et al.,(1998). Number of erythrocytes were determined by hemocytometer method (Stevens, 1997).

Statistical analysis

The data obtained from the above experiment were subjected to one way analysis of variance (ANOVA) followed by post-hoc testing using Duncan mean, performed with SPSS version 16. The data are presented as mean±standard error of the means.

RESULTS

Table-1 Changes in the Haematological parameters level in a freshwater fish *Oreochromismossambicus*treated with chronic concentration of Diehtylphthalate

	RBC	WBC	HB	MCV	MCH	MCHC
CONTROL	0.546±0.024 ^{c}	26.100±0.057 ^{c}	2.100±0.057 ^{c}	1.240±1.154 ^{b}	44.66±0.284 ^{a}	35.133±0.633 ^{a}
5ppm	0.756±0.008 ^{b}	60.326±0.336 ^{b}	3.093±0.103 ^{b}	1.700±0.577 ^{a}	42.53±0.523 ^{c}	23.300±1.361 ^{c}
15ppm	0.910±0.011 ^{a}	63.766±0.338 ^{a}	4.063±0.036 ^{a}	1.230±1.154 ^{c}	43.33±0.290 ^{b}	33.566±0.938 ^{b}
F	126.521***	5.628***	187.87***	721***	7.919***	39.528***
P	0.000	0.000	0.000	0.000	0.000	0.000

****Significant at p<0.001*

In a column , figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

The variation in hematological parameters of the fish *Oreochromismossambicus* to chronic toxicity DEP is presented in Table-1. Significant increase in meanRed Blood Cells (RBC),meanWhite Blood Cells (WBC), mean Haemoglobin, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) , Mean Corpuscular Haemogliobin Concentration (MCHC) was observed in the blood of DEP treated fish when compared to the untreated ones . Furthermore , dose dependent increase was depicted in the hematological parameters of the DEP treated fishes . In comparison to the control (0.546±0.024), mean RBC count significant (F=126.521

,P<0.001)elevated at 5-ppm (0.756 ±0.008) and 15ppm (0.910±0.011), DEP at 5ppm and 15ppm registered mean WBC count of 60.326±0.336 and 63.766±0.338,respectively , which was found to significantly higher (F=5.628,P<0.001) when compared to the unexposed fish. (26.100±0.057).Mean HB concentration was significant elevated (F=187.87 ,P<0.001) in the DEP treated fishes (5ppm:3.093±0.103;15ppm:4.063±0.036) when compared to the unexposed ones (2.100±0.057). DEP induced significant increase in mean MCV count of *Oreochromismossambicus* (5ppm:1.700±0.577; 15ppm:1.230±1.154). which was found to significantly higher than DEP untreated ones (1.240±1.154). Control fishes registered mean MCH level of 44.66±0.284, which significantly increased (F=7.919,P<0.001) on exposure to DEP (5ppm:42.53±0.523;15ppm:43.33±0.290). Similarly,mean MCHC level also significantly (F=39.528,P<0.001) elevated in *Oreochromismossambicus*on exposure to DEP (5ppm:23.300±1.361 ; 15ppm:33.566±0.938) DEP unexposed *Oreochromismossambicus*registered mean MCHC count of 35.133±0.633.

DISCUSSION

The impacts of DEP on the hematological profile of tilapia have been assessed in the present investigation.Increase in WBC evinced in this studyagress with that of Joshi et al., (2002), who have observed increase in mean WBC count in fish *Clariasbatrachus* exposed to lindane and malathion .Changes in the leucocytes system manifest in the form of leucocytosis with heterophilia and lymphopenia, which are characteristics of leucocytic response in animal exhibiting stressRamesh and Saravanan(2008). In the present investigation, significant increase in mean WBC count could be attributed to hypersensitivity of leucocytes to DEP due to immunological reaction to produce antibodies to cope up with stress induced by DEP.

The present study disagrees with that of Ramesh andSaravanan (2008) who have evinced decrease in RBC count during the acute treatment of the fish *Cyprinuscarpio* with chlorpyrifos and have ascribed it to severe anemic state or hemolysing power of toxicant particularly on the red cell membrane.The present result is not in good accord with the findings of Ramesh and Saravanan (2008) who have reported decrease in the HB content of the blood of chloropyrifos treated *Cyprinuscarpio* under acute conditions. They have ascribed it to rapid oxidation of haemoglobin to methaemoglobin or release of O_2radical brought about by the toxic stress of chloropyrifos.

The present findings is in consistent with that of OliveriaRibeiro et al ., (2006) who have observed increase in mean RBCs counts in the fish (*Hopliasmalabaricus)* exposed to methyl mercury. The RBC may also be affected by other pollutants as reported by Allin and Wilson (2000) in *Oncorhynchusmykiss* after an acute exposure to aluminium and by Adhikari et al .,(2004) in *Labeorohita* exposed to sub-lethal level of cypermethrin and carpofuran. In addition, according to Chowdhury et al., (2004), other mechanisms of toxicity may be associated with the O_2^-carrying capacity such as the inhibition of iron absorption and defective iron metabolism shortening the life span in erythrocytes, as observed for cadmium exposure Liu et al., (1999).

Increased MCV evinced in to DEP treated fishes *Oreochromismossambicus*agress with that of OliveriaRibeiro et al ., (2006) who have also observed increase of MCV in *Hopliasmalabaricus* exposed to MeHg and have explained that it could be due to the presence of large amount of older or larger red blood cells as described by Hardig and Hoglund 1983;Adhikari et al ., 2004 also reported effects on MCV values in *Oncorhynchusmykiss*exposed to other pollutants such as pesticides.Twice increase of leucocytes number in the MeHgexposed fish *Hopliasmalabaricus* evinced by OliveriaRibeiro et al., (2006) is in line with the present result. On contrary,Kumar et al ., (2011) have observed significant decline in the blood cell count (RBC ,WBC and Haemoglobin (HB) in endosulfan exposed fish tilapia *Oreochromismossambicus.*

The present observation coincides with that of Carvalho and Fernandes (2006) who have observed increase in RBC count and HB concentration and decreased in MCV at 20^o C (pH 4.8), while at 30^{o}C (pH 4.5) decrease in RBC and HB concentration in copper exposed *Prochilodusscrofa*, Further they have observed (pH 8.0) , RBC were higher and MCH,MCHC were lower than there values found in fish at pH 7. Subsequent exposure to copper reduced RBC and increased MCV and MCH. The present result is well supported by Chokkalingam and Kavitha et al ., (2010) who have evinced increased WBC,MCV and MCH count up to 20th day and 25th,day respectively in arsenate treated fishes and thereafter declined till 35th day when compared to the untreated ones. On contrary to the present result they have noted a slight decrease in MCHC content in arsenate treated fish throughout the study period when compared to the untreated ones.

The present finding disagrees with that of Allin and Wilson (2000) who have observed decreased number of RBC of WBC in *Oncorhynchusmykiss*on exposure to aluminium .The increase in MCV may also result from an increase in immature RBC Carvalho and Fernandes(2006). The significant increase of MCHC values during acute treatment of DEP might be resulted from sphaerocytosis as suggested by Sobecka (2001). In the present study the significant elevation in WBC count during sublethal treatment might have resulted from stimulation of immune system by DEP and to protect the fish against toxicity. Similarly,significant (P<0.001) increase in WBC count was evinced in methyl parathion exposed rats for a period of 4 weeks (5ppm:8.4±1.1;10ppm:11.5±1.8) when compared to the control (5.3±0.4). Leucocytosis observed in the present investigation indicates an immune system to protect the fish against infection that might have caused by chemical and secondary infections. Leucocytes which may be directly proportional to the severity of the causative stress condition may be attributed to an increase in leucocyte mobilisation.

CONCLUSION

The results of the present investigation reveal that under experimental condition, blood parameters of tilapia were sensitive to diethylphthalate exposure. These findings permit us toconclude that DEP is highly toxic to fish. Hence, the presence of DEP in waterways could have adverse impact on the survival of the fish. Therefore it is necessary to monitor, the level of DEP in aquatic environments.

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