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An exposure study of endocrine disruptors on haematology of fresh water fish *Labeo rohita*

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Abstract

Synthetic chemicals released in water bodies resulting in exposure to fresh water organisms and enter in food chain. Phthalates and parabens are those chemicals now being studied for their endocrine disrupting potentials. This study was designed to determine the haematological effects of phthalate, parabens and their mixtures on fresh water and commonly eaten fish *Labeo rohita*. To healthy fish (*Labeo rohita*) of juvenile age, experimental dose of ester of phthalate, paraben and their mixture were given as treatment group for 30 days. Haematological parameters were studied. A significant fluctuation was seen in erythrocytes (0.025±0.005), packed cell volume (0.15±0.05), haemoglobin (0.3±0.1*), platelets (12 ± 2*), mean corpuscular haemoglobin (726.5±623.5), and mean corpuscular haemoglobin concentration (7050±6950) as compared to control. Dimethyl phthalate (DMP) and dibutyl phthalate (DBP) treated fish showed a statically significant decrease in erythrocytes (0.025±0.005*; 0.08±0.07*) and platelets (12 ± 2*; 71±1.5*) as compared to fish of other groups. Fluctuations in blood parameters may lead to further abnormalities in an organism.

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Introduction

In highly populated areas, synthetic or manmade chemicals discharge in huge amounts in rivers or other water bodies in the form of industrial effluents, agricultural and urban settlements emission waste (Frenzilli *et al.*, 2009) and thus results in the contamination of water bodies leading to exposure of most freshwater organisms to these toxic chemicals (Sumpter, 2009). Some of these synthetic chemicals were proved to be endocrine disruptors.

The mode of action of endocrine disruptors in the body is to block hormones and cause disruption in the normal physiological functions. They may cause interference in endocrine system resulting in adverse systemic and immune effects in vertebrates. Phthalates and parabens are included among the many chemicals now being studied for their endocrine disrupting potentials. These have low water solubility and high lipid solubility making way for bioaccumulation in adipose tissue.

Phthalate esters have recently attracted special attention of the scientific community, regulatory agencies and common man as a result of their high production volume, widespread use (Darbre and Harvey, 2008; Sepperumal and Saminathan, 2013).

Estrogenic activity of esters of parabens has also been proved in vitro and in vivo experimental studies (Boberg *et al.*, 2010). Adverse reproductive effects of BuP and PrP have been reported in some animal studies (Oishi, 2002a, b) but not others (Hoberman *et al.*, 2008), with some association with sperm damage (Meeker *et al.*, 2011) and altered thyroid hormones (Koeppe *et al.*, 2013) in humans.

Among the aquatic organisms, fish occupy an important position in the field of aquatic toxicology (Di Giullio and Hinton, 2008).Fish appear to possess the same biochemical pathways to deal with the toxic effects of endogenous and exogenous agents as do mammalian species. Blood is the only tissue that can be removed from an organism, without causing any lethal damage to it and its complete test is possible for diagnosis of disease. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels (Kori-Siakpere and Oboh, 2011). Haematological parameters have been employed in assessing the condition of the fish as they indicate the nutritionalstatus and overall health indication of the fish (Akinrotini *et al.*, 2012). This study aimed to determine the haematological effects of phthalate, parabens and their mixtures on fresh water fish *Labeo rohita*.

Materials and methods

The adverse effect of ester of phthalate and paraben reported in literature. To treat the fish in experimental groups Dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), methyl paraben (MP) and propyl paraben (PP) were chosen.

The healthy fish (*Labeo rohita*) of juvenile age (n= 100) were collected from fish pond. Those fish were kept in aquariums for one week for acclimatization.

Then fish were distributed in 9 groups and kept in 9 different aquariums as shown in Table 1. Water of each aquarium was changed on daily basis. 10 fish were kept in each aquarium. Temperature, pH and electrical conductivity of aquarium water were noted down. Experimental dose of ester of phthalate, paraben and their mixture were given to them as treatment group for 30 days. 10 fishes were kept as their control group. Commercial feed was given to them 2 times daily.

Chemicals

Dimethyl phthalate, diethyl phthalate dibutyl phthalate, methyl paraben and propyl paraben were purchased from Sigma Aldrich. Heparinised syringes and EDTA tubes were also purchased.

Sample collection

After 30 days of treatment, blood samples were taken from the caudal peduncle vein of the fish by using heparinised syringes in EDTA tubes. Tubes were labelled properly.

Blood analysis

Different blood parameters as erythrocytes (RBCs), white blood cells (WBCs), packed cell volume (PCV), haemoglobin (Hb) were evaluated along with red blood cell indices such as Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The concentration of Hb and blood cells count were immediately estimated by using haematology analyser. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using the formulae reported by (Dacie and Lewis, 1991).

Statistical analysis

Data were tabulated and mean standard error of mean or standard deviation of population was calculated. Data was analysed by using SPSS package and the statistical differences were calculated by using one way analysis of variance (ANOVA) at the $P \leq 0.05$ level of significance indicated as superscript * in Table 2. The statistical significance as compared to control was determined by using Tukey HSD Post-hoc Test.

Results and discussion

Haematological parameters study is the easiest and cheapest method of defining the immediate toxic effects of chemicals on fish.

Table 1. Distribution of fish in different groups.

SN	Fish Groups	Chemical of exposure
1.	A	Control
2.	B	Dimethyl phthalate
3.	C	diethyl phthalate
4.	D	dibutyl phthalate
5.	E	methyl paraben
6.	F	propyl paraben
7.	G	DMP+DEP
8.	H	MP+PP
9.	I	DMP+DEP+MP+PP

So this study was designed to observe the effect of different endocrine disruptors and their mixture on the haematological parameters of juvenile *Labeo rohita* fish. Level of analysed blood parameters was reported in Table 2.

The significant decrease in RBC, PCV, Hb and PLT were noted in this study. While MCH and MCHC values showed increase as compared to control.

DMP and DBP treated fish showed a statically significant decrease in RBC and platelets as compared to fish of other groups. A significant fluctuation was seen in platelets of all treated group fish except group G. DBP exposed fish showed an abrupt elevation in MCHC values which was sign of autoimmune haemolytic anaemia, where the Hb more concentrated inside the red cells.

It was supposed that the decrease in Hb% in this study may be due to anaemia caused by subjected toxic chemicals as consequences of decrease rate of production of erythrocytes or heavy loss of these cells during exposure period. Reduced level of RBC is sign of haemolysis or anaemia. Reduction in PCV is also an indicator of anaemia which was also observed in this study. Similar results of significant lessening in RBCs, PCV and Hb were noted by Latif *et al.* (2014) in *Labeo rohita* fish exposed to sub lethal dose of naphthalene, copper sulphate and lead nitrate. And similar increase in MCH, MCV was observed in juvenile *Labeo rohita* fish by Latif *et al.* (2014) due to copper sulphate. Kumar, *et al.* (2011) also observed significant decline in RBC,WBC and Haemoglobin (HB) in endosulfan exposed fish Tilapia. Ahmad *et al.* (2015) also reported diminution in such parameters in *Labeo* fish given heavy metals dose.

Table 2. Haematological parameters of *Labeo rohita* treated with phthalates and parabens esters.

Blood Parameters	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H	Group I	Significance P
RBC×10^6/µl	0.2±0.04	0.025±0.005*	0.19±0.11	0.08±0.07*	0.035±0.015	0.05±0.03	0.12±0.02	0.05±0.03	0.05±0.03	0.0367*
HbmM/l	1.3±0.3	0.3±0.1*	1.1±0.5	1.1±0.3	0.6±0	0.8±0.1	0.75±0.05	0.95±0.25	0.6±0	0.0438*
PCV%	1±0.2	0.1 ±0	1.15±0.65	0.4±0.4	0.15±0.05	0.2±0.1	0.6±0.1	0.25±0.15	0.3±0.2	0.0348*
MCH	64±2	116.5±6.5	62.5±12.5	726.5±623.5	210±90	268.5±181.5	63.5±6.5	250±100	187.5±112.5	0.218
MCHC%	127.5±2.5	300±100	99±21	7050±6950	450±150	566.5±333.5	127±13	500±200	360±240	0.183
MCV%	50±0	41.5±8.5	63±1	76.5±23.5	45±5	43.5±6.5	50±0	50±0	56±6	0.06
PLT	283±133	12 ± 2*	55±13*	71±1.5*	27±14.5*	42±10.5*	87±2.5*	99±44	79±0.5*	0.008*

Values are Mean ± SD, n =3, *Significant at p <0.05, RBC = Red blood cells, Hb= Haemoglobin, MCH= Mean corpuscular volume, PCV%= Packed cell volume, MCHC = Mean corpuscular Haemoglobin concentration, MCV= Mean corpuscular Haemoglobin, PLT= platelets.

But contrary to that an increase in the RBC count, Hb and PCV compared to the control fish was noted insame fish exposed to different concentrations of diazinon (Zubair, 2011) and in fresh water fish treated with sub lethal dose of DEP (Sepperumal and Saminathan, 2013).

As compared to control, increase in MCV and PCV was defined by Sepperumal and Saminathan (2013) in fresh water fish treated with sub lethal dose of DEP which was comparable to this study and explained that it could be due to the presence of large number of older or larger red blood cells.

Conclusion

Fluctuations in haematological parameters were noted due to exposure of selected endocrine disruptors in juvenile fish *Labeo rohita*.

This imbalance in haematology may further lead to abnormality and malfunction in different systems of fish. This fish is commonly used as a winter food in Pakistan and this exposed fish due to contamination of pollutants in water bodies; taken as food, resulting in ultimate disruption in fish eaters.

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