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Research Article

Study of the haematological and biochemical parameters of *Clarias batrachus* (Linn.) exposed to sublethal concentration of dioctyl phthalate

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Abstract: Dioctyl phthalate (DOP) is widely used in plastic, medical devices and personal care product at various concentrations. Therefore a study was designed to determine the acute toxicity of DOP on haematological and biochemical parameters in fish, *Clarias batrachus*. Fishes were exposed to sublethal concentration of DOP 2.9ml/l and the alteration of haematological and biochemical parameters were studied at the end of 30 days. During the above treatment period the parameters like RBC, haemoglobin and Alanine aminotransferase(ALT) level decreased while WBC and serum enzyme like Aspartate aminotransferase(AST); Alkaline phosphatase(ALP) level increased.

Keywords: Sublethal concentration, Dioctyl phthalate, *Clarias batrachus*, Acute toxic

Introduction

Dioctyl phthalate (DOP) is one of the most important phthalate used as plasticizers in poly vinyl chloride, Cosmetics, detergents, Soap, lubricating oils and in some medical devices. DOP is insoluble in water (23-340µg/l) but because of readiness with which it forms colloidal solution, its true solubility in water is to be 26-50µg/l and has a very low volatilization rate (WHO, 2003). Since there is a growing concern that phthalate esters may be a menace to health and to our ecological system. There is a need to review the evidence on the toxicity and possible health threats, which these esters present directly or indirectly to man. DOP enters into the aquatic environment from industries manufacturing plastic products and can expose potential fish and also human health hazards. Fish are widely used to evaluate the health of aquatic system and physiological changes in fishes serve as biomarkers of environmental pollution (Winger *et al.,* 1990). Haematological parameters of fish are closely related to the response of fish to environmental and biological factors (Fernandes and Mazon 2003). Therefore, analysis of blood indices is a valuable guide to assessing the condition of the fish, as it provides a reliable index of their physiological condition, and is important in fish aquaculture (Alyakrinskyaya and Dolgova 1984). A number of haematological indices are used to assess the functional status of the blood stream and have been used as an indicator of metal pollution in aquatic environment (Shah and Altindag 2005). Measurement of blood parameters is commonly used as a diagnostic tool in biomonitoring by which acute and chronic pathophysiological changes attributable to nutrition, water quality and disease are detected (Adams *et al.,* 1996). Fish blood plasma biochemistry can be used to detect the health status of the fish (De Pedro *et al.,* 2005).Thus the present study aims to gain insight into the changes induced in haematological as well as biochemical parameters of *Clarias batrachus* on sublethal exposure to DOP.

Materials and Methods

Collection and maintenance of fish

DOP toxicity was assessed using *C.batrachus* as an aquaculture model. In this experiment we collected 30 mature adult *C. batrachus* obtained from local fish farm, Chaibasa, W. Singhbhum, Jharkhand, India and brought to avoid any injury and disinfected by giving a bath of 2 minute in 0.05% $KMnO_4$ solution thereafter, they were transferred to glass aquarium for two weeks for acclimatization in the laboratory condition. Fish were fed two times daily with earthworms or tubifex or Blood worms. The size of the fish varied from 26.5±5.0cm in standard length and 68.3±2.0g in weight. They were reared in aquaria containing 50 L dechlorinated tap water which was provided with continuous aeration. The water was renewed every other day. The mean temperatures, pH of the water used were 27.2^{0}C and 6.58 respectively.

Dioctyl phthalate (DOP) is a widely used plasticizer. It is slightly yellow transparent oily liquid soluble in ethanol, ether, mineral oil and majority of organic solvent.

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All chemicals were analytical grade. Dioctyl phthalate ($C_{24}H_{38}O_4$, CAS registry number 117-81-7) purchased from National Enterprises, Kolkata. DOP was dissolved in ethanol in ratio 1:1 to prepare the desired degrees of concentration of chemical for toxicity test.

Sublethal Exposure Experiments:
To study the effect of Sub lethal concentration of Dioctyl phthalate on the haematological parameters and biochemical parameters, 30 fishes are divided into 3 groups.

Group A Control Normal diet for 30 days.

Group B Exposure I 1/5th dose (2.9ml/l Dioctyl phthalate +2.9ml/l ethanol) of LC50 of chemical for 30days.)

Haematological Analysis
Blood samples were collected via caudal vein puncture as described by Kori-Siakpere *et al.,* (2005). Fish was held by the person to collect the blood in a slanting and/or vertical position with the ventral part facing the person. Blood samples were collected with sterile 5 ml syringe and 21G needle. The needle was introduced on the ventral mid line between the anal opening and the beginning of the anal fin to assess the caudal vein beneath the vertebral column. The first portion of the collected blood was dispensed into heparinised tubes for haematological analysis. Red blood cells (RBC) and White blood cells (WBC) counts were estimated using a Neübauer haematocytometer with Hendricks (1952) diluting fluid for RBC counts and Shaw (1930) solution for WBC counts as described by Hesser (1960). Haemoglobin (Hb) concentrations were estimated using the Sahli-Hellige haemoglobin method as described by Hesser (1960). Packed cell volume (PCV) was estimated using a micro-haematocrit method as described by Hesser (1960). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were estimated as follows (Stockham and Scott 2002):
MCV = (Packed cell volume as percentage/RBC in millions) ×10 µm3

MCH = (Hb in grams/RBC in millions) ×10 pg
MCHC= (Hb in grams/Packed cell volume) ×100 g per 100mL.

Biochemical Analysis
The second portion of the collected blood was dispensed into non-heparinised tubes. Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum biochemical parameters. The serum biochemical parameters like Aspartate aminotransferase (AST) alanine aminotransferase (ALT) and cholesterol activities were estimated using standard kit (Med source ozone biochemical Pvt. Ltd.

Table 1: Haematological parameters of *Clarias batrachus* exposed to sub lethal dose of DOP.

Haematological Parameters	Control	Group I
Heamoglobin %	9.61±0.36	5.08±0.77
RBC×10⁶/mm³	2.89±0.9	1.7±0.24
WBC×10³/mm³	8.59±0.27	13.9±0.42
PCV (%)	29.5±0.89	18.22±2.30
MCH (pg)	33.37±0.06	32.69±6.79
MCHC (%)	32.79±0.59	27.50±0.7826
MCV (µg)	104.98±2.82	116.68±4.09

Statistical Analysis
All the data obtained in the study was calculated by differences in the means and are presented as mean with standard error of mean.

Results

The changes in haematological and biochemical parameters of the fish *Clarias batrachus* exposed to acute toxicity of DOP are presented in Table 1 and Table2 respectively. During acute (2.9ml/l) treatment RBC, haemoglobin and Alanine aminotransferase (ALT) level decreased while WBC and serum enzyme like Aspartate aminotransferase (AST); Alkaline phosphatase (ALP) level increased in the treated fish. fish exposed to DOP show abnormal behavior changes like agitated or erratic swimming, rapid air gulping, restlessness, sudden quick movement,light pigmentation in the dorsal and lateral part and rapid opercula movement.

Table 2: Biochemical parameters of *Clarias batrachus* exposed to sub lethal dose of DOP.

Biochemical Parameters	Control	Group I
CHOLESTEROL(mg/dl)	210.32± 18.65	224±28.75
ALP(U/L)	24.96±0.70	85±2.32
ALT(U/L)	49.17±4.84	24±2.25
AST(U/L)	155±12.72	525±8.26

AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; ALP – Alkaline phosphatase. Each value is mean ± SD of 3 observations.

Discussion

The impacts of DOP on the haematological and biochemical profile of *Clarias batrachus* have been assessed in the present investigation. The treatment with dioctyl phthalate (DOP) in the present investigation showed remarkable haematological stress markers at higher level of DOP. The decrease in RBC count, haemoglobin concentration and hematocrit level observed in this study may be due to the disruptive action of the DOP. Alterations in the haematological parameters were brought about by DOP as a hemolytic condition due to increased lipid per oxidation of RBC. Our results are in line with those found by Arun *et al.,* (1999) in RBC in vitro. The present study agrees with that of Ramesh and Saravanan (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. Our studies provide

evidence that DOP effect on erythrocyte hemolysis. Increase in WBC evinced in this study agrees with that of Joshi et al., (2002), who have observed increase in mean WBC count in fish *Clarias batrachus* exposed to lindane and malathion. In the present investigation, significant increase in mean WBC count could be attributed to hypersensitivity of leucocytes to DOP due to immunological reaction to produce antibodies to cope up with stress induced by DOP. The significant elevation in WBC count during sub lethal treatment might have resulted from stimulation of immune system by DOP and to protect the fish against toxicity. Haematological findings after exposure to DOP revealed toxic manifestations with evident effects after sublethal exposure. The alterations of blood parameters were attributed to a decrease in the erythrocyte count indicate the developing anemia. The anemia could be due to the destruction of RBC production which triggered by the influx of DOP. The increase of WBC shows that, a typical defensive response of the fish against a toxic invasion.

The results of the biochemical profile of *Clarias batrachus* (Table 2) are used as a tool to assess the health status of fishes. Through biochemical constituents of the fish blood, the metabolic disturbances of fishes could easily be assessed. Increased significant activities of serum enzymes (AST and ALP) in the treated fish also resulted from cellular damage in these fish, which might have arisen from the toxic effect of the DOP. This is because serum enzymes are cytoplasmic in nature and are only released into blood circulation after cellular damage (Sallie *et al.,* 1991; Palanivelu *et al.,* 2005). Increased liver enzyme activity would result in leakage of these enzymes into the serum resulting in increase in the serum level.

Conclusion

It can be concluded that DOP was moderly toxic to fish and causes haematological and biochemical changes in fish. *Clarias batrachus* are more susceptible to pollutant; so their use on/near fish farm or in area close to aquatic environment could have adverse impact on the survival of the fish. Therefore it is necessary to monitor, the level of DOP in aquatic environments.

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