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THE IMPACT OF DIETHYL PTHALATE ON THE BIOCHEMICAL PARAMETERS IN *CHANNA PUNCTATUS*

KEYWORDS

Channa punctatus, Diethyl phthalate, physiological, biochemical and haematological parameters.

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ABSTRACT The physiological, biochemical and haematological changes in Channa punctatus after exposure to sublethal concentra tions of the Diethyl phthalate have been investigated. The toxicity of the five-ppm diethyl phthalate with respect to behaviour and haematological parameters of the fish C. punctatus has been studied. Red blood cell (RBC) count and haemoglubin (Hb) content were decreased 0.9% with the increasing concentrations of the diethyl phthalate. However, the white blood cell (WBC) count was increased 1% with increasing concentrations of the diethyl phthalate. A dissimilar relationship was established with respect to RBC and WBC. The constant increase in the differential count clearly indicates that the heavy metal stress certainly stimulate the white blood cells to produce more at all times of exposure. Biochemical changes of protein, lipid and carbohydrate were recorded. Also, such fish when consumed as food leads to the deposition of the heavy metal in the soft tissues of the human body leading to exposure to a health effects.

INTRODUCTION

Water harbours all types of biotic and abiotic components including chemicals essential for life processes. The effects of heavy metals and trace elements in water have been well known since the episodes of the Minimata and Nigata bay incidents and with the existence of itaital disease caused due to mercury pollution in Japan. Because of these disasters the study of toxicity of heavy metal to the aquatic life is very much required. Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. Heavy metals are dangerous because they tend to bioaccumulation. As trace elements, some heavy metals like copper, zinc are essential to maintain the metabolism of the human body. Copper has been used to control fungal diseases in plants. High concentrations of this heavy metal were detected in some aquatic ecosystems, collecting runoff water and it is also highly concentrated in ground water[1]. There are also, anthropogenic sources of environmental contamination by copper including mining, smelting, foundries, municipal waste incinerators, burning of coal for power generation and a variety of copper based products in building and construction. Cadmium (Cd) is a well-known heavy metal toxicant with a specific gravity 8.65 times greater than water. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. The target organs for Cd toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones.

The toxicology of diethylphthalate has been the subject of extensive study. With other phthalates, it shares the characteristic of being among the least toxic of substances in industrial use. As the discussion in this section will indicate, human or clinical studies reporting serious direct physiological insult as a result of diethylphthalate exposure show only rare, transient, and mostly minor occurrences in high dose situations or with sensitive individuals. These include irritation from heated diethylphthalate, central nervous system depression after heavy exposure, along with rare dermal sensitivity in individuals with a pre-disposition for dermal sensitization.

Channa punctatus is one of the most preferred edible species in India where the present study was carried out. However, its performance in terms of growth is slower compared to other species in the multispecies culture system. The most of synthetic organic pesticides of organophosphates, carbomates and organochlorides are extremely toxic to non-target species of freshwater fauna, which damage the population dynamics, complex food-web and food web energetic [2]. Fishes are particularly very sensitive to the water contamination. Hence, pollutants such as insecticides, herbicides may significantly affect some physiological and biochemical processes when they enter into the organs of fishes [3]. More over the insecticides mainly impacts on liver of fish and also decline glycogen content in liver and the intestine of *Ophiocephalus punctatus* exposed to sub lethal concentration of cypermethrin, these values of glycogen showed disturbance in carbohydrate metabolism due to toxic stress[4]. The mode of action of toxicants and causes for death of poisoned aquatic animal is better understood from biochemical investigations besides mortality studies. Several workers have investigated the impact of various heavy metals on biochemical constituents of various aquatic organisms[5]. Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution. The present study aims to document the toxicity of diethyl phthalate is on biochemical and haematological parameter of *Channa punctatus.*

MATERIALS AND METHODS

For the experimental purpose, the healthy live *Channa punctatus* were collected from the local fish farm at Orathanadu. The collected fish were brought to the laboratory without injury in order to avoid dermal infection and they were made to acclimate themselves to the conditions prevailing in the lab for about a week. *Channa punctatus* were fed with groundnut oil cake, coconut oil cake, rice bran; they were given feeds for 7 to 15 h. The food found unused by them was cleared periodically from that tank, during the acclimation period. The chlorine free water was changed daily. The feeding was stopped one day prior to the experiment.

In a preliminary study the toxic and sublethal levels of 2 to 20 ppm diethyl phthalate were found out for 96 h exposure. The fish were observed for behavioural changes and mortality at 96 hours and 30 days of exposure were recorded and tabulated employing each of the two test media. The 15 ppm concentration at which 100 percent mortality was observed within 96 hours was considered as the lethal concentration (96 h LC_{100}) and the 5 ppm concentration at which 100 percent survival was observed by the end of 96 hours was considered

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as the sublethal concentration (96 h LC_0). The LC_{50}, the lethal concentration, which kills 50% of individuals at 96 h exposure, was found out and the data obtained were tabulated. For this present study diethyl phthalate was taken and this was prepared at different concentrations.

The experiments were carried out in such a manner that the *C. punctatus* were subjected to 2 to 20 ppm different sublethal concentrations in order to assess the effect of diethyl phthalate on selected biochemical and haematological parameters. Before the actual starting of the experiments, the test fishes were divided into 3 groups of the same weight one control and two experimental. They were selected from the stock tank and transferred into the test chamber with test solution of various concentrations of diethyl phthalate viz., 5 and 10ppm. Each group consisted of 10 fish as per aquarium. These groups were actually subjected to both short (96 h) and long term (30 days) exposure periods. Both long term and short tem exposure were given to all the groups involving the 5 & 10 ppm concentrations of diethyl phthalate. A control third group (diethyl phthalate free water) was also maintained in the above said manner. After the exposure period was over, fish was taken out and sacrificed for the analysis of selected haematological parameters viz., RBC, WBC, Hb, MCV, MCHC and MCH with respect to short term and long term exposure periods of fish to the different concentrations of diethyl phthalate. After 30th day analysis of biochemical and enzymes was estimated in muscle of fish. The estimation of glucose[6], protein [7], Alanine transaminase and Aspartate transaminase [8], Lipase [9] were estimated.

RESULTS

Survival Capacity: The study recorded that 96 h LC_{50} value of diethyl phthalate was 15 ppm. Percentage of mortality of *Channa punctatus* is varied with effect of diethyl phthalate concentrations and exposure time. The mortality of fish was increased with increasing concentrations of diethyl phthalate. No mortality was observed up to 10 ppm.

Haematological parameters: With respect to haematology, the study illustrative of the fact that there was a gradual decrease in the haematological parameters such as RBC number, Hb content and gradual increase in WBC number, such was actually caused by the use of the chemicals. The diethyl phthalate shows significant concentration dependent. The reduction of red blood cells was noticed at lower concentration and also at short term (Acute) exposures i.e. in 5 and 10 ppm for 96 h. The same individual were treated at a higher a concentration of 5 and 10 ppm even though there was a short term exposure results a considerable number of decreased RBC. At the same times for a long period, the maximum reduction was observed.

It is evident that the normal blood parameter values of RBC count was 2.81 X 10^6/cmm, WBC 9641 X 10^3/cmm, Hb 10.41 g/100 ml; PCV 30.41%; MCV 108.58 ulX10^9; MCH 37.15 P.gm and MCHC 34.22%. The RBC counts found in the 5 ppm diethyl phthalate treated fish after an exposure of 96 h was 2.71 X 10^6/cmm. Later when the fish was introduced at high concentration at 10 ppm, its count was 2.61X10^6/cmm with a decrease of 7.15%. When the fish was introduced of choronic exposure period at higher concentration of 10 ppm the RBC count was 2.56 x 10^6 cmm with a decrease 8.93%. Likewise, the same results are also true for haemoglobin contents. For a prolonged exposure period of different concentrations of diethyl phthalate medium, the Hb content was decreased in *C. punctatus* by 6.8% at low concentration and by 9.7% at higher concentration. During the experiments, white blood cell count (WBC) increased more and more at prolonged exposure period. The consistent increase in the WBC indicates that the chemicals stress certainly stimulates the production of more WBC's at all time of longer exposures.

There are variations in the values of MCV, MCH and MCHC when the fish are exposed to 5 and 10 ppm of diethyl phthalate for 96 h and 30 days (Tables 1 & 2). It has been suggested that enumeration of different cell ratio count provides a useful diagnostic procedure to assess physiological stress in the fish as well as the water quality. There are variations in the values of enzymes and biochemical content when the fish are exposed to 5 and 10 ppm of diethyl phthalate for 96 h and 30 days. The biochemical and enzymes results presented here have clearly demonstrated that the elevated chemical concentrations for diethyl phthalate substances (Table 3).

DISCUSSION

Chemicals are present in aquatic environment from mining activities and industries that use these metals in various processes[10]. Aquatic ecosystems polluted with chemicals and heavy metals, may therefore threaten human nutrition and health directly. Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution. The survival of aquatic animals depends on not only the biological state of the animals and physico-chemical characteristics of water but also on kind, toxicity, type and time of exposure to the toxicant. In the present study, the mortality increased with an increase in concentration of diethyl phthalate and also the duration of the exposure. Table 1 depicts the percentage mortality for different exposure periods at different concentrations of diethyl phthalate. LC_{50} value of diethyl phthalate for the fish *Channa punctatus* was determined.

A capacity of diethyl phthalate destruction fish and aquatic animals is largely a function of its toxicity, exposure time, does rate and persistence in the environment. Immediately after transfer to the test solution, *C. punctatus* became hypersensitive and showed a rapid of opercular movements accompanied by occasional gulping of air. The higher concentration, the more pronounced was this behaviour. After several minutes of exposure the individual lost their equilibrium. They were floated with complete cessation of movements and finally dead.

The haematology of fishes has gained recognition, as an applied science. Haematology tests have become important diagnostic tools in medicine. Recent studies have shown that the haematological parameters may be equally valuable, in indicating the disease or the stress in the fish. The composition of blood of fishes varies with the changing conditions of the environment and responds immediately to any change in water quality because of intimate contact. Out of varied haematological parameters differential red blood cells counts are of immense physiopathological importance. In the present investigation, an attempt has been made to elucidate the effects of diethyl phthalate with different sublethal concentration on certain physiological properties of the blood of *C. punctatus. Channa punctatus* exposed to sublethal concentrations of diethyl phthalate resulted in a significant decrease in RBC's count leading to anaemia as a result of inhibition of erythropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in haemopoietic organs. Natarajan[11] reported a reduction in Hb content. RBC count and PCV values resulting in hypochronic anaemia due to deficiency of iron and decreased utilization for Hb synthesis.

The anaemic condition recorded in the present study could be due to the destruction of mature RBC or inhibition of erythrocyte production. Such decreases in RBC and anaemic suspense have been observed [12] in *Sarotherodon mossambius* after exposure to lethal concentration of submithion. Chouhan [13] in *Puntius ticto* treated with herbicide. Lal [14] in *H. fossilis* exposed to Malathion. It is evident that in the present study the reduction in number of RBC and Hb content and decrease in MCV values might have caused microcytic anemia as suggested in *Glossogobicus giuris* after exposure to sublethal concentration of Malathion. Baskaran[15] have opined that a reduction in the number of RBC and Hb content with an increase in MCV and MCHC values might cause macrocytic anaemia in *Oreochromis mossambicus.* Since the Hb and RBCs are oxygen-

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carrying devices, the quantitative decrease in their levels might have led to the rearrangement of the oxidative metabolism with a concomitant decrease in the tissues of respiratory potential.

The fish facing asphyxia of undergoing physical exercise and facing hypoxic stress required increased amount of Hb to cope with the decreased oxygen availability. This consequently is partially achieved by immediately releasing mire and more cells, which also bring more erythroblasts in circulation. In the present study also, the RBC and Hb content are more or less the same due to the tolerance to short term exposure, at longer exposure period, depletion or reduction of RBC numbers and Hb content was obtained.

The long- term exposure to diethyl phthalate treated fish reduced the red blood cell count and haemoglobin value. This indicates that he high doses of diethyl phthalate produce anaerobic condition and limits the oxygen carrying capacity and there by decrease the mobility. Most of the blood corpuscles were very thin after long-term exposure and the hypochromic cells naturally contained the decreased concentration of haemoglobin. Total leucocytes count showed an increase in their number in the higher concentrations, but at lower concentration, there is a gradual increase in their number. In the present investigation at higher concentration and longer exposure period produces an increase in total WBC and MCHC count as in *G. hiuris* suggested in *Oreochromis mossambicus* after exposure to malathin and Ekalak Ec-25 respectively. Achuttan Nair[16] suggested that this leucocytosis was the result of direct stimulation of the immunological defenses due to the presence of toxic substance or may be associated by induced tissue damage. A linear relationship was established with respect to diethyl phthalate and total leucocytes. The constant increase in the differential count clearly indicates that the copper stress certainly stimulate the white blood cells to produce more at all times of exposure. It has been suggested that the enumeration of differential cell ratio counts provide of useful diagnostic procedure to assess the physiological stress in the fish.

A significant hyperglycaemia was also recorded after exposure to this wastewater e.g. control fish had a mean plasma glucose of 56.81 mg/100 cm3 while the-treated fish exhibited an increase in the levels of plasma glucose to 65.31 and 81.01 mg/100 cm3, respectively. This means that the fish were subjected to some sort of hypertoxic stress[17]. In this study, tissue biochemicals were generally influenced by this chemical that may be attributed to the relative changes in the mobilization of protein and carbohydrates. Changes in the biochemical concentrations may be a result of increased production of chemical, which is a sequestering agent. On the other hand, the elevation of enzymes that runs parallel to a decrease in muscle biochemical content may be on indication of a gluconeogenetic response. This additional source of biochemical may support the fish with the required energy highly demanded to manage with the presence of a potentially harmful substances such as diethyl phthalate.

Table 1. Effect **of sublethal concentration at 5 ppm of diethyl phthalate on selected haematological parameters in *Channa punctatus***

S. No	Blood parameters	Control	Acute 96 h exposure	Chronic 21 days exposure
1	RBC X 10^6/cmm	2.81 ± 0.52	2.71 ± 0.75	2.61 ± 0.59
2	WBC X10^3/cmm	9641 ± 0.12	9791 ± 0.85	9796 ± 0.73
3	Hb g / 100ml	10.41 ± 0.11	10.36 ± 0.76	9.71 ± 0.55
4	PCV %	30.41 ± 0.72	29.51 ± 0.76	28.61 ± 0.85
5	MCV µl X 10^9	108.58 ± 0.12	109.26 ± 0.85	110.01 ± 0.85
6	MCH (Pg)	37.15 ± 0.72	38.34 ± 0.29	37.31 ± 0.11
7	MCHC (%)	34.22 ± 0.75	35.09 ± 0.23	33.92 ± 0.35

Table 2. Effect **of sublethal concentration at 10 ppm of diethyl phthalate on selected haematological parameters in *Channa punctatus***

S. No	Blood parameters	Control	Acute 96 h exposure	Chronic 21 days exposure
1	RBC X 106/cmm	2.81 + 0.52	2.66 + 0.75	2.56 + 0.59
2	WBC X 103/cmm	9641 + 0.12	9991 + 0.85	10111 + 0.73
3	Hb g / 100ml	10.41 + 0.11	10.31 + 0.76	9.41 + 0.55
4	PCV %	30.41 + 0.72	29.11 + 0.76	23.11 + 0.85
5	MCV l X 109	108.58 + 0.12	109.82 + 0.85	110.18 + 0.85
6	MCH (P g)	37.15 + 0.72	38.87 + 0.29	36.97 + 0.11
7	MCHC (%)	34.22 + 0.75	35.38 + 0.23	33.46 + 0.35

Table 3: Effect **of sublethal concentration at 5 ppm of diethyl phthalate on selected biochemical and enzyme parameters in *Channa punctatus***

S. No	Parameters	Control	5 ppm diethyl phthalate		10 ppm diethyl phthalate	
			Acute 96 h exposure	Chronic 30 days exposure	Acute 96 h exposure	Chronic 30 days exposure
1	Total protein	24.8	22.4	15.8	16.3	12.9
2	Carbohydrate	10.6	10.3	9.1	9.2	8.3
3	Phosphatase	18.8	16.6	15.9	16.1	14.5
4	Protease	6.9	5.8	5.6	5.5	4.6
5	Lipase	1.09	1.6	1.1	1.4	0.96
6	AST	4.53	3.9	3.3	3.6	2.37
7	ALT	0.45	0.56	0.75	0.69	0.97

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