Immunotoxic Stress of Cadmium Exposure in Fresh Water Air Breathing Fresh Water Teleost *Heteropneustes fossilis* (Bloch)

Verma,Vinod Kumar, Department Of Life Sciences And Biotechnology, C.S.J.M. University Kanpur (U.P.)

Abstract

Cadmium has no evidence that it is either biologically necessary or beneficial elements but it is used in many products, including batteries, pigments, metal coatings, and plastics, and it is found in cigarette smoke. At low concentrations it is toxic to all life, including microorganisms, plants, fish, frogs, birds, and mammals. In the present investigation the exposure suppressed the immune system of fresh water teleost *Heteropneustes fossilis* by changing the numbers of differential leucocyte counts (DLC) at different concentrations (Group B; 90 ppm and Group C;180ppm) at different time period (7, 14, 21, and 28 days) as compare to control group (Group A). In differential leucocyte counts the lymphocytes were reduced 81% (Control group) to 63% and 64% in the exposed groups at maximum exposure period (28 days). While the neutrophils were increased maximum at 28 days exposure period at 180 ppm of cadmium. Monocytes counts showed increased in previous results and further deceased in later results from 4% (Control group) to 3% in Group C at 28 days exposure period. The eosinophils percentages showed the allergic response in their values also, it was increased maximum 7% in Group B at 28days exposure period as compare to control group which was 2. Basophils were also increased from 1% (Control group) to 3% in Group B at 14 days exposure period. So the present results indicate that the alteration in DLC values represents that heavy metal cadmium can suppress the immunity of fish after exposure.

Keywords: DLC,Cadmium, *Heteropneustes fossilis*

Introduction

Anthropogenic activities and modern industrialization are increasing the heavy metals in the environment which are causing adverse effects on human health. Thousands of people are being affected globally due to contaminated water, air, soil and food. Concentration of heavy metals in water resources, air, and food is assessed with this regard (Mousavi *et al.*, 2013; Ghorani-Azam *et al.*, 2016; Luo *et al.*, 2020). Toxic effects of heavy metals affect different body organs like immune system, birth defects, nervous system disorders, gastrointestinal and kidney dysfunction, and cancer are examples of the acute toxicity (Fernandes Azevedo *et al.*, 2012; Cobbina *et al.*, 2015;; Gazwi *et al.*, 2020). Cadmium is a non-essential heavy metal and highly toxic to all living organism including man. People who work with this metal are reportedly exposed to both acute and chronic Cd toxicity. Pneumonitis, pulmonary edoema, gastro-enteric discomfort, pain, and prostration can all occur with acute Cd poisoning. According to Jiang et al. (2015), Richter et al. (2017), and Cao et al. (2018), chronic poisoning is characterised by renal tubular failure, proteinuria, osteomalacia, anaemia, liver damage, and hypertension. Haematological indices have been acknowledged as useful tools for assessing the physiological status of fish, the changes in which depend on the species, age, sexual maturity cycle, and diseases of the fish. In 2009, Vinodhini and Narayanan; in 2012, Summarwar and Verma; in 2014, Soundararajan et al.; and in, Mallesh et al. 2015. Gabriel *et al*., 2004 reported that cadmium exposure leads to accumulation in fish tissue and after some time causes many serious disorders in metabolism, changes in behavior, abnormalities in movement, and effects on blood in african catfish *C. geriepinus.* Cadmium toxicity to freshwater fishes has been reported like anaemic condition was reported in Channa punctatus and *Oreochromis mossambicus* exposed to different doses of Cadmium by Karuppasamy et al 2004 and Wu *et al.* 2006. Schuwerack,*et al.* (2003) were reported haemological stress like leukocytosis, neutrophilia and eosinophilia in Cyprinus carpio exposed to sublethal concentrations of cadmium. Naglaa *et al.* (2019) also observed the significant changes in eosinophils counts after exposure with cd in african cat fish. Effects of cadmium exposure resulted as an immunosuppressant in common carp (*C. carpio*), *Oreochromis aureus*, *O. niloticus* and *Ictalurus melas* by Patrick, *et al.* (1993), Viola *et al.* (1996) and Małgorzata, (2005). In the present investigation was aimed to evaluate the immunotoxic responses by evaluating leucocytes profile of fresh water air breathing fresh water teleost *Heteropneustes*

fossilis (Bloch) through cadmium exposure

Material & Methods

For investigations on immunotoxicity, catfishes called *Heteropneustes fossilis* (10–20 gm) were employed. Fish for the experiment were sourced from Bithur fish Market in the Kanpur district. The healthy fish were acclimated to the environment of the lab. For each experimental group, a total of 64 fishes of various sizes and body weights were chosen. For the trials, a control and two chronic levels of cadmium (Cd) were planned, as shown in table 1

.Table-1.: Concentration of Cd in different groups

No.	Group	Exposure to Cd (Cadmium)	Concentration /ppm
1	A	Control group.	0.00
2	B	Low concentration	90
3	C	Intermediate concentration	180

In order to conduct the haematological study, thin blood smears were produced on clean slides, rapidly air-dried, and then preserved in methanol for 2–5 minutes. The fish's caudal vein was used to draw blood for the investigation. Giemsa (Qualigens) was then used to stain the blood films in phosphate buffer solution (pH 6.8). Using a gentle brush, the solutions were evenly combined in a ratio of 1:7, stained for 40 minutes, and then rinsed under running water for 2-4 minutes to remove any remaining stain. The slides were then left to dry while standing on end. Slides that were positive and dry were mounted in DPX. Using a manual or computerised blood cell counter, the cells were counted in a strip running the entire length of the film under a high power microscope. Leucocytes were counted and classified as small lymphocytes, large lymphocytes, monocytes, neutrophils, eosinophils, and basophils based on the morphological features of the nucleus after at least 100 cells had been counted (Wintrobe 1981).

Results

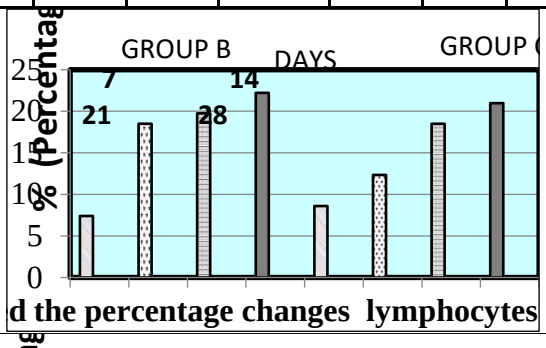
White blood cells are a major component of the body's immune system which are composed of and granulocytes (lymphocytes and monocytes) (Fig.1-A & B). While non-granulocytes (neutrophils, eosinophils, and basophils). (Fig.1- C, D & E) We examined the DLC in the blood of fish after exposure of fish to cadmium at different concentrations and time exposure. Granulocyte, the lymphocyte showed maximum fall 63% with 22.22% percentage decreased in minimum concentration and higher exposure period (180ppm of Cd; 28 days) as compare to control values (81%) (Table-2; Graph-1). Monocytes were significantly increased in initial investigation but later it showed decreased in their values, maximum percentage increase was 75% and as compare to control groups at 90 ppm at 21 days exposure period of cadmium (Table-2; Graph-2). The values of neutrophils were significantly increased 125% at maximum concentration (180 ppm of cd) during maximum exposure period (28 days) (Table-2; Graph-3). Eosinophils were affected maximum with 250% of increased at 90ppm of cd and 28 days exposure period. (Table-2; Graph-4), While the basophils were not significantly effected in all exposure time and concentrations and showed some fluctuation in their results (Table-2; Graph-5).

Table: 2- Shows the changes in DLC in the blood of *Heteropneustes fossilis* after exposure with Cd.

D.L.C.	Group A (Control)	Group B (90 ppm Zn)				Group C (180 ppm Cd)			
		7 Days	14 Days	21 Days	28 Days	7 Days	14 Days	21 Days	28 Days
Lymphocytes	81	75	66	65	63	74	72	66	64
Monocytes	04	05	05	07	04	05	04	04	03

Neutrophils	12	18	23	23	26	18	21	26	27
Eosinophils	02	01	03	05	07	03	03	04	04
Basophils	01	01	03	00	00	00	00	00	02

7 14 21 28



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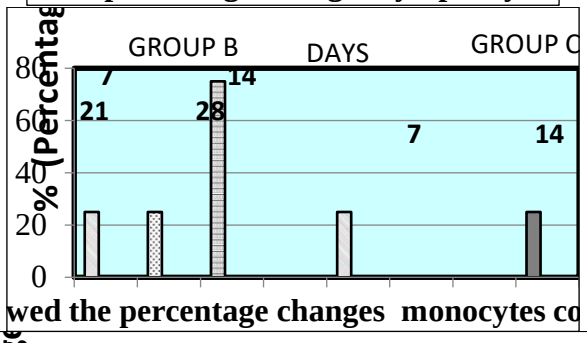

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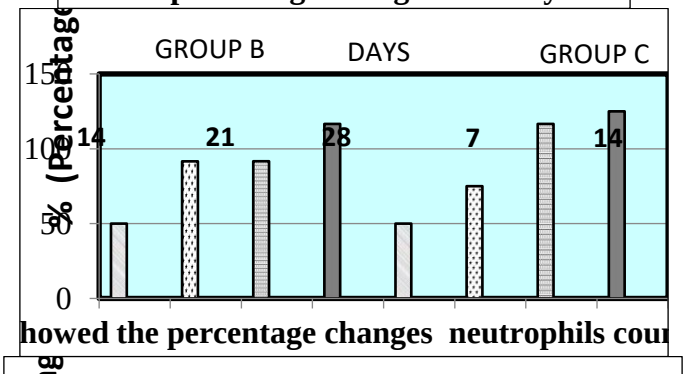

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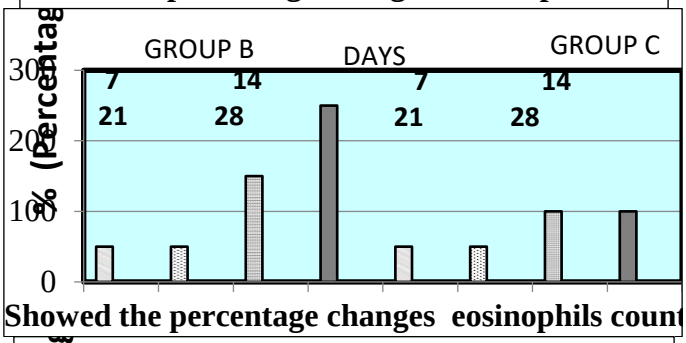
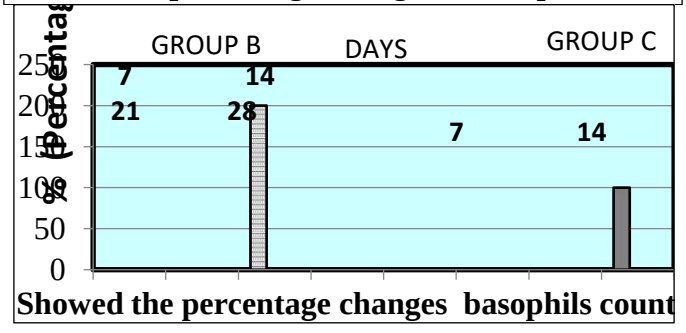


Showed the percentage changes eosinophils count



Showed the percentage changes basophils count

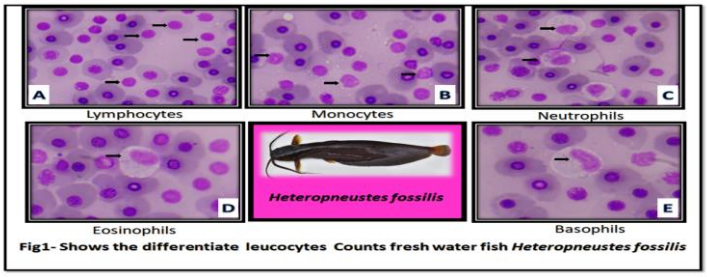


Fig1- Shows the differentiate leucocytes Counts fresh water fish *Heteropneustes fossilis*

Discussion

Toxic substance when introduced into the aquatic environment shows physico-chemical changes in water and affects the organisms of physiological processes by changing through absorption, transport, distribution, metabolic transformation, accumulation, excretion and intoxication. (Tao *et al.*, 1999). Cadmium has been recognized as one of the most deleterious nonessential heavy metals which are used in industries all over the world (Stoeppler, 1991). It is most toxic metals in the aquatic environment which possesses high toxicity if allowed to accumulate in metabolically active tissues. (Sorensen 1991). One trustworthy biomonitoring tool for determining the amounts of heavy metal pollution in the aquatic environment is fish. The underwater ecosystem's current state of nursery water can be predicted by analysing the biological characteristics of fish (Rashed, 2001; Lamas *et al.*, 2007; Mohamed and Osman, 2014; Authman *et al.*, 2015).

The haematological parameter in fish can significantly change in response to chemical stressors. However, there alterations are nonspecific to a wide range of substance. As a result, they make an excellent tool for in situ or in vitro toxicological research (Al-Akel and Shamsi 2000; Cicero, Barrella, and Rotundo 2014; Hamidipoor *et al.* 2015; Seriani, Franca, *et al.* 2015) Fish health can be determined by leukocyte counts, and a decrease in lymphocytes is typically seen as a stress reaction (Chen *et al.* 2002). Lemly (2002) also noted variations in the total number of leukocytes and thrombocytes in fish from polluted settings. Hence, fish blood serves as a reliable non-destructive biomarker for evaluating water pollution. In this present investigation the signification variation in the differentiate leucocyte counts was observed in Indian fresh water fish *H. fossilis* due to the different concentrations of cd at 7, 14, 21, and 28 days. Lynphocytes were reduced 63% maximum in Group B during 28 days exposure period as compare to control group (84%). Viale and Calamari (1984) reported that the cd slightly reduced the humoral immune system which increase mortality in catfish, Ictalurus melas, while Witeska *et al.* (2006) identified that lymphocyte were slightly increased and neutrophil counts were decrease in their numbers after exposure with cd in fresh water fish *Tinca tinca. According to* Singh *et al.* (2008) investigation, Channa punctatus subjected to heavy metals would result in an increase in neutrophils. Akbari (2010) reported that cadmium, elevated the number of lymphocytes while decreased the number of monocytes and neutrophils in *rutilus rutilus caspicus* exposed to sublethal concentrations of cadmium for a period of 4 days while other author Khalesi *et al.* (2016) observed in their study, the number of lymphocytes remained unchanged and neutrophils were significantly increase with cadmium exposure as compared to the control.

Naglaa *et al. (*2019) also identified alteration in leucocytes numbers could be attributed by stimulation of the immune system due to oxidative stress caused by cadmium in *Clarias gariepinus* resulted variation in lymphocytes, neutrophils and lymphocytes numbers. The decreased values of monocytes and lymphocytes in treated fish exposed to thiomethoxamn might be due to the weaker immunological response of fresh water fish labeo rohita in Pakistan. Jabeen *et al.* (2021) observed a prominent variation in the quantity of neutrophils increased, a significant decreased in lymphocytes and monocytes in treated groups as compared to non-treated group.

Conclusion

In conclusion, fish that have been obtained cadmium contaminated water by urban as well as industrial effluent have blood collected from exposed fish, with higher percentages change of

lymphocytes, neutrophils, monocytes, eosinophils and and lower levels of basophils. It appears that the heavy metal cadmium causes of these modifications. Differential WBC counting in particular, as well as haematological characteristics in general, were thought to be reliable indicators of exposure to environmental stressors.

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