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Immunological Effect of Cadmium in *Heteropneustes fossilis* Bloch.

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Abstract: The present study was carried out to evaluate the effect of short-term exposure to high concentration of cadmium in fish blood. *Heteropneustes fossilis* were subjected for 3,6,12,24,48,72 and 96 hours to10 mg/l of cadmium. Blood was sampled immediately after the end each exposure period. In the white blood cell system a decrease in total leucocytic count took place caused by a considerable drop in lymphocyte count. The percentage of neutrophils increased. However, metal exposure showed a decrease in reactive oxygen radical production. All these alterations indicated short-term exposure to cadmium in the white blood cell system produces a considerable immunosuppressive effect.

Key words: Cadmium toxicity · White blood cell · *Heteropneustes fossilis*

INTRODUCTION

The aquatic ecosystems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities [1-6]. Heavy metal contamination may have gross biological impact on aquatic organisms [7-9] including fishes which are the inhabitants and cannot escape from the detrimental effects of these pollutants [10-12]. The heavy metals such as lead, mercury and cadmium are known to cause public health hazards [13]. Among heavy metals, cadmium has been chosen for the present study because it is a wide spread metal pollutant of high toxicity not only to warm blooded vertebrates, but also to aquatic animals including fishes [14]. The largest source of cadmium release to the general environment is the burning of fossil fuels such as coal or oil or incineration of municipal waste materials [15- 18]. Cadmium may also escape into the air, from zinc, lead or copper smelter [19]. It can enter waster from disposal of wastewater from households or industries [20]. Fertilizers often contain some cadmium [21]. In this context, the present investigation has been designed to study the immunological effect of cadmium in catfish *Heteropneustes fossilis* exposed to high concentration of cadmium chloride.

MATERIALS AND METHODS

Irrespective of the sex, healthy specimens from *H. fossilis* of 36-38 g body weight and 18-20 cm length belonging to a single population were collected locally and were confined to large plastic aquaria bearing tap water for 30 days in the laboratory for acclimation. They were fed with mined goat liver on everyday d for 3 hrs h before the renewal of the medium. Water was renewed after every 24 h with routine cleaning of the aquaria leaving no faecal matter, dead fish if any or unconsumed food. Seven groups of 10 fish each were exposed separately to cadmium chloride 10 mg/l solution prepared in tap water. The experimental medium was prepared by dissolving cadmium chloride 10 mg/l in tap water having dissolved oxygen 6 ppm, pH 7.5, water hardness 40.44 mgL^{-1} and water temperature 28±2°C [22]. Each group was exposed to 50 L of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaria containing 50 L tap water (without the addition of cadmium chloride) as controls. Feeding was not allowed in the experimental as well as control groups. After the expiry of 3, 6, 12, 24, 48, 72 and 96 hours of exposure, fish from each group were sampled and the blood was collected from live fish by heart puncture from each group. The blood of control fish was also sampled immediately. The following parameters were evaluated: white blood cell counts (WBC) and number of lymphocytes, neutrophils and thrombocytes calculated basing on the blood smear analysis and the reactive oxygen radical production by phagocytes as NBT reduction test. The data obtained were subjected to standard statistical analysis based on random sampling. Duncan's multiple range test [23] was performed. Values of $p < 0.05$ were considered statistically significant.

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Table 1: Showing the percentages of WBC counts, number of Lymphocytes, number of Neutrophils, number of Thrombocytes and NBT in control *H. fossilis* and after 10 mg/l cadmium chloride treatment for 3, 6, 12, 24, 48, 72 and 96 hours

	Experimental							
Parameters (in percentage)	Control	3h	6h	12h	24h	48h	72h	96h
WBC counts	100	82	64*	105	66*	53*	47*	32*
Lymphocytes	100	89	85	80*	76*	68*	65*	51*
Neutrophils	100	500*	1100*	1150*	1200*	600*	400*	300*
Thrombocytes	100	42*	47*	49*	55*	43*	40*	42*
NBT	100	95	106	87	80*	74*	68*	42*

Note: Experimental values are compared with control values; Based on Duncan's Multiple Range test.

*$p < 0.05$; Average of control value is considered as 100%

RESULTS AND DISCUSSION

A decrease in WBC was observed at all exposure periods when comparing to the control groups (Table 1). It is known that cortisol secreted during stress reaction shortens the life span of lymphocytes and promotes their apoptosis [24,25] and reduces their proliferation [26], so a decrease in lymphocyte count, as well as in their activity are often observed effects of stress, irrespectively of the stressing agent. Siwicki and Studnicka [27] observed a 20% drop in WBC of common carp subjected to a 30 minute chemical stress with 1% solution of trichlorfon, while Elsaesser and Clem [28] reported a 50% decrease in WBC and a considerable drop in lymphocyte percentage in *Ictalurus punctatus* after a 15 minute transport. Leukopenia is also a common reaction of fish to metal exposure. According to Dick and Dixon [29] and Vosyliene [30], a decrease in leukocyte count following an acute metal exposure is rather a nonspecific stress reaction caused by a metal induced stimulation of kidney chromaffine cells and cortisol secretion than a specific toxic action of metals upon the cells. However, the results of the *in vitro* exposure of lymphocytes indicated that both reactions may be involved [31-34]. An increase in neutrophil count occurred in the present study. This may probably due to cortisol induced this hormone prevents neutrophil migration into the tissues inhibiting inflammative response and extends their life span by inhibition of apoptosis [24]. A four-fold increase in neutrophil percentage in fish subjected to transport was observed [28].

The intracellular killing activity of phagocytes was significantly reduced. Due to this all non-specific immune functions become suppressed. Neutrophilia is an often observed result of metal exposure [30, 34, 35-38]. This reaction is also explained with stress-induced increase in cortisol level [30, 39]. Thrombocyte count was decreased compared to the control groups. This may be due to the stress of cadmium. Under stress conditions blood clotting is often accelerated, but it is not always accompanied by significant increase in thrombocyte count. According to Al-Akel and Shamsi [40], cortisol may affect fish thrombocytes in a similar way as lymphocytes, reducing their number.

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