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RESEARCH ARTICLE

CHELATING PROPERTIES OF *Denolex elata* ON HAEMATOLOGICAL PARAMETERS AGAINST EXPOSED TO CADMIUM TOXICITY IN THE FRESH WATER FISH *Labeo rohita* (HAMILTAN)

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ABSTRACT

The effect of cadmium on the biochemical and haematological parameters of *Labeo rohita* were observed, after exposure to sublethal concentrations of cadmium (51.82 μg/l) for a period of 24, 48, 72 and 96 hrs. The number of white blood cells (WBC) and glucose levels were increased. The numbers of red blood cells (RBC), haemoglobin content, haematocrit, concentration was significantly higher ($p>0.05$) in the experimental group, compared with the control group. The above results of examination of the blood parameters indicate a marked physiological effect of cadmium in fish.

INTRODUCTION

The release of cadmium into the environment increased considerably in most industrialized countries during the second half of the last century. This has led to increased danger to health, not only in exposed workers, but also in the general population of these countries (Anderson, 1992.) Fish exposed to high concentration of cadmium quickly develop lack of calcium and low blood hemoglobin. Haematologica parameters is a reliable indicator of the physiological condition of the fish. This has been incentive to the scientific interest and development of fish haematology as clinical tool in monitoring fish health programme (Gill *et al.,* 1991). Koca *et al.* (2008) reported sex, season and maturity stages are known to heamatological values. The heamatological characteristics of fishes are an integral part of evaluating their health status (Martins, 2008). It is notable for Hb, Er and PCV levels of blood due to their responsiplites for transportation of nutrition, oxygen and metabolic wastes (Min *et al.,* 2008). Ralio *et al.,* (2003) reported that the blood parameter of diagnostic importance are erythrocyte and leucocyte counts, haemoglobin, haematocrit and leucocyte differential counts would readily respond to incidental factor such as physical stress and environmental stress due to water contamination . Similarly the significant increase of WBC count may be due to generalized immune response environmental pollutants like arsenic can influence fish immune system particularly with head kidney and spleen compromises the health and survival of fish (Ghosh, 2006). Measurement of blood chemistry parameters is commonly used as diagnostic tool in biomonitoring by which acute and chronic pathophysiology changes attributable to nutrition, water quality, and disease are detected (Adams *et al.,* 1996). Changes in blood glucose have been suggested as useful general indicator of stress in teleost (Ramesh and Saravanan, 2008). It also acts as a pathological reflector of the whole body. Hence haematological parameters are important in diagnosing the functional status of the exposed animal to toxicants (Joshi *et al.,* 2002). In this context, the present study deals with the effect of sublethal concentration of cadmium on the biochemical and haematological parameters of the fingerlings fish *Labeo rohita*.

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MATERIALS AND METHODS

Healthy *Labeo rohita* were procured from the freshwater farm located in Puthur, Nagappattinam district. They were acclimatized for a maximum period of 15 days in the laboratory condition. The fish each measuring 8.0 to 10.0 cm in length and weighing 10 to 15 g were used for the experimental studies. *Labeo rohita* fingerlings were exposed to sublethal concentration of cadmium 51.82 mg/kg for a period of 96 hrs. The sublethal and control group were sacrificed for blood parameters. Blood samples were collected from the candal vein of live fish from both control and treated group. A portion (1 ml) was mixed well in a clean dry vial containing EDTA anticoagulant (1.5 μg /l) according to McKnight (1966) to evaluate the RBC, WBC, haemoglobin, and haematocrit etc. The red blood corpuscles (RBC) and white blood corpuscles (WBC) were counted by Neubauer's haemocytometer using Hayem's and Tuerk's solution as a diluting fluid, respectively. Haematocrit values were measured by Wintrope's method and estimation of haemoglobin (Hb), blood sample was treated with N/10 HCl and the colour of the acid haematin was matched with the given standards using Sahli's haemoglobinometer. The mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated by following standard formula (Dacie and Lewis, 1991).

$$MCV = \frac{PCV/1000\,\text{ml blood}}{\text{RBC in millions}/mm^3} = \text{fl}$$

$$MCH = \frac{\text{Hb in g}/1000\,\text{ml blood}}{\text{RBC in millions}/mm^3} = \text{pg}$$

The data obtained from the control and experiment were subjected to statistical analysis by student't' test.

RESULTS AND DISCUSSION

The quantitative changes of haematological parameters like RBC, WBC, Hb, haematocrit, MCH, MCV, have been observed in the fish *Labeo rohita* in control and sublethal concentration of cadmium (0.0 mg/l) exposed after 24, 48, 72 and 96 hrs and are given in Table 1.

In the present study the RBC, haemoglobin and haematocrit values were decreased gradually till 96 hrs. Reduction in RBC content may be caused either by the inhibition of erythropoiesis or by the destruction of red cells. In the present study, the significant decrease in RBC counts during sublethal study is due to anemic condition and haemolysis caused by cadmium toxicity. The quantitative changes of biochemical and haematological parameters like RBC, WBC, Hb, haematocrit, MCH, MCV, glucose, protein, and cholesterol have been observed in the fish *Labeo rohita* in control and sublethal concentration of cadmium (51.82 ug/l) exposed after 24, 48, 72 and 96 hrs and are given in Table 1. In the present study the RBC, haemoglobin and haematocrit values were decreased gradually till 96 hrs. Reduction in RBC content may be caused either by the inhibition of erythropoiesis or by the destruction of red cells. In the present study, the significant decrease in RBC counts during sublethal study is

Table 1. Variations of Haematological parameters RBC ($x10^6/mm^3$), WBC ($x10^3/mm^3$), Hb(g/L) PCV (%), MCV (fl), MCH (pg) and MCHC (%) of the *Labeo rohita* exposed to cadmium and *Delonix elata* for 120 hours.

PARAMETERS	GROUPS	HOURS OF EXPOSURE			
		24	48	72	96
	I	3.60 ± 0.18	3.35 ± 0.13	3.50 ± 0.17	3.55 ± 0.14
	Control				
	II	3.40 *± 0.14	3.30** ± 0.16	3.44^{NS} ± 0.02	2.70** ± 0.13
RBC	Cadmium	-5.5	-1.49	-1.79	-23.94
($x10^6/mm^3$)	III	3.45^{NS} ± 0.17	3.32* ± 0.19	3.46^{NS} ± 0.17	3.04** ± 0.13
	Cadmium + *Delonix elata*	-4.16	-0.89	-1.14	-0.15
		-1.47	-0.60	-0.58	-12.59
	IV	4.57** ± 0.18	4.60** ± 0.23	4.65** ± 0.18	4.55** ± 0.27
	Delonix elata	26.94	37.81	32.85	28.16
	I	540 ± 27.0	538 ± 26.9	542 ± 27.1	538 ± 26.9
	Control				
	II	730** ± 29.2	728** ± 29.12	735** ± 29.4	732** ± 36.6
WBC	Cadmium	35.18	35.31	35.60	36.05
($x10^3mm^3$)	III	650** ± 26.0	645** ± 32.85	655** ± 32.75	660**± 39.6
	Cadmium + *Delonix elata*	20.37	19.88	20.84	22.67
		10.95	11.40	10.98	9.83
	IV	580** ± 29.0	575**± 28.75	852**± 23.82	577 **± 28.85
	Delonix elata	7.40	6.87	7.38	7.22
	I	13.8 ± 0.69	13.0 ±0.65	13.5 ± 0.67	13.2 ± 0.66
	Control				
	II	11.8** ± 0.70	11.4** ± 0.57	11.6* ± 0.69	11.2** ± 0.56
Hb	Cadmium	-14.49	-12.30	-14.07	-15.15
(g/L)	III	12.8* ± 0.64	12.5^{NS} ±0.62	12.8^{NS} ± 0.60	12.0* ±0.72
	Cadmium + *Delonix elata*	-7.24	-3.84	-0.64	-0.72
		-8.47	-12.20	-10.34	-7.14
	IV	14.8** ± 0.88	14.4 ** ± 0.86	14.1 ** ± 0.84	14.0** ± 0.56
	Delonix elata	7.24	10.76	4.44	6.06
	I	34.18 ±1.74	34.40 ±1.72	34.02 ±1.70	34.5 ± 2.07
	Control				
	II	31.7**± 1.96	32.4** ±1.62	31.9* ±1.27	31.6** ±1.89
	Cadmium	-6.03	-5.81	-6.23	-9.17
	III	32.7*±1.96	33.6^{NS} ±1.68	33.0^{NS} ±1.65	32.9* ±1.31
	Cadmium + *Delonix elata*	-2.87	-2.32	-2.9	-4.63
PCV		-3.47	-3.70	-3.44	-4.11
(%)	IV	40.06** ± 2.40	37.4* ±1.87	39.6 **±1.98	40.5** ± 2.02
	Delonix elata	15.06	8.72	16.40	17.39
	I	130.61± 6.53	131.89 ±1.49	132.77± 7.6	132.91±6.64
	Control				
	II	113.82** ± 5.69	112.61** ± 5.63	113.10* ± 4.52	117.03* ±7.02
	Cadmium	-12.85	-14.61	-14.81	-11.94
MCV	III	120.60** ± 6.03 -	120.90** ± 6.04	121.81^{NS} ± .30	119.93* ± 7.19
(fl)	Cadmium + *Delonix elata*	7.66	-8.33	-8.25	-9.76
		-5.95	-7.36	-7.70	-2.56
	IV	142.38** ±7.11	137.56** ± 6.87	140.46** ± 5.61	139.37** ± 5.57
	Delonix elata	9.01	4.29	5.79	4.86
	I	30.25 ±1.51	30.45 ±1.52	31.28 ±1.56	31.11±1.55
	Control				
	II	28.91**± 1.44	27.87** ±1.39	27.52** ±1.37	28.56 **±1.42
	Cadmium	-4.42	-8.47	-12.02	-8.19
	III	29.97*±1.19	29.88* ±1.19	28.8 ** ±1.15	29.50** ±1.47
MCH	Cadmium + *Delonix elata*	-0.92	-1.87	-7.89	-5.17
(pg)		-3.66	-7.21	-4.94	-3.29
	IV	32.31**±1.61	31.70** ±1.58	32.56^{NS} ±1.95	32.16** ±1.28
	Delonix elata	6.80	4.10	4.09	3.37

due to anemic condition and haemolysis caused by cadmium toxicity. Toxicants are known to have multiple haematological effects such as haemolysis and anemia (Arujun *et al.,* 2002. Decreased Hb level may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production (Ahmad *et al.,* 1995). In the present investigation shows a decreased level of RBC count, Hb concentration PCV and MCHC of blood in cadmium treated fish and increased MCV MCH values.

The reduction of RBC count and Hb content may be due to the destructive action of cadmium on erythopoietic tissue as a result of which the viability of the cell might have been affected. Decrease in RBC count, Hb content and PCV were symptoms and fish suffer anemia (Koprucu *et al.*, 2006*)* In addition increase in MCV, MCH and decreased MCHC values indicate that the anemia was of a macrocytic type (Talas *et al.,* 2009). Similar result was observed in acute effect of diazinon on carp (Svoboda *et al.,* 2001). Swelling of RBC'S due to hypoxic condition in the toxicant treated organisms may lead to a significant increase in MCV values as suggested by Wepener *et al.,* (1992). The increase in MCV may also result from an increase of immature RBC (Carvalho and Fernandes, 2006). A Reduction of hematological values indicated anemia in the pesticide exposed fish. This may be due to erythropoiesis, hemosynthesis and osmorequlatory dysfunction or due to an increase in the rate of erythrocyte destruction in hematopoietic organs (Jenkins *et al*., 2003) Cadmium can have two mode of action on blood cells. It may either induce oxidative stress, as a hydrophobic compound it may accumulate in cell membranes and disturb membrane structure (Michelangeli *et al* 1990). Reduction of Hb content could be due to either an increase in rate at which Hb is destroyed or a decreased in the rate of Hb synthesis .Decreased Hb content may be consequence of changes in the number of circulating erythrocytes. Similar findings were reported in goats (Khan *et al.,* 2009). In the present investigation cadmium treated fish (group2), WBC count slightly increased and recovery group remarkable increase in WBC count.WBC as key components of innate immune defense and leukocytes are involved in the immunological function in the organisms (Kavitha *et al.,* 2010*)* has been reported increasing the WBC count during sublethal treatment of arsenic on *Catla catla*, Increasing WBC might be resulted from stimulation of immune system and to protect the fish against toxicity.

In the present investigation cadmium treated group glucose level increased when compared to other group. Serum glucose level was increased in the treated group at 120 hours compared to control group. Increasing of blood glucose due to increase the synthesis of adrenocortico tropic hormone and glucagon in the suprarenal gland and decrease in the synthesis of insulin due to the increase hepatic glycogen is rapidly converted into glucose and passes into systemic circulation. There by a rapid increase in blood glucose (Prusty *et al.,* 2011). Suggested that cadmium-induced hyperglycemia in *R. quelen.*
The WBC count of the experimental fish *Labeo rohita* shows a prompt increase after exposure to 24, 48, 72 and 96 hours of cadmium toxicity (Table 1). The WBC showed greatest sensitivity to changes in the environment and the most important of leucotyes were lymphocytes (Karuppasamy and Subathra, 2005). This increase of WBC might be due to the increase in population of leucocyte which indicates an immune system to protect the fish against infections under cadmium stress. The increase is also for the removal of cellular debris necrosed tissue at a quicker rate as reported by Anupama and Neera (2005) in *Channa punctatus* under zinc stress. An increase in leucocyte count was reported by Garg *et al*. (1989) in Heteropneustes fossilis after exposure in manganese. A similar increase was later reported in *Channa punctatus* exposed to copper and chromium (Singh, 1995). The increased level of MCV and MCH was recorded throughout the experimental period which may indicate a condition of macrocytic anaemia in the cadmium exposed fishes. The increase MCV and MCH value with decrease in MCHC perhaps is due to toxic substances in the medium causing differences. Ruperelia *et al.* (1992) recorded increase in MCV and MCH in blood of cadmium exposed Oreochromis. Increase in MCV values in all exposures may be considered an index of RBC destruction and endomosis. The fish immersed in 0.018 mg/l solution of cadmium behaved identically as those in control groups. There was no signs of stress symptoms (anxiety, excessive mucus secretion, changes in the respiratory rhythm, etc.).

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