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Biochemical Studies on Common Carp (*CyprinousCarpio*, L.) exposed to Environmental Pollution cadmium and treated with probiotics

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ABSTRACT

Heavy metals are recognized as cumulative toxic substances causing serious health hazards to man depending on their concentration. Forty Common Carp (CyprinousCarpio, L.) were collected from Abbassa Sharkia government and fed commercial fish diet. Thirty fish were exposed to cadmium chloride 1p.p.m.) and 30◦ temp. For 21 days. Ten fish were kept without treatment (control). Hematological analysis of the exposed group demonstrated a marked elevation in serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum glucose, urea, creatinine, sodium, potassium, and phosphorus, while serum calcium, haemoglobin and PCV were reduced . Kidney, spleen, and gills, which were reflected on the biochemical and hematological parameters. Heavy metals induced cumulative effect; therefore, equivalent lesions of fish may occur in humans. Moreover, immune suppression could play an important role in predisposing for further infections conditions. After 30 days' treatment with probiotic it returns to normal parameters

***Key words:** Pollution cadmium, Common Carp, probiotics*

INTRODUCTION

Four out of the six top contaminants in the world reported by Pure Earth (2015) belong to the class of trace elements, namely lead, mercury, chromium and cadmium. Many of these elements like lead or mercury have been known for their toxic properties for a long time and therefore, at least in richer countries, enormous efforts have been taken to decrease their release

into the environment [1]. Amongst others, the European Union has set maximum levels for cadmium, lead, mercury, and nickel as priority substances regarding aquatic environments in the directive on environmental quality standards in the field of water policy [2] and in many European countries the criteria demanded in this law have been met already. However, millions of people's health is threatened by the known toxic effects of contamination with trace elements, mostly in low- and middle-income countries as precautionary measures to diminish contamination with trace elements are inadequate [3].

On the contrary, the novel use of silver is booming right now in high-income countries, due to increasing applications in many aspects of daily life, commonly in form of nanoparticles, with rarely known consequences to the aquatic environments [4]. There is nearly no control of silver emission into the environment which may evolve into a serious problem even in high-income countries in the future. Surveillance of trace elements in fish has two benefits. Fish have been recognized as bioindicators for environmental contamination for a long time, providing an integrated insight into the status of their environment over longer periods of time.

This is particularly valid for most metals, as they show very long biological half-life. Therefore, elevated tissue concentrations can occur even if the exposure is not continuous [5]. On the other hand, fish is an important food source for humans in many parts of the world and therefore monitoring trace element levels in them is also of important concern in ensuring food safety. Especially regarding mercury, fish used for alimentation is believed to be the main source of this element for humans [6].

The term probiotic was firstly used to denominate microorganisms that have effects on other microorganisms [7]. Probiotics can provide some solutions to this problem through different mechanisms or properties such as the production of inhibitory compounds such as bacteriocins, competition for adhesion sites with opportunistic or pathogen microorganisms, competition for nutrients with other bacteria or an improvement of the immune status (e.g. increase of production of immunoglobulins, acid phosphatase, antimicrobial peptides, improvement of cellular ctivities, etc.) [8-15]. Use of microbial probiotics to promote health maintenance and disease prevention and control is now widely accepted as the new ecofriendly alternative measures for sustainable aquaculture [16]

MATERIALS AND METHODS

Experimental design

Total of forty Common Carp100-200 gm body weight of each was acclimatized a tized to laboratory conditions for two weeks before use. They were divided into control group (10 fish) and experimental group *(30* fish) that was exposed to cadmium chloride at a concentration of 1 p.p.m. and 30◦ temp. for 21 days Blood samples were collected from the caudal vein after 7 and 21 days of exposure. Serum for biochemical analysis and heparinized blood for haematological investigations were obtained from each sample.

Biochemical analysis

Test kits of Bio Merieux (France) were used for evaluation of serum glutamic pyruvictransminase and glutamic oxaloacetic transaminase [17]. Serum glucose was assessed according to Trinder [18]. Serum urea and creatinine were determined using kits of Bio Merieux (France). The concentration of cadmium, sodium, potassium and calcium were detected by using atomic spectrophotometry according to Forstner [19].

Haematological examination

Blood haemoglobin (Rb) was assessed by Drabkin [20]. Haematocrit value was carried out by using microhematocrit capillary tubes, centrifuged at 1200 r.p.m. for 5 min.

Bacteriological examination

Bacterial isolation was done from skin, liver and kidney of fish on blood tryptose agar, MacConcky agar and tryptic soy agar plates. The plates were incubated aerobically and anaerobically.

The bacterial isolates were identified morphologically and biochemically, according to Nomiyama [21]. The serum IgM was measured according to Fudaet al. [22]. Antisera for fish were prepared by immunizing rabbits as previously described by Fuda et.al [22]. The procedure for labelling antibody fragment with enzyme was performed.

Elisa assay procedure: Assay was carried out in 96-well polystyrene ELISA microtiter plates (Titertex, Horsham, P A). The microtiter plates were coated with rabbit antigrey mullet IgM and were fractionated by DE-52 at a concentration of 40 μ g/ml in 0.01 MPBS. A volume of 150 μ l was dispensed into each well and incubated for 4 hrs at 4°C.

Blocking was achieved after one washing with 200 μ l of 0.01MPBS + 0.1 % 20 μ l per well and two washings with 200 μ l of PBS +1% PBS. 0.01% thiomerosol was added to each well and incubated for 2 hrs at room temperature. Incubation of samples and standards after washing was carried out as described above. 100 μ l of sample and standard were placed into the appropriate wells in the microtiter plates and incubated at room temperature.

Incubation with peroxidase labelled antibody after washing was done as described above, each well received 150 μ l of peroxidase labelled antibody 1:1600 in PBSBSA, followed by incubation for 12 hrs at room temperature .The probiotic bacterium, Lactobacillus rhamonsus (ATCC 53103) was cultured in MRS broth at 26.8C for 48 h, centrifuged and washed with sterile PBS 2 times.

Bacterial pellets were measured in PBS and their densities were determined. Under sterile conditions, the bacteria were manually incorporated into commercial dry pellets at rates of 108 and 1010 CFU/g in feed for low and high LAB diets, respectively. Fish fed only commercial dry pellets served as a control. Fish were fed approximately 0.8% of body weight once a day. The probiotic groups ingested an average of 3.8 x 106 and 3.8 x 108 cells day-1.

RESULTS

Serum biochemical analysis

Fish exposed to cadmium chloride (1 p.p.m) showed a significant increase of SGPT and SGOT activity with pronounced elevation of urea and creatinine by 1st, 2nd, 3rd week of exposure. High level of sodium and potassium in serum of exposure fish was noticed (Table 1). Hyperglycaemia and hypocalcaemia were noticed along the experimental period with- marked elevation of serum cadmium (Table 2).

Table-1: Effect of cadmium chloride 1.p.m. on kidney and liver function of Common Carp

Exposure time	**SGOT U/L**	**SGPT U/L**	**Urea mg/dl**	**Creatinine mg/dl**	**Sodium Meg**	**Potassium Meg**
1st week (control)	89.0 ± 0.12	19.7 ± 1.5	2.99 ± 0.17	0.65 ± 0.62	120 ± 0.47	3.99 ± 0.05
1st week of exposure	90.00 ± 2.10	22.5 ± 0.63	3.10 ± 0.24	0.71 ± 0.01	121 ± 0.86	3.60 ± 0.02
2ndst week control)	87.00 ± 0.10	20.1 ± 1.18	2.80 ± 0.18	0.65 ± 20	115.3 ± 3.9*	3.23 ± 0.58
2ndst week of exposure	120 ± 0.35*	27 ± 1.9*	3.81 ± 0.11**	0.80 ± 0.15**	130 ± 0.60**	5.1 ± 0.07
3rd week (control)	91 ± 2.0	19.01 ± 0.06	2.80 ± 0.17	0.62 ± 0.20	120 ± 3.2	3.7 ± 0.06
3rd week of exposure	130 ± 2.06*	30 ± 1.06*	4.0 ± 0.20*	0.90 ± 0.10**	140 ± 6.6	5.05 ± 0.11
After 30th days treatment with probiotics	90.0 ± 0.17	20.7 ± 1.75	3.29 ± 0.27	0.76 ± 0.72	124 ± 0.57	4.19 ± 0.07
* Significant P< 0.05; ** highly significant P < 0.01						

Haematological profile

Reduction of Hb concentration and P.C.V value were observed (Table 2).

Bacteriological examination

Pure culture of *Streptococcus spp., Staphylococcus spp. Agrobacterium spp., Flavobacterium spp. and Lactobacillus spp.* were isolated from the internal and external organs of exposed fish (Table 3).

Determination of fish IgM

There was a significant decrease in total protein and IgM level from the first week of exposure until the end of last week (Table 4). **After treatment with probiotic** it returns to normal parameters.

Table-2: Some hematological and biochemical changes in Common Carp exposed to cadmium chloride.

Exposure time	**P.C.V %**	**Hemo globin gm/dl**	**Gluco se mg%**	**Cadmi um p.p.m**	**Calci um mg/d l**	**Phos phon is mg/d l**
1st week (control)	20.00 ± 0.08	8.0 ± 0.2	60 ± 1.00	0.04 ± 0.01	5.00 ± 0.22	4.0 ± 0.05
1st week of exposure	16.9 ± 0.05	8.0 ± 0.01	65 ± 0.40	0.07 ± 0.010	3.80 ± 0.67	5.8 ± 0.29
2ndst week control)	20 ± 0.20	7.8 ± 0.30	58 ± 0.06	0.05 ± 0.060	5.0 ± 0.81	3.9 ± 0.60
2nd week of exposure	14 ± 1.07	6.00 ± 0.90**	68 ± 1.03**	0.11 ± 0.03*	3.8 ± 0.53*	6.0 ± 0.10*
3rd week (control)	21.0 ± 1.52	7.9 ± 0.06	60.2 ± 0.60	0.04 ± 0.01*	4. ± 0.70	3.4 ± 0.01
3rd week of exposure	14.9 ± 0.7	6.01 ± 0.20*	8o ± 0.02*	0.14 ± 0.0 2*	3.0 ± 0.80*	5.3 ± 0.3*
After 30th days treatment with probiotics	21.00 ± 0.06	8.7 ± 0.3	62 ± 1.20	0.05 ± 0.01	5.6 ± 0.32	4.2 ± 0.07
* Significant P< 0.01; ** highly significant P < 0.05						

Table 3: Bacteriological recovered in Common Carp exposed to cadmium chloride (1 p.p. m).

Bacterial strain	External surface	Internal organs	Internal organs liver	Gills
FlavobacterIum spp.	$6x10^7$	$5.2x\ 10^6$	$5X10^3$	$6.3X10^8$
Staphylococcus spp.	$5.8x10^5$	$4.5x10^4$	$6\ x10^3$	$4\ x10^6$
Streptococcus SPP.	$4.5x10^7$	$8x10^5$	$6.1x10^6$	$2.9x10^7$
Lactobacillus spp.	$3.\ x10^3$	$4.\ x10^6$	$1.9x10^3$	$2.1x10^6$

Table 4: Influence of cadmium chloride 1 p.p.m on 1gM and protein level.

Exposure period	1gM/old	Total protein/neg/dl
Control	0.95 ± 0.10	6.84 ± 0.20
1st week of exposure	0.78 ± 0.22**	6.00 ± 0.89*
2st week of exposure	0.70 ± 0.80*	5.42 ± 0.20*
3st week of exposure	0.65 ± 0.40 *	5.2 ± 0.40*
After 30th days treatment with probiotics	0.98 ± 0.13	7.0 ± 0.20
* Significant P< 0.01; ** highly significant P < 0.05; ± Standard errors		

DISCUSSION

Aforementioned data of exposed fish to cadmium chloride (1 p.p.m) for 3 weeks revealed an elevation of serum GPT, GOT, urea and creatinine. These findings are in agreement with previous results. Elevation of urea and creatinine beside liver enzymes in cadmium-exposed fish may be attributed to liver and kidney injury. Reduction of calcium level in serum may have resulted from its increased excretion in urine through inhibition of calcium ATPase enzyme. On the other hand, increase of the phosphorus level in serum of exposed fish was noticed. Cadmium chloride toxicity leads to disturbance in blood electrolytes followed by skeletal changes [17, 23]. Hyperglycaemia was observed in the present work which coincides with that obtained in Rainbow traut and salmogaidneri [23]. The blood glucose level was affected by the rate of carbohydrate metabolism under hypoxia and stress conditions. Hyperglycaemia is attribute to stress stimuli followed by rapid secretion of both glucocorticoids and α -techolarnines from the adrenal tissue [24]. Regarding to haematological profile exposed fish,

haemoglobin and P.C.V. values were decreased. These results agree with previous findings [16, 21]. The erythropenia resulted from reduction of Hb concentration and P.C.V. value in Kwait Mullet due to disturbance of osmoregulatory mechanism accompanied with destruction of gill membrane and failure of gas exchange [25]. Cadmium interfered with sulpha-hydride groups of essentials enzymes [23, 26]. Heavy metals are recognized as cumulative substances leading to serious health hazards to man and animals [6-9].

In the present study, a significant decrease of IgM and total protein during the experimental period were observed. Reduction of IgM level indicated that the cadmium chloride toxicity leads to suppression of immune system of exposed fish which become susceptible to any infective agents [15, 20]. There is a significant decrease in IgM level in fish exposed to cadmium chloride if compared with control which may have resulted from high cortisol secretion that was indicated by hyperglycaemia in exposed fish.

Microscopical examination of fish exposed to cadmium chloride for 21 days revealed a congestion of all internal organs and friable bloody liver. These findings are in agreement with those mentioned by other authors [27-33].

As an essential trace element, the uptake of zinc is highly regulated by the organism [34]. It has been reported that Cd blocks Zn-containing enzymes [35]. It could be concluded that cadmium chloride at 1p.p.m induced deleterious effects in fish such as damage of liver, Kidney, spleen, and gills, which were reflected on the biochemical and hematological parameters. Heavy metals induced cumulative effect; therefore, equivalent lesions of fish may occur in humans. Moreover, immune suppression could play an important role in predisposing for further infections conditions. After treatment with probiotic it seems to be normal parameters.

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