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Effect of Sub-Acute Exposure to Nickel on Hematological and Biochemical Indices in Gold Fish (*Carassius auratus*)

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Abstract

The increasing use of heavy metals in industry in the modern world unfavorably affects the aquatic environment. Acute toxicity of nickel to the fresh water fish carassius auratus was determined using Probite analyze, Fish were exposed to selected concentration of nickel and the mortality data were determined after 24, 48, 72 and 96 hours. LC50 values for 24,48,72 and 96 hours were calculated with the 95% fiducially limits. The 24,48,72 and 96 hours LC50 values of nickel to the fish were 161.78 ± 3.05, 130.58 ± 2.32, 110.19 ± 1.57, 100.39 ± 0.54 ppm respectively. White blood cell (WBC) count, hemoglobin (Hb) and hematocrit (Hct) level were significantly reduced at experimental concentrations ($p<0.05$). Red blood cell (RBC) count, mean corpuscular hemoglobin (MCH), cortisol and glucose levels in nickel treated groups were significantly higher than the controlled group at experimental periods ($p<0.05$) but significant differences were not found in mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) ($p>0.05$). In summary, nickel intoxication resulted indicated that hematological and biochemical parameters can be used as an indicator of nickel related stress in fish on exposure to elevated nickel status.

Keywords: Heavy metal; Nickel; Contamination; Toxicity

Introduction

One of the most prominent natural resources is water that its regular supply is extremely much essential for the survival of all living animals. The variations impurities display in water sources are expressed through water quality changeful which are broadly classified as biological and Physicochemical. In the different industries greatly used heavy metals in some industries of the new world adversely affect the aquatic animals after eject of the waste material into water [1]. The improvement coefficient of this metal in the food chain arrived extreme. Once it's absorbed by the animals and people through food chain in the form of fish, shrimp shell fish and plants, it would collect in the corresponding functional organs of the animals and human body. The important action of the environmental toxicologist is to assess impartially the endanger obtained from the presence of such materials. These materials may also alter the quality of water and thus desecrate the fisheries management [2,3].

Nickel is greatly utilized in industry and is a common aquatic pollutant. In natural waters Ni^{+2} is the important chemical species. In aquatic ecosystems nickel interacts with very many inorganic and organic compounds and occurs as soluble salts adsorbed onto materials of various chemical origin [4] many of these interactions are additive or synergistic in producing adverse effects, and some are antagonistic. The toxicity of nickel to aquatic life was intensively investigated during previous decades, and a considerable amount of experimental data has been compiled and reviewed [5]. Fish are used as test organisms in aquatic toxicology because of their top position in the trophic chain and their role as food for humans.

Hematological values are progressively used in fish as indicators of the physiological or sub-lethal stress response to endogenous or exogenous alterations and are more quickly reflected in the poor condition of fish than in other commonly measured [6].

Material and Method

Experimental fish and laboratory condition

Acute toxicity tests were conducted on goldfish (10.5 ± 1.0 g and 8.7 ± 1.2 cm) obtained from commercial fish farms, Gorgan, Iran. Only healthy fish, as obtained by their activity and external appearance, were maintained alive on board in a fiberglass tank. Samples were transferred to a 400 L aerated tank with 200 L of test medium. All samples were acclimated for 10 days in 10 aerated fiberglass tanks at 19°C under a constant 12:12 L:D photo period. Acclimatized fish were fed daily a formulated feed. Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of water quality. The average values for aerated and dechlorinated tap water used during the both acclimation period and experiments were pH 8.17 ± 0.40, dissolved oxygen 8.23 + 0.17 mg/L, temperature of 22.26 ± 1°C and total hardness 274+1.57 mg/L as $CaCO_3$. Water was renewed daily, and the water quality parameters mentioned above were measured twice a week during the acclimation period and sub-acute toxicity test.

Acute toxicity test

Only healthy fish, as indicated by their activity and external appearance, were maintained alive on board in a fiberglass tank. Fish were transferred to a 400 L-aerated tank equipped with aeration with 200 L of test solution. The acute toxicity test was conducted following

the Organization for Economic Cooperation and Development guideline under static renewable test conditions. Groups of 21 fish were exposed to various concentrations of nickel for 96 h. Values of mortalities were measured at 24, 48, 72 and 96 h, and dead fish were immediately removed by dip net to avoid possible deterioration of the water quality. The LC50 values were calculated by EPA Probit Analysis V. 1.5 for 24, 48, 72 and 96 h [7].

Sub chronic test

Following the toxicity test, in order to investigate the effect of nickel on hematological and biochemical parameters of the three fish, two concentrations (40% and 80% of 96-h LC50) were considered. In this stage, 120 randomly selected Carasius auratus from acclimation tanks and randomly graded into several experimental 400-L tanks expose to concentrations of 40% and 80% of 96-h LC50 for a period of seven days to hematological, plasma glucose and cortisol analysis. For sub-acute toxicity assay, the exposure water in the tank was changed daily and freshly prepared solution was added to maintain the concentration of nickel at a constant level. Moreover, during the experiment, water was continuously monitored. A control test without nickel was conducted under the same conditions.

Biochemistry and hematology

Blood samples were collected from both the control and experimental fishes that survived the 7 Days sub-acute exposure period. The blood samples were taken by cutting posterior caudal vein using ethylenediaminetetraacetate (EDTA) as anticoagulant [8]. Blood, 2.0 ml, was decanted in heparinzed bottles for determination of blood parameters. The microhaematocrit method of Snieszko (31) was used to determine the hematocrit (Ht). Hemoglobin (Hb) concentration was measured with Hb test kit using the cyanmethemoglobin method [9]. Determinations of the number of white blood cell (WBC) and red blood cell (RBC) tests were performed immediately on fresh blood. The number of blood leukocytes and erythrocytes was counted by diluting heparinized blood with Gimsa stain at 1:30 dilution, and cells were counted using a hemocytometer Neubauer under the light microscope [10]. The derived hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae as described by Jain (29): MCV was calculated in femtoliters=Ht/RBC x 10; MCH was calculated in picograms=Hb/RBC x 10; and MCHC=(Hb in 100 mg blood/Hct) x 100. For the biochemical tests, the blood was placed in tubes and allowed to clot at room temperature (approximately 22°C) for 30 min. Serum was removed from the clotted sample after centrifugation at 2795 g for 5 min and frozen at 80°C until analysis. The determination of plasma glucose were carried out using diagnostic kits (Pars Azmoon Co., Iran) at 546 nm and 38°C by the glucose oxidase method [11]. Glucose were measured photometrically according to a method modified based on the quantification of NADH after a glucose oxidation catalyzed by glucose dehydrogenase also cortisol was determined directly from serum using an ELISA kit (DRG Diagnostics, Mountainside, NJ, USA) as described by Shaluei et al.[7].

Statistical analyses

For each index, Experimental data and those of control were tested by means of analysis of variance (ANOVA). Standard deviation (SD). Significance was set at P=0.05. All analysis was performed using SPSS software (version 18.0).

Results

The results of the acute toxicity test for the nickel on gold fish are presented in Table 1. No mortality was observed in the control group during the experiment. Fish mortality increased significantly when the concentration and the time of exposure were increased. As expected, the 96-h LC 50 values decreased with increase in exposure time. This indicates an increase in toxicity with exposure duration. Prior to death, fish exhibited rapid gill movement, nervous manifestations, erratic swimming, loss of equilibrium and inability to remain upright.

	Concentration (ppm) (95% of confidence limits)			
point	24 h	48 h	72 h	96 h
LC1	43.32 ± 3.05	32.74 ± 2.32	25.13 ± 1.57	21.49 ± 0.54
LC10	68.54 ± 3.05	49.28 ± 2.32	35.33 ± 1.57	30.83 ± 0.54
LC30	114.49 ± 3.05	99.44 ± 2.32	52.21 ± 1.57	65.59 ± 0.54
LC50	161.78 ± 3.05	130.58 ± 2.32	110.19 ± 1.57	100.39 ± 0.54
LC70	180.35 ± 3.05	154.43 ± 2.32	132.11 ± 1.57	120.19 ± 0.54
LC90	199.78 ± 3.05	171.59 ± 2.32	153.42 ± 1.57	150.95 ± 0.54
LC99	211.45 ± 3.05	199.57 ± 2.32	171.13 ± 1.57	161.53 ± 0.54

Table 1: Lethal Concentrations (LC1-99) of nickel (mean ± Standard Error) depending on time (24-96 h) for goldfish.

Hematological parameters

Results of hematological parameters (RBC, WBC, Hb, Hct, MCH, MCHC and MCV) of the test and control Goldfish expose to 40% and 80% of LC50 are shown in Table 2.

Glucose and cortisol

The average plasma glucose levels and cortisol in the unexposed control group of Goldfish was 45 ± 1.19 mg/dl and 27 ± 1.04 ng/ml respectively. As it is obvious from Figures 1 and 2, there was a significant increase in the glucose levels of the treated groups when

compared with their respective controls (P<0.05). Glucose plasma levels in gold fish exposed to 40 and 80% of 96 h-LC50 were 57 ± 1.02 and 68 ± 1.08 and also cortisol plasma levels in gold fish exposed to 40 and 80% of 96 h-LC50 were 34 ± 1.02 and 41 ± 1.06, respectively. There was a significant increase in gold fish exposing 80% of 96 h-LC50 than those exposed to 40% of 96 h-LC50. In fact, the value of glucose decreased with increasing the concentration.

Fish	Treatments	RBC(106 cells/l)	Hb(g/l-1)	Hct(%)	WBC(cells/l)	MCH (pg/cell)	MCHC(g/l-1)	MCV(μm^3/cell)
Goldfish	Control	0.68 ± 0.10 a	7.41 ± 0.04 a	17.24 ± 0.03 a	8656.13 ± 217.15 a	117.1 ± 0.19 a	47.31 ± 0.14 a	297 ± 2.31 a
	40% of LC50	0.75 ± 0.02 b	6.00 ± 0.05 b	14.30 ± 0.05 b	7533.33 ± 88.19 b	95.33 ± 0.05 b	45.91 ± 0.03 a	295 ± 1.19 a
	80% LC50	0.80 ± 0.03 c	4.90 ± 0.10 c	11.86 ± 0.05 c	6400.27 ± 88.19 c	90.74 ± 0.10 b	45.62 ± 0.04 a	294 ± 1.35 a

Table 2: Some hematological parameters values of exposed to nickel in rows and Significant differences from control values (P<0.05). Each value expresses the means ± standard error of three observations. Letters show different compare to control group (n=21).

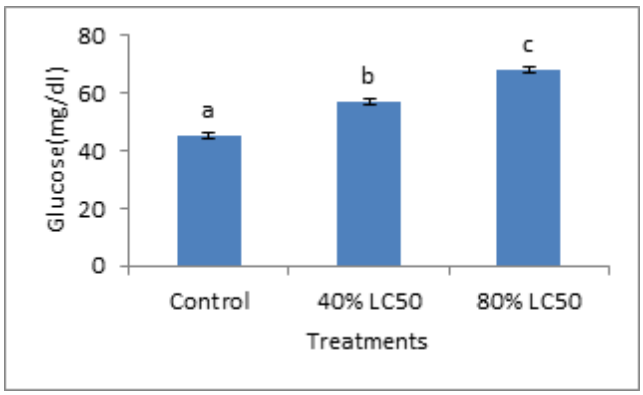

Figure 1: Serum cortisol levels in goldfish exposed to sub lethal concentrations of nickel for 7 days.

Discussion

It is apparent from the outcome that the heavy metal concentration has a direct effect on the LC50 values of the respective fish. LC50 acquired in the recent study correspond to assess that have been published in the literature for other species of fish. The distinction in acute toxicity may be due to alters in water quality and trial species [12]. The impressionability of fish species to a particular heavy metal is a very main factor for LC50 stages. Fish that are very sensitive to the toxicity of one material may be less or even not sensitive to the toxicity of another metal at the many level of that metal in the ecosystem. Unlike, a metal which is very toxic to a fish species at low concentrations may be less or even non-toxic to other species at the same or even higher concentrations [12].

Serum glucose levels of gold fish exposed to sub-lethal concentrations of nickel for 7 days increased with enlarging concentrations (80% LC50) of nickel in the water. Vinodhini et al. [13] in common carp with exposing of fish to heavy metals showed that the concentrations of serum glucose were significantly elevated. Serum glucose levels also increased with increasing concentrations of Cd in Mugil cephalus [14]. It was indicated that serum glucose stages in fish were affected by many stress parameter, including heavy metals [15]. Exposure to nickel indicates to draw out a temporary stress reply in gold fish. Significantly increased plasma cortisol were evident on 7 day in the both concentration (40 and 80% LC50), also increased plasma glucose levels were observed on 7 day exposure. The coalition of elevated plasma cortisol and glucose levels is frequently observed following exposure of fish to water pollutants or other stressors, and the relationship very likely is causal: the primary response leads to the secondary via stimulation by cortisol of gluconeogenesis [16-18].

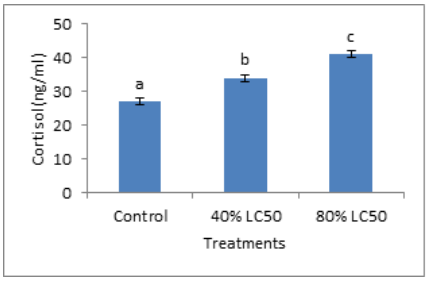

Figure 2: Cortisol levels in goldfish exposed to sub lethal concentrations of nickel for 7 days.

In sub efficient or stressful situation the chromaffin cells release catecholamine hormones, adrenaline and noradrenaline toward blood circulation [19] Those stress hormones in connection with cortisol circulate and exalt glucose production in fish through glucogenesis and glycogenolysis pathways to cope with the energy demand produced by the stressor for the "fight of flight" reaction. Glucose is then released toward blood circulation and enters into cells through the insulin action [20]

The Ht, Hb, RBC, WBC, MCV, MCH and MCHC of the fishes exposed to nickel are indicated in Table 2. Significant different were watched between the different blood indices with various concentrations of toxicants. The results indicate that changes in hematological parameters of fish may be owed to nickel and are predestine both by the concentrations of the heavy metals in the water and time of exposure and both these factors can cause inverted and non-inverted changes in the homeostatic system of fish [21].

Our results in the hematological indices shown that no significant change was recorded in the mean corpuscular volume (MCV) and mean corpuscular hemoglobin content (MCHC) but there was significant change in the mean corpuscular hemoglobin (MCH) especially at higher concentrations (80% LC50). However, small vacillation was recorded in the MCV and MCHC when compared with

the control. Cells released from the spleen, which is an erythropoietic organ would have the lower MCV values when compared with the control. A similar observation was made for Cyprinus carpio after cadmium exposure (30). The significant different in the MCH of the trial fish when compared with the control may be resulted to the decrease in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis [22].

Results of the hematological part, indicated that the mean WBC, Ht, and Hb of fish in the control trial were 17.24 ± 0.03 (%), 8656.13 ± 217.15 (cells/l) and 7.41 ± 0.04 g/dl respectively. Decrease in these factors was observed in the trial fish as the concentration increases as a reply to the 7 day exposure to nickel. The sunset were significant at higher concentration (80% LC50) ($P<0.05$). The decrease in WBC count of the treatment groups may be due to the release of epinephrine during stress which is capable of causing the contraction of spleen [23]. Our results demonstrate that hemoglobin and hematocrit counts could be ascribed to the dissolve of erythrocytes. Similar reductions given by Vinodhini et al. [13]. When exposed fish to polluted environment under in vitro conditions. Therefore, the significant decrease in these factors is an indication of severe anemia caused by exposure of the experimental fish to nickel in the water. Vinodhini et al. [13] observed that various fish species after zinc exposure increased hematocrit levels. They ascribed such an increase in hematocrit values to increase in the size of the erythrocytes as being exhibited for chromium and zinc treated rainbow trout. Observed depression in hematocrit and hemoglobin count linked with decreased and deformed erythrocytes are obvious signs of anemia [24]. Unlike our results Srivastava and Mishra [25] reported that in *Cotisa fasciatus* after acute exposure to sublethal concentration (15 ppm) of lead RBC count were decreased, also reported results similar to ours haematocrit values and haemoglobin content were decreased. However, they recognized haemolytic anaemia on the basis of RBC lysis. The number of leukocytes in fish can also vary between individuals of a single species, depending on the conditions under which the sample of blood is taken or on the physiological conditions of the fish. This individual variation within a species is thought to be too great as to render impractical the use of white blood cell count as a diagnostic tool in fish diseases [26]. The lessen number of WBC may be the result of bio concentration of the checked metal in the kidney and liver. Other authors have associated the cause to hindering of granulopoiesis or lymphpoiesis, induced by primary or secondary changes in haematopoietic organs [23]. Similar our study Annune et al. [27-30] reported a significant increase in RBC count of *C. gariepinus* when exposed to Zinc treatment. They indicated the RBC elevation to blood cell reserve integrated with cell shrinkage as a result of osmotic alterations of blood by the action of the metal. Results of the present investigation indicated that the sub-acute nickel concentrations tested is a toxic substance in goldfish and may cause several changes in the serum biochemical parameters [31,32].

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