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Immunological, hematological and biochemical changes induced by short term exposure to cadmium in catfish (*Clarias gariepinus*)

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PEER REVIEW

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Comments

Generally, this is a potential research work in which authors have demonstrated that the hematological, biochemical and immunological changes in catfish (*Clarias gariepinus*) experimental doses exposed to cadmium. Anemia and increases in biochemical and hematological parameters were observed in higher dose cadmium exposed fish.
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ABSTRACT

Objective: To investigate the hematological, biochemical and immunological changes in catfish (*Clarias gariepinus*) (*C. gariepinus*) experimental exposed to cadmium.
Methods: *C. gariepinus* were exposed to different concentrations of cadmium (Cd) (0, 2, 5, and 10 mg/L) for 3 weeks. Blood samples were collected for assessing some hematological, biochemical and immunological studies at the end of experiment.
Results: The results showed marked normocytic normochromic anemia, leukocytosis, neutrophilia and lymphopenia in 5, 10 mg/L in cadmium exposed fish. Also the blood level activities of ALT and AST significantly increased, as well as glucose, creatinine, urea, potassium and uric acid. Meanwhile total protein, albumin and sodium were significantly decreased at 5, 10 mg/L of cadmium exposed fish. The immunological parameters in cadmium exposed experimental dose groups decreased serum bactericidal activity, lysozyme, neutrophils adhesion test as well as decreased resistance to *Aeromonas hydrophilla* with increasing exposure dose seemed to correspond with suppressive of non-specific immune functions.
Conclusions: The treatment of *C. gariepinus* with cadmium under the same conditions had immunosuppressive and decrease diseases resistance in a dose-dependent effect.

KEYWORDS
Cadmium, Immune response, Hematological parameters, Biochemical parameters, Catfish (*Clarias gariepinus*)

1. Introduction

Dissolved metals occur naturally in trace amounts in the aquatic environment; however, through industry they may be transported, concentrated, changed into other forms and are reintroduced into the aquatic system as contaminations. Consequently, fishes in contaminated areas are often exposed to much higher concentrations or to chemical forms different than those that are normally in the environment[1].

Cadmium (Cd) is a naturally occurring metallic element that is used for electroplating and galvanization processes in the production of pigments in batteries, as a chemical reagent, and in miscellaneous industrial processes[2]. The cadmium in ionic, colloidal, complexes or particulate forms is taken up by aquatic organisms. In fish, cadmium is mainly taken in through the gills while accumulation via their food seems less important[3,4].

Cadmium toxicity in freshwater fish has been extensive investigated. Anemia was documented in *Oreochromis mossambicus* and *Channa punctatus* intoxicated with different doses of cadmium[5,6] respectively. Schuwerack *et al*[7] reported leukocytosis, neutrophilia and eosinophilia in *Cyprinus carpio* (*C. carpio*) exposed to sublethal concentrations of cadmium. Alteration of some serum

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biochemical parameters attributed to liver, gill and kidney dysfunction reported by Oner *et al*[8] in *Oreochromis niloticus* (*O. niloticus*) intoxicated with cadmium. Cadmium can induce immunosuppressant in common carp (*C. carpio*), *O. niloticus*, *Ictalurus melas* and *Oreochromis aureus* which documented by[1,9-11] respectively.

The present studies to investigate the cadmium toxicity in *Clarias gariepinus* (*C. gariepinus*) are scanty. The aims of the study were to examine the hematological, biochemical and immunological changes in *C. gariepinus* exposed to short-term high levels of cadmium.

2. Material and methods

2.1. Experimental fish

One hundred and twenty, apparently healthy African catfish weighing (100±20) g were obtained from local farm, maintained in glass aquaria filled with dechlorinated tap water supplied with continuous aeration. The photoperiod was maintained on a 12:12 h light/dark schedule. The fish were acclimatized to laboratory conditions for 15 d before the start of experiment. The temperature was kept at (24±2) °C throughout the experiment. About half of the water was changed daily in all experimental aquaria. Fecal matters were siphoned out once daily. All fish were fed twice daily at 2% of their body weight along the period of experiment.

2.2. Experimental design

A total number of 120 African catfish were randomly divided into four equal groups, each containing 30 fish. The first group served as a control. The other three groups were subjected to sublethal concentration of cadmium 2 mg/L (Cd1), 5 mg/L (Cd2), and 10 mg/L (Cd3) in water all over the experimental period according to Jana and Bandyopadhyaya[12].

At the end of experiment, 3 weeks post exposure, five fish were randomly sampled from each group in 2 replicates. Blood samples were collected by heart puncture in air-dried, sterile test tubes (2 mg EDTA/mL) to study the non-specific defense mechanism, erythrogram, total and differential leukocytic count and neutrophil adhesion test. The remaining whole blood samples were centrifuged at 3 000 r/min for 5 min and serum was stored at 80 °C to be used for serum biochemical parameters, bactericidal activity and lysozyme assay. All groups were challenged with *Aeromonas hydrophila* (*A. hydrophila*) and mortality rate was recorded.

2.3. Hematological and biochemical studies

The erythrogram, (erythrocytes count, hemoglobin concentration, PCV value, blood indices, MCV, MCH and MCHC), total and differential leukocytic counts were performed according to Stoskoph[13]. Serum biochemical parameters (ALT, AST, total protein, albumin, glucose, urea, creatinine and uric acid) were estimated following standard methods using commercial kits (Spinreact, Spain). Sodium and potassium were estimated using a flame photometer (Sherwood 410, model UK).

2.4. Immunological studies

2.4.1. Superoxide anion production

The superoxide anion production of blood phagocytes was measured according to[14] with some modifications of[15]. In summary, 100 μL buffer containing poly-L-lysine solution (0.2% Sigma) were pipette into flat bottom 96-well microtitre plates. Whole blood (100 μL) was added to each well and incubated at 37 °C for 2 h, then washed with Hanks balanced salt solution (HBSS). Then 100 μL of NBT (1 μg/mL HBSS) was added containing 10^4 *Streptococcus iniae* cells. After incubation for 30 min at 37 °C, the reaction stopped by adding 100 μL of methanol and the medium was removed. The formazone in each well was dissolved with 120 μL of 2 mol/L KOH and 140 μL of DMSO and measured using a plate reader (Bio TEC, ELX800G, USA) at 630 nm, with 405 nm as reference.

2.4.2. Bactericidal activity

Serum bactericidal activity was done following the procedure of[16]. An equal volume (100 μL) of serum and bacterial suspension 2×10^8 (CFU) was mixed and incubated for 1 h at 25 °C. Blank control was also prepared by replacing serum with sterile PBS. The mixture was then diluted with sterile PBS at a ratio 1:10. The serum-bacterial mixture (100 μL) was plated in blood agar and plates were incubated for 24 h at 37 °C. The number of viable bacteria was determined by counting the colonies grown in nutrient agar plates.

2.4.3. Serum lysozyme

Serum lysozyme was determined using turbidimetric assay by the method of[17]. Briefly, the lysozyme substrate was 0.75 mg/mL of Gram positive bacterium *Micrococcus lysodeikticus* lyophilized cells (Sigma, St. Louis, MO) was suspended in 0.1 mol/L sodium phosphate/citric acid buffer, pH 5.8. Plasma or mucous (25 μL) was placed in triplicate into a microtiter plate and 175 μL of substrate solution was added to each well at 25 °C and reduction in absorbance at 450 nm read after 0 and 20 min using microplate ELISA reader (Bio TEC, ELX800G, USA). The units of lysozyme present in plasma or mucous (μg/mL) were obtained from stander curve made with lyophilized hen egg white lysozyme (Sigma).

2.4.4. Neutrophils glass-adhesion

Neutrophils glass-adherent, using nitroblue tetrazolium assay, was determined according to[18]. Briefly, within 15

min after blood samples were collected, one drop of blood using heparinized capillary hematocrit tubes was placed onto a 22-mm square coverslip. The coverslips were placed individually in Petri-dishes humid chambers and incubated for 30 min in room temperature (25 °C) to allow the neutrophils to stick to the glass. After incubation, the coverslips were gently washed with PBS (pH 7.4) and the cells were transferred upside down to a microscope slide containing a 50 μL drop of 0.2% filtrated NBT solution (Fluka Buchs, Co. Switzerland). After other 30 min of incubation, the positive, dark-blue stained cells were counted under the microscope. Two coverslips were examined for each fish. Three random fields were counted on each slide. The six fields were averaged. The mean and standard error of the mean of the fish lots were calculated.

2.5. Disease challenge

The challenge test was performed in 2 replicates (10 fish/replicate) where 20 fish from each aquarium of both control and cadmium treatment groups were inoculated with pathogenic *A. hydrophila*. The inoculation was done via *i.p.* route using 0.5 mL culture suspension of pathogenic *A. hydrophila* containing 10^8 bacteria/mL that had been previously isolated from naturally infected catfish and identified according to standard bacteriological tests, as well as for their pathogenic studied. The challenged fish from each aquarium were observed for 7 d in order to record the mortality percent.

2.6. Statistical analysis

The mean and standard error were calculated for each variable. The data were analyzed by analysis of variance (ANOVA) to identify the significantly different groups at (P<0.05) by one way ANOVA with post-hoc LSD multiple comparison test using SPSS software statistical program (SPSS for windows ver.15.00, USA).

3. Results

Erythrogram results in our work revealed a significant decrease in RBCs count, Hb and PCV values in Cd2 and Cd3 groups in compare to control group with a non significant change in group Cd1. MCV, MCH, MCHC results clarifies non significant changes in all groups under investigation. This previous results suggest presence of normocytic normochromic anemia in groups Cd2 and Cd3 (Table 1).

Total leukocyte count and neutrophil count showed a significant elevation in Cd2 and Cd3 groups with a higher elevation in group Cd3 than group Cd2 when compared to control group. Lymphocyte count revealed a significant increase in group Cd3 than control group and a non significant change in other groups. Monocyte, esinophile and basophile count showed no significant changes in all groups (Table 2).

Table 1

Erythrogram picture (mean±SE) in catfish (*C. gariepinus*) exposed to cadmium.

Groups	RBCs million/μL	Hb g/dL	PCV %	MCV fL	MCH pg	MCHC %
Control	2.51^{a}±0.14	8.98^{a}±0.34	28.9^{a}±1.12	115.1^{a}±6.80	35.8^{a}±1.75	31.2^{a}±1.15
Cd1	2.34^{a}±0.16	8.04^{a}±0.52	27.2^{a}±1.86	115.8^{a}±5.42	34.2^{a}±1.34	29.3^{a}±1.08
Cd2	1.81^{b}±0.12	6.38^{b}±0.26	20.9^{b}±1.18	115.5^{a}±7.25	35.2^{a}±1.34	30.5^{a}±2.13
Cd3	1.76^{b}±0.13	6.42^{b}±0.34	20.12^{b}±1.32^{a}	113.6^{a}±3.79	36.7^{a}±1.92	29.9^{a}±1.21

Dissimilar superscript letters in the same column show a significance (P<0.05). RBCs: red blood cells, Hb: hemoglobin, PCV: packed cell volume, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

Table 2

Leukogram picture (mean±SE) in catfish (*C. gariepinus*) exposed to cadmium.

Groups	TLC 10^3/μL	Neutrophil 10^3/μL	Lymphocyte 10^3/μL	Monocyte 10^3/μL	Esinophil 10^3/μL	Basophile 10^3/μL
Control	28.64^{c}±2.01	8.98^{c}±0.59	17.48^{a}±1.04	1.62^{a}±0.15	0.56^{a}±0.04	0.0±0.0
Cd1	27.45^{c}±2.21	9.24^{c}±0.68	16.06^{a}±1.20	1.64^{a}±0.18	0.51^{a}±0.05	0.0±0.0
Cd2	32.58^{b}±2.98	14.28^{b}±0.61	16.03^{a}±1.12	1.78^{a}±0.16	0.49^{a}±0.03	0.032±0.032
Cd3	38.51^{a}±2.10	19.58^{a}±0.98	14.53^{b}±0.98	1.92^{a}±0.21	0.48^{a}±0.03	0.0±0.0

Dissimilar superscript letters in the same column show a significance (P<0.05). TLC: total leukocytic count.

Serum biochemical parameters in our study showed a significant increase in ALT and AST activities in group Cd2 and Cd3 than control group with a non significant change between the both groups. Total protein, albumin and sodium results revealed a significant decrease in group Cd3 when compared to control group and non significant changes in other groups. Urea, creatinine, uric acid and potassium results revealed a significant increase in group Cd3 when compared to control group and non significant changes in other groups. Glucose value clarifies a significant increase in all cadmium groups than control group with higher elevation in group Cd2 and Cd3 than group Cd1 with a non significant variance between Cd2 and Cd3 groups (Table 3).

Our immunological results in cadmium exposed groups revealed a marked reduction in measurement of superoxide

Table 3

Some biochemical parameters (mean±SE) in catfish (*C. gariepinus*) exposed to cadmium.

Groups	ALT U/mL	AST U/mL	TP g/dL	Albumin g/dL	Glucose mg/dL	Urea mg/dL	Ccreatinine mg/dL	Uric acid mg/dL	Sodium mEq/L	Potassium mEq/L
Control	38.0^{b}±3.21	108^{b}±6.14	3.38^{b}±0.28	1.34^{b}±0.10	64.8^{b}±4.3	9.80^{b}±1.02	0.95^{b}±0.10	1.82^{b}±0.20	136.8^{b}±6.40	3.42^{b}±0.20
Cd1	46.2^{b}±5.14	121^{b}±9.15	3.51^{b}±0.32	1.28^{b}±0.12	96.4^{c}±5.3	11.40^{b}±0.84	0.88^{b}±0.11	1.76^{b}±0.18	131.8^{b}±5.38	3.48^{b}±0.31
Cd2	64.2^{a}±4.48	152^{a}±8.41	3.01^{ab}±0.28	1.12^{ab}±0.15	134.0^{a}±9.42	12.02^{b}±1.10	0.99^{b}±0.09	1.98^{ab}±0.16	124.0^{b}±6.20	3.58^{ab}±0.29
Cd3	71.2^{a}±6.14	168^{a}±9.84	2.74^{a}±0.21	0.81^{a}±0.11	128.0^{a}±7.10	15.60^{a}±1.12	1.26^{a}±0.12	2.45^{a}±0.28	102.0^{a}±5.14	3.96^{a}±0.18

Dissimilar superscript letters in the same column show a significance (P<0.05). ALT: alanine aminotransferase, AST: aspartate aminotransferase, TP: total protein.

anion, bactericidal activity and neutrophil glass adhesion assay in group Cd3 with a non significant change in other groups in compare to control group. Lysozyme value show a significant decrease in groups Cd2 and Cd3 than control group with non significant change between the both groups. In accordance with these results the mortality rate increased in all groups exposed to cadmium with higher mortality rate recorded in group Cd3 than other groups (Table 4).

Table 4
Some immunological parameters (mean±SE) in catfish (*C. gariepinus*) exposed to cadmium.

Groups	Superoxide anion (O.D)	Bactericidal activity % (CFU)	Lysozyme μg/mL	Neutrophil glass adhesion	Mortality %
Control	0.18^{b}±0.05	24.1^{b}±1.50	9.98^{b}±0.28	12.3^{b}±1.14	63.1^{c}±4.1
Cd1	0.19^{b}±0.07	22.4^{b}±1.98	9.57^{b}±0.31	10.2^{b}±0.98	70.0^{cb}±6.1
Cd2	0.16^{b}±0.04	20.8^{b}±1.42	8.12^{a}±0.24	11.4^{b}±1.12	83.0^{b}±3.1
Cd3	0.10^{a}±0.02	14.1^{a}±1.42	8.01^{a}±0.21	8.4^{a}±0.94	93.0^{a}±2.1

Dissimilar superscript letters in the same column show a significance (P<0.05).

4. Discussion

Our result showed normocytic normochromic anemia in Cd2 and Cd3 exposed to 5 and 10 mg/L of cadmium respectively in compare to control group. The kidney is principle hemopoietic tissues in teleost fish[19]. Cadmium induces renal damage in silver *Crucian carp* fish documented by[20]. Our result in accordance with[5,6] who observed anemia in *Oreochromis mossambicus* and *Channa punctatus* respectively. In the same line anemia after exposed to heavy metals was recorded in *Leporinus obtusidens*, *Hypentelium nigricans*, *Hoplias malabaricus* and *Channa punctatus* by Gioda *et al.*, Schmitt *et al.*, Oliveira *et al.* and Tyagi and Srivastava[21-24] respectively.

The leukogram in our work revealed leukocytosis, neutrophilia and lymphopenia in group Cd3 in compare to control group. Cadmium persuades neutrophilia in *C. carpio* was reported by[7]. Leukocytosis in *Hoplias malabaricus, Channa punctatus, Prochilodus scrofa* and *C. carpio* was demounted by[23-26] respectively.

Liver transaminase enzymes (ALT and AST) were elevated in both groups Cd2 and Cd3 in compared to control group. This elevation could be attributed to liver damage. Liver damage included swollen and ruptured parenchymal cells, loss of cord structure, vacuoles filled with cellular debris, focal necrosis, and a significant increase in Kupffer cells as a result of cadmium intoxication reported by[27] in *Chondrostoma nasus*. Oner *et al*[8] observed increase serum ALT and AST in *O. niloticus* long term exposed to cadmium. In the same aspect ALT plasma level significantly increased in *C. carpio* exposed to cadmium[28]. Meanwhile Teles *et al*[29] reported insignificant increase ALT in *Anguilla anguilla* caged in heavy metals polluted sites.

Hypoproteinemia and hypoalbuminemia observed in group Cd3 only and could be due to liver and kidney damage. Oronsaye[30], documented kidney damage in *Gasterosteus aculeatus* exposed to cadmium. In contrast to our result Oner *et al*[8] observed insignificant change in total plasma protein in *O. niloticus* intoxicated with cadmium.

Hyperglycemia recorded in all cadmium exposed groups. Stress in fish accompanied with hyperglycemia due to increase glycogenolysis[13]. Teles *et al*[29] observed increase cortisol blood level in *Anguilla anguilla* caged in heavy metals polluted sites. Hyperglycemia reported by[8] in *O. niloticus* exposed to cadmium, as well as by[31,32] in *Coregonus clupeaformis* and *C. carpio* were exposed to heavy metals.

The elevation of urea in group Cd3 was reported in this study. Urea in fish is produced by liver, it is excreted primarily by the gills rather more the kidney[13]. The elevation of urea in our work may be attributed to gill dysfunction. Gill damage as a result of cadmium intoxication reported in *Gasterosteus aculeatus* by[30]. In the same aspect, Oner *et al*[8] reported increased blood urea in cadmium exposed fish (*O. niloticus*).

Regarding to kidney function test, creatinine significant increased in group Cd3 only. Renal damage of the sea bass *Dicentrarchus labrax* and marine bony fishes exposed to cadmium were approved by[33-34]. Yang and Chen[32] recorded elevation creatinine blood level in *C. carpio* exposed to gallium.

Uric acid is formed by fish from exogenous and endogenous purines. It is converted in the liver to urea for excretion by the gills[13]. Elevation of uric acid levels in higher dose cadmium treated group Cd3 could be attributed to liver damage induced by cadmium. Shi *et al*. documented liver damage in *Carassius auratus* exposed to cadmium[35].

Hyponatremia and hyperkalemia in high dose cadmium exposed group Cd3 could be attributed to gill dysfunction[13]. Gill damage has been reported in *Puntius gonionotus, Sparus aurata* and by[36,37]. In the same line, sodium and chloride levels as well as plasma osmolality were significantly reduced in *C. carpio* exposed to cadmium[28].

Our immunological results showed immunosuppressive in cadmium exposed groups Cd2 and Cd3 compared with control group. Superoxide anion production, bactericidal activity, and neutrophils adhesion cell in group Cd3 as well as serum lysozyme in both groups Cd2 and Cd3. Immunosuppressive effect of heavy metals in fish has been documented. NBT reduction assay and serum lysozyme was significantly decreased in Javanese carp (*Puntius gonionotus*) exposed to copper toxicity[38]. Kidney lysozyme was decreased in common carp exposed to cadmium[39]. Also Betoulle *et al*. observed reduce phagocyte oxidative burst activity by gallium in *C. carpio*[40]. Mortality rate in cadmium exposed group challenged with *A. hydrophila* is dose dependent. Increase mortality rate could be attributed to immunosuppressive effect of cadmium on catfish (*C. gariepinus*). Similarly, decrease diseases resistance was

recorded in zebrafish (*Brachydanio rerio*) and Javanese carp (*Puntius gonionotus*) challenged with *Listeria* infection and *A. hydrophila*[41,38] respectively.

We concluded that cadmium had different organs damage and immunosuppressive effect in catfish (*C. gariepinus*) and subsequent decrease diseases resistance.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Cadmium is a nonessential heavy metal but it has accumulative polluting effect, and causes toxicity to aquatic organisms even in minut concentrations. Therefore, it is regarded as one of the most toxic elements in the environment. As shown the previous literatures, cadmium toxicity in freshwater fish has been extensive investigated. Specially, alterations of some serum biochemical parameters in liver, gill and kidney of fish were examined. In this study, hematological, biochemical and immunological changes in catfish after exposure to sub-lethal doses of cadmium (Cd). This study has a potential significant about determination of Cd toxicity in the most fish.

Research frontiers

The manuscript describes the hematological, biochemical and immunological changes in *C. gariepinus* were exposed to different concentrations (0, 2, 5, and 10 mg/L) of cadmium. Blood samples were collected to determine hematological, biochemical and immunological parameters.

Related reports

The occurrence of cadmium in considerably toxic amounts was reported by earlier workers in various aquatic ecosystems. Cadmium is reported to cause anemia in a variety of fish species at low as well as high concentrations after entering into the organism of fresh water fishes through the gills. The literature data shows that they have evidence in anemia formation and higher biochemical and hematological parameters after exposure to 10 mg/L dose of cadmium.

Innovations and breakthroughs

This scientific study support and suggest a dose-dependent effect of Cd had immunosuppressive and decrease diseases resistance.

Applications

This scientific study support and suggest the treatment of *C. gariepinus* with cadmium under the same conditions had immunosuppressive and decrease diseases resistance in a dose-dependent effect.

Peer review

Generally, this is a potential research work in which authors have demonstrated that the hematological, biochemical and immunological changes in catfish (*C. gariepinus*) experimental doses exposed to cadmium. Anemia and increases in biochemical and hematological parameters were observed in higher dose cadmium exposed fish.

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