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Ameliorative Effect of Ascorbic Acid on Hematological Parameters Due to Cadmium Toxicity in *Cyprinus carpio*

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Abstract: The present study showed the protective effects of Vitamin Cby reducing the toxicity of cadmium on the hematological parameters in common carp during exposure period. The results of present study showed significant differences at P≤0.05 between treatments with cadmium and control, ascorbic acid only and ascorbic acidic cadmium on hematological parameters. The results also showed the effect of Vitamin C (ascorbic acid) to reduce cadmium toxicity after 7 to 14 days after chronic exposure to $CdCl_2$ increased white blood cells number, mean corpuscular volume, Mean Corpuscular Haemoglobin and decreased RBC, Hb content, PCV and the mean corpuscular hemoglobin concentration as compared to control were found. The exposure of cadmium with ascorbic acid the WBC, RBC decreased and Hb contents, RBC, the packed cell volume PCV increase values of these parameters as that in control groups when compared with cadmium exposure groups.

Keywords: Ascorbic acid, Cadmium toxicity, Common carp, Hematological parameters

The Heavy metals concentration trend to increase more and more in the all environment spheres especially in the aquatic environment. Fishes have been recognized as good accumulators of organic and inorganic pollutants (Eneji et al 2011). Aquatic organisms such as fish accumulate metals from water or sediment with concentrations higher many times than the concentrations of these metals in the water, also they can concentrate metals at different levels in different organs, and these heavy metals acquired by the chain of food as a result of pollution will be posed chemical hazards and threatening to human. However, low levels of heavy metals, such as copper, cobalt, zinc, iron and manganese are crucial for activity of enzyme activity and many other biological processes. Other heavy metals, for example cadmium and lead are not recognized to have essential role in living organisms and they are toxic even at low concentrations, while the essential metals also developed to become toxic at high level of concentrations, the consequences of heavy metal pollution can be hazardous to humans through food (Sani 2011).

Fish is the most susceptible aquatic animals to the heavy metals. The physical and chemical parameters of the environment in which the fish resides appeared to affect the rate of bioaccumulation of trace elements (Cao et al 2010). Normally, fish may be considered as one of the healthiest and cheapest source of protein due to it's higher cysteine amino acid composition than most other sources of protein. The effects of exposure to any hazardous constituent depend on the dose, exposure time, exposure mode, and presence of other chemicals (Adekola et al 2007). Therefore, heavy metals monitoring in aquatic environment are very important, especially for breeding of fish. Increased loads of toxic metals in waste water may increase the risk of water contamination in the current study, the hematological parameters were studied to evaluate the cadmium chloride impacts on carp fish.

Red blood corpuscle RBC and hemoglobin can be used as an indicator foranemia, f1uid volume disturbances. In general, heavy metals have acute effect in hematological parameters and cause reduction in RBC and Hb. Further, anemia could be happened due to plasma increasing volume caused by disturbed water balance and will decrease RBC production (Saraswathi et al 2003). In addition, as the haemoglobin (Hb) is the main constituents of blood, which transport of oxygen throughout the blood capillaries, decreasing the haemoglobin level might reduce the biological activity of fishes due to stress syndrome. Researchers have reported alteration in RBC and Hb level (Garg et al 1989, Magare and Patil 2000), and also in the present investigation on the protective effect of ascorbic acid to reduce the toxicity of cadmium on hematological parameters in *Cyprinu scarpio.*

MATERIAL AND METHODS

Experimental fishes: The freshwater common carp *Cyprinu scarpio* with a size range of 16-20cm and, weighing 54 ±4 g irrespective of their sex, have been chosen as the test organism in the present study. Before the exposure

experiment fishes were acclimatized to the laboratory conditions in large fiber glass tanks with unchlorinated ground water for 3 days at a room temperature of 28 ± 20C°. In order to avoiding the carp fishes overcrowding as its benthic in nature, five fishes putted in each tank. After every 24h, water was changed completely. To prevent the escaping of fishes, tanks were covered with net. No feed used during the experiment period (14 days).

Chemicals and reagents: Cadmium chloride ($CdCl_2$) and vitamin C (L-Ascorbic acid) were obtained from the environmental laboratory at the Collage of Science, Babylon University, then the $CdCl_2$ was dissolved in H_2O (1 mg l^{-1}) and the L-Ascorbic acid was added with a ratio of 50 mg l^{-1} (Al Maamouri 2011). The other chemicals (reagent grade) were obtained from local scientific distributors in Iraq.

Animals and experimental design: 60 healthy adult individuals of common carp *Cyprinu scarpio* (60 ± 5 g) were obtained from a registered fish farm in Babylon province. 3 days period of time was used for fish acclimation. Then it spread in glass aquaria with (dimensions, 90 × 30 × 50 cm) filled with 120 L fresh water(de-chlorinated); the carp fishes were kept in a laboratory, setting with a photoperiod, light (12 hours) and dark cycle (12 hours). Furthermore, temperature was maintained of 28 ± 2°C and then water aquarium was aerated. Water dissolved oxygen level (6.5 mg l^{-1}), pH (6.9 ± 0.4), and electrical conductivity (219 ± 2 µm ho cm^{-1}) of aquaria water which measured using multi meter; conditions were closely monitored and kept stable during the experiments. They were subdivided into four groups with 15 individuals for each one. Control group were given tap water only .Second group were given Vitamin C at a dose 50 mg l^{-1}. Third groups were given cadmium at a dose 1 mgl^{-1} and fourth groups were (Vitamin C and cadmium) given Vitamin C 50 mg l^{-1} prior three hours of cadmium in concentration 1 mg l^{-1} administration. *Cyprinu scarpio* was exposed to sub-lethal concentration of Cadmium for 14 days. For sub-lethal exposure experiment, three replicates were maintained. The water was changed every day, so that the concentration of cadmium chloride remained the same during the experimental period.

Haematological studies: Blood was taken at the end of 7 day and 14 day by catching each fish very gently using a small net, one by one with minimal disturbances. Each fish wrapped in a clean and dry towel was trapped, the posterior half of its body was cleaned with coarse clean paper. The blood samples were collected using severance of caudal peduncle and placed in Eppendorf tubes holding 1% (EDTA) (Mgbenka et al 2003). Haematological parameters were evaluated using the methods of Hesser (1960) and Blaxhall and Daisley (1973).

Red Blood Corpuscular (RBC) measurement: Counting of RBC was done with a Neubauer chamber, Sohn and Henry (1969). Red glass bead pipette was used for indicting the counting chamber (triplicate).

Haemoglobin (Hb) content: Cyanmethemoglobin method was performed for determination of Hb (Dacie and Lewis 1968). 0.02 ml of blood sample was pipetted into Darbkinp's reagent (5ml) and shaken then allowed to settle for 10 min. after that, absorbance was measured at 540 nm wavelength with spectrophotometer against a reagent blank.

Estimation of Packed Cell Volume (PCV): Evaluation of PVC was achieved using micro-haematocrit method (Snieszko 1960).Non-clotted blood specimens (Heparinized) were collected in even bored capillaries. It was indorsed to run 1/2 - 3/4 lengths of capillary tube closed with sealing wax on opposite sides and then assigned to a high speed micro-haematocrit centrifuge (12000 rpm) for 5 min.

White Blood Corpuscles (WBC) measurement: WBCs were measure during the method described by Donald Hunter and Bomford (1963).

Statistical analysis: Data analysis was done using SPSS ver. 23, and present as mean SD. It was considered as significant when P value was 0.05.

RESULTS AND DISCUSSION

The results of presents study were to determine the impact of cadmium toxicity on *C. carpio* and ameliorative effect by L-ascorbic acid on the levels of hematological indices, the hematological indices in presents study showed significant differences at p≤0.05 among the treatments. Ascorbic acid alone has no effect on all hematological markers as compared with treatments of cadmium

RBCs: Red blood cells number in control was 3.5 and 3.3x 10^6 mm^{-3} at 7 and 14 days respectively. While in cadmium exposure, its number was significantly decreased (2.62–1.46) 10^6 mm^{-3} at 7 and 14 days, respectively as compared with treatment groups with ascorbic acid which its numbers significantly increased at 7 and 14 days (3.12-3.3) 10^6 mm^{-3}, respectively (Fig. 1).

WBC: The number of white blood cells in cadmium exposure significantly increased at 7 and 14 days and reached to 4.32–5.87x 10^6 mm^{-3}. While it was decreased significantly in treatment with ascorbic acid along with cadmium exposure to be (3.87 – 3.11 x 10^6/ mm^3). The WBC for the control treatment was (3.22- 3.17x 10^6 mm^{-3}) (Fig. 2).

Hb: The Hb level in treatment with ascorbic acid with cadmium exposure reached at 7 and 14 days (9.1-8.9 g 100 ml^{-1}) as compared with control groups at 7 and 14 days (9.6-10.8) g 100 ml^{-1}, respectively. The level of hemoglobin percent in cadmium exposure was significantly decreased at

7 and 14 days to reach (8.3-7.4) g 100 ml^{-1}(Fig. 3).

PCV: The packed cell volume PCV% in the control was (34.6-33.6%) at 7 and 14 days and its percentage in cadmium treatment were significantly decreased from 29.4%-26% and vitamin C+ cadmium treatments were from 32.9-33.8% at 7 and 14 days, respectively (Fig. 4).

The percent of MCV in cadmium exposure was significantly increased at 7 and 14 days and reached to 112.21-178.08 μm^3 While its concentration in treatment with ascorbic acid plus cadmium exposure was significantly decreased at 7 and 14 days (108.33-97.57 μm^3) as compared with control groups at 7 and 14 days (98.85-108.33 μm^3), respectively (Fig. 5).

The MCH in cadmium exposure was 31.67-44.8 pg, while in treatment with vitamin C+ cadmium MCH was reached 29.16-31.98 pg and in control was ranged between 3085-30.35 pg at 7 and 14 days exposure respectively (Fig. 6). The MCHC in cadmium exposure was significantly increased at 7 and 14 days and reached 28.23 and 16.85%, respectively. While its concentration in treatment with ascorbic acid plus cadmium exposure decreased at 7 and 14 days (26.92-26.23%) as compared with control groups at 7 and 14 days (31.21-25.8%), respectively (Fig. 7).

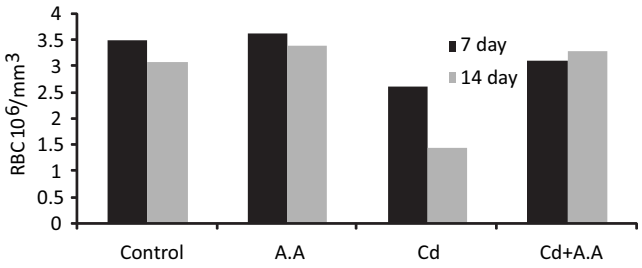

Fig. 1. Red blood corpuscular counts 10^6 mm^{-3} in bloods of *C. carpio* during period of exposure

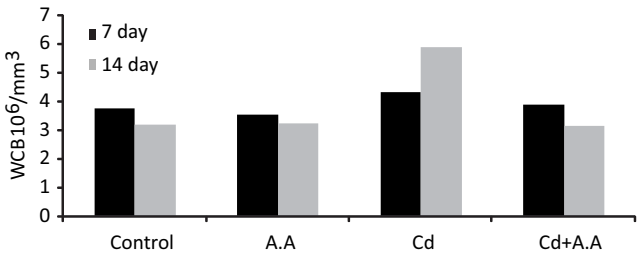

Fig. 2. White blood cells counts 10^6 mm^{-3} in bloods of *C. carpio* during exposure period

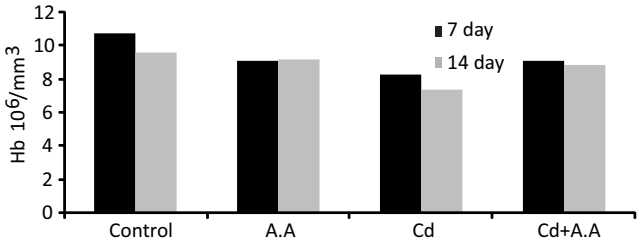

Fig. 3. Hb 10^6 mm^{-3} in bloods of *C. carpio* during period of exposure

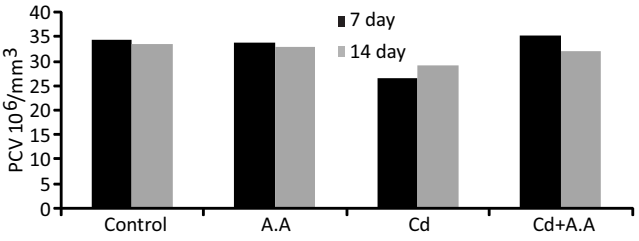

Fig. 4. PCV 10^6 mm^{-3} in bloods of *C. carpio* during period of exposure

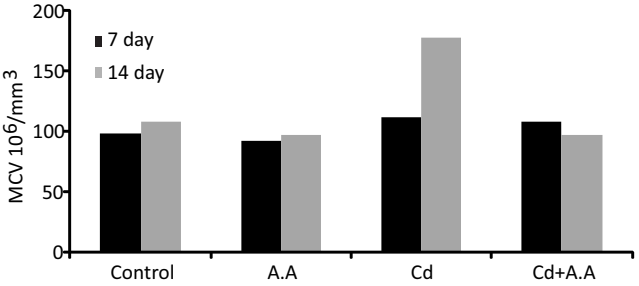

Fig. 5. MCV 10^6 mm^{-3} in bloods of *C. carpio* during period of exposure

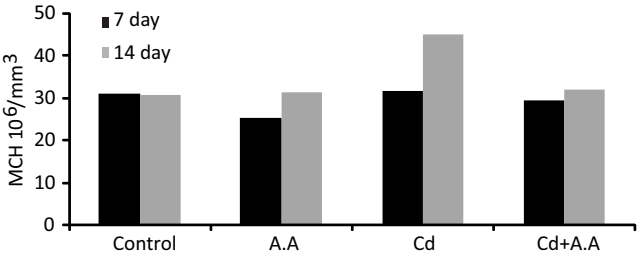

Fig. 6. MCH 10^6 mm^{-3} in bloods of *C. carpio* during period of exposure

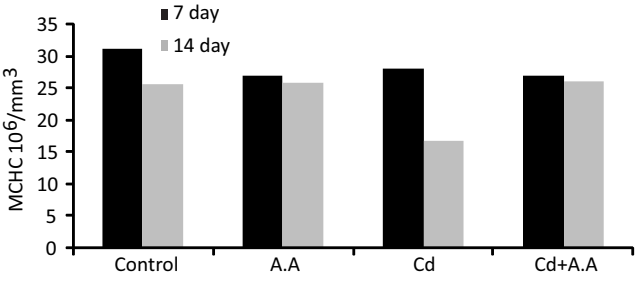

Fig. 7. MCHC% 10^6 mm^{-3} in bloods of *C. carpio* during period of exposure

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