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The effect of Ascorbic acid (Vitamin C) on lead – induced alterations on RBCs of common carp, Cyprinus carpio

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ABSTRACT

Lead is reported as heavy metal that affects the blood profile of aquatic species. The present study was performed to examine the hematological toxicity ad to investigate whether Vitamin C can protect RBC and WBC count against lead poisoning in Cyprinus carpio (Common Carp). During the present experimental period, all the test groups were exposed to various concentrations of lead nitrate (5, 10, 15 and 20 ppm) 450 mg/l of Vitamin C. During the current research period, RBC count, WBC values were analyzed to access the changes in other allied blood parameters. The fishes reared for the period of 15, 30, 60 and 90 days with well-oxygenated lead nitrate-free water were treated as controls and the blood samples were collected from all the treatments at set durations. Parameters assessed included survival rate, mortality, hematological profile. All fish survived, indicating that common carp are able to cope with these low metal concentrations, at least during a one-week exposure. The sub-lethal concentrations of lead nitrates were recognized to radically change the haematological values in common carp, while it was observed that Ascorbic acid plays an interpretative role to sustain the blood parameters and thereby the health of fish. The outcomes during the current research tenure led to some vital outcomes, which may be indispensable for monitoring the prime health conditions of the fish common carp.

Keywords: Common carp; heavy metal; Vitamin C; Haematological profile.

Introduction

Over the past three decades, heavy metals concentration trend to increase more and more in all environmental spheres, especially in the aquatic environment. there is an increasing global concern over the public health impact attributed to direct and indirect environmental pollution, particularly, the global burden of disease. Since the world health organization (WHO) estimates that, about a quarter of diseases faced by mankind today is due to prolonged exposure to environmental pollution [37]. The aquatic ecosystem is continuously under threat as it is the final receptor and sink of waste streams. As the human population grew, the demand for food made available to everyone has risen. The production of fish and shellfish has been much greater than before as both are the first–rate supplement of protein. Internationally, humans get (25%) of their protein from fish [5]. Fish living in a polluted environment can accumulate these metals via food, or via direct uptake from water through the gills [28].

Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments [25]. Polluted water has a direct impact on living organisms and biological equilibrium. The reaction and survival of organisms exposed to heavy metals depend not only on the biological state of the organisms but also on the toxicity and exposure time and type of the toxicant [36]. There are various pollutants leading to heavy metal pollution, development of industry, mining activities and agriculture gave rise to an increase in these metal pollution in the aquatic environment Sources of food and feed contamination are soil, industrial polluted air and agricultural sources such as lead-containing pesticides, phosphate fertilizers and food processes Bersenyi (2003). The world's major dilemma because these elements are entering the aquatic ecosystem through various sources like the use of insecticides, pesticides, fertilizers, and leaching [10]. Trace elements may enter the body of fish through the skin, gills, and mouth in the form of particles taken as food by the fish. Once they are engulfed, they pass from the bloodstream and are delivered to the liver or bones as a storage product [27]. As reported by Bansal (1979) in fishes the route of heavy metals is either through the gills or mouth hence into the blood organs and organ system to investigate firstly their physiological changes and metabolic alterations. As blood is the pathophysiological reflector of the body and therefore haematological parameters is an asset in diagnosing the functional and structural status of body organs exposed to toxicants. Decrease in haematological profile in malathion-exposed freshwater carp, *Cyprinus carpio* reported by Ramesh and Mahavalaramanujam, (1992). Generally, as the concentration of metals increases in the environment, fish accumulate higher levels in their tissues [2]. When the intake is not balanced with excretion processes and detoxification mechanisms, metals can show their toxic effects Handy (2003). Gills, considering that they are in direct contact with the aquatic environment, are the main entrance for dissolved substances, Heath, (1995). These substances can subsequently reach different organs such as the liver, which is the main organ for metal detoxification, through the circulatory system. When the carrying capacity of the liver is exceeded, it can be stored in other tissues such as muscle. Metal accumulation in the muscle is generally low because it is not a metabolically active tissue, but it is important in transferring the metals through the food chain [35]. Metals present in the aquatic environment can be taken up via common uptake routes, and interact with each other, and this interaction can affect bioaccumulation and toxicity [21]. For example, Cd and Zn have a comparable electron configuration and a high affinity for molecules containing -SH groups, therefore competition between $Zn2+$ and $Cd2+$ ions is expected for the uptake [7]. Moreover, Cd uptake can also be reduced by the presence of Cu [21]. Fishes have been recognized as good accumulators of organic and inorganic pollutants [38]. 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 are acquired by the chain of food as a result of pollution will be posed chemical hazards and threatening to human contamination of water by pesticides can have deleterious effects on aquatic biota, particularly in fishes reported by [22].

Material and Methods

Experimental Fish

Common carp, Cyprinus carpio Linnaeus (1758) belongs to the Cyprinidae family. It's a freshwater species but is present in brackish water also. *Cyprinus carpio* is large minnow species exotic native to Europe and Asia. It's distributed around the world and is hardy in nature and can tolerate a wide variety of conditions through the IUCN Red list status has listed common carp under the "vulnerable" category. Medium-sized freshwater fish, measuring around 12-18cm and weight about 80- 120 grams,) *Cyprinus carpio* were collected from a local fish market and acclimatized in laboratory conditions for 15 days. The Physico-chemical parameters by methods [4].

Determination of LC50

Seven different concentrations of Lead nitrate were used to access its effect on the mortality of *Common carp* and to determine the LC50 of the Lead nitrate, during the present experimental period. 10 mg/l to 60 mg/l Lead nitrate was used to assess the rate of mortality at each concentration. 60 mg/l was seen to be LC100 for Lead nitrate. 50% mortality was recorded in experimental groups exposed to 30-40 mg/l of Lead nitrate. Hence the acute 96h LC50 value for the present experimental fish, *Common carp* was calculated to be 35 mg/l (ppm).

Experimental design

Fishes were divided into various groups during the present experimental period. All the test groups were exposed to various concentrations of lead nitrate (5, 10, 15 and 20 ppm) and the other group received lead nitrate concentration and 450 mg/l of Vitamin C at the end of the experiment, fishes were sacrificed and the samples of blood were taken from the caudal vein of fish. Fishes showing no respiratory movement and response to tactile stimuli were considered dead and removed immediately. Percent mortality was calculated and the values were transformed into a probit scale and analyzed as per Finney (1971).

Results and Discussion

Since previous studies showed that fishes are the most sensitive to aquatic toxicants and they are the best bio-indicators for the aquatic environment. In the recent past [**33**] have found heavy metal accumulation in five organs of *Labeo rohita, Cirrhinus mrigala* and *Catla catla* captured from different industrialized areas of Lahore. Similarly [**12**] found toxic effects of dense ores in fish and higher animals.

Fish blood parameters are important in diagnosing the structural and functional status of the fish exposed to toxicants. Blood analysis is ought to be the most affirmative tool to assess the status of the health of any animal. In the case of fish, blood is the best indicator of the stress factor, revealed through fluctuation in parameters. During the present study period, the common carp exposed to various sublethal concentrations of lead nitrate were investigated for RBC (X 106/μL) and (WBC) of course the heavy metals are change the blood parameters depending upon the quantity of the heavy metals and the duration of exposure.

Ascorbic acid (vitamin C) as a reducing agent has reduced oxidative stress induced by heavy metals; results of this research show that. Application of vitamin C has been achieved at a significant level to reduce the effects of heavy metal intoxication in all researched parameters (MDA, GST, AST, ALP and TP). Ascorbic acid is a water-soluble vitamin and is required for collagen and bone formation [**14**]. Vitamin C indicates a positive role in detoxification behaves as an efficient chelator and is an antioxidant that plays an important role in the intracellular oxidation-reduction system and in the binding of free radicals produced endogenously [**13**] and [**24**]. The present work revealed that the sub lethal concentrations of lead nitrates radically changed the haematological values in common carp, while as it was observed that Vitamin C plays a critical role to stabilize the haematological parameters and thereby the health of fish. The outcomes during the current research tenure led to some vital outcomes, which may be indispensable for monitoring the prime health conditions of the fish.

Haematological changes in Common carp exposed to various concentrations of Lead nitrate and the protective role of Vitamin C

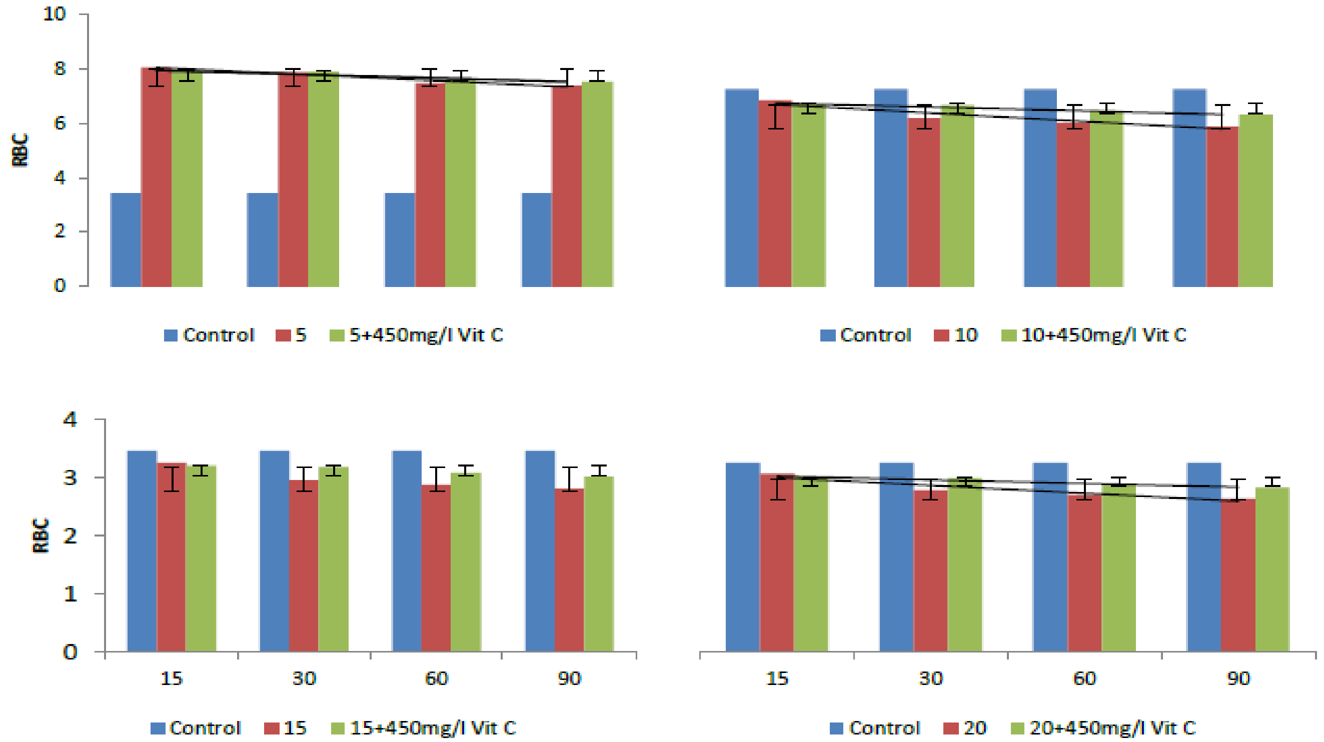
During the current research period, RBC count, and WBC values were analyzed to access the changes in allied blood parameters. The fishes reared for the period of 15, 30, 60 and 90 days with well-oxygenated lead nitrate-free water were treated as controls and the blood samples were collected from all the treatments at set durations. Experimental period all the test groups were exposed to various concentrations of lead nitrate (5, 10, 15 and 20 ppm) alone and with lead nitrate and 450 mg/l of Vitamin C. The protective role of Vitamin C has been identified by many workers including *Chew, 1995; Sae-Yong et al., 2002; Khara et al., 2016 and Nourain et al., 2019.* The sub-lethal concentrations of lead nitrates were recognized to radically change the haematological values in common carp, while it was observed that Vitamin C plays a critical role to stabilize the haematological parameters and thereby the health of fish. The outcomes during the current research tenure led to some vital outcomes, which may be indispensable for monitoring the prime health conditions of the fish.

Tabel 1: Statistical interpretation in RBC's of *Cyprinus carpio* with respect to exposure of various concentrations of Lead Nitrate and Vitamin C as Protector

Lead Nitrate Conc. PPM	Control	Duration of Exposure			
		15 days PE	30 days PE	60 days PE	90 days PE
5.0		3.35±0.04(↓2.89)^{a,b}	3.19±0.22(↓7.53)^{b,c}	2.98±0.41(↓13.6)^{a}	2.88±0.52(↓16.5)^{a,b}
5.0 with Vit C 450mg/l		3.29±0.21(↑4.63)^{c,d}	3.24±0.14(↑6.08)^{a,b}	3.10±0.23(↑10.2)^{ab}	3.05±0.24(↑11.6)^{c,d}
CoVar		0.005	0.033	0.110	0.162
10.0		3.25±0.35(↓5.79)^{a,c}	2.95±0.29(↓14.5)^{a}	2.87±0.21(↓16.8)^{a,d}	2.80±0.22(↓16.5)^{b,d}
10.0 with Vit C 450mg/l	3.45±0.12	3.19±0.15(↓7.53)^{a,c}	3.17±0.31(↑8.11)^{a,c}	3.07±0.24(↑11.0)^{a,d}	3.00±0.14(↑13.0)^{b,c}
CoVar		0.02	0.125	0.168	0.211
15.0		3.05±0.36(↓11.6)^{b,c}	3.01±0.12(↓12.7)^{a,d}	2.82±0.11(↓18.3)^{bd}	2.65±0.21(↓23.2)^{a,c}
15.0 with Vit C 450mg/l		3.17±0.37(↑8.11)^{a,c}	3.12±0.11(↑9.56)^{a,c}	3.07±0.32(↑11.0)^{b,c}	2.90±0.22(↑15.9)^{b,c}
CoVar		0.08	0.096	0.198	0.32
20.0		2.85±0.36(↓17.4)^{b,d}	2.63±0.25(↓23.7)^{a,c}	2.45±0.45(↓29.0)^{bc}	2.21±0.14(↓35.9)^{b,d}
20.0 with Vit C 450mg/l		3.05±0.11(↑11.6)^{a,c}	2.85±0.21(↑17.4)^{a,d}	2.72±0.24(↑21.2)^{b,c}	2.68±0.14(↑22.3)^{c,d}
CoVar		0.18	0.336	0.50	0.768

(↓): Percent depression; (↑): Percent Stimulation a, b, c, d Values with no common superscript differ significantly ($P<0.05$)

Fig. 1: Graphical representation of fluctuation in RBC content of *Cyprinus carpio* on exposure to different concentrations of Lead Nitrate and protective role of Vitamin C



Effect of treating Red Blood Corpuscles (x 106/µL)

Depicts the variations in RBC content in *Common carp* exposed to various concentrations of Lead nitrate after 15, 30, 60 and 90 days and is statistically presented in **table 1** depicts the statistical analysis of the variations in red blood cell counts in exposure groups treated with 450 mg/l Vitamin C. The mean±SD value of RBC (Red Blood Corpuscles) expressed in x 106/µL was 3.45±0.12 in the control group. The fishes exposed to 5.0 ppm Lead nitrate (16.6% of 96 h LC50) for a period of 15 days showed a significant (P<0.01, 0.001) decrease in RBC count 3.35± 0.04 (↓2.89), as compared to the control group, which was more or less similar in vitamin C treated group (3.29± 0.21 (↑4.63)), recording a covariance of 0.005 between the groups. The fishes showed a significant (P<0.01, 0.005) depression in RBC count post 30 days exposure 3.19± 0.22 (↓7.53), which showed a significant (P<0.05, 0.01) decrease to the value of 2.88± 0.52 (↓16.5) in fishes exposed for a period of 90 days. The protective role of vitamin C depicted in test groups was identified by the increased RBC counts in the protected groups as compared to the exposure groups only. Although there was a significant depression in RBC count in protected groups also, the values were improved as compared to the test groups. As is evident from the table, a significant (P<0.005, 0.001) increase was observed in RBC count (3.05± 0.24 (↑11.6)) in fishes protected with Vitamin C, 90 days PE, recording a covariance of 0.162 between the groups. Generally, as the dose and duration of lead exposure increased, the red blood cell count decreased and the count decreased very meagerly in test fishes protected by vitamin C, however, the duration showed a negative impact on the red cell count. The graphical demonstration of changes between the exposure groups based on duration is presented in **Figure 1**, which depicts a correlative change in RBC count via a linear trend line between the test groups and the visual impact of duration on the fluctuations in red cell count.

The test group exposed to 10 ppm (33.0% of 96 h LC50) lead nitrate over the duration of 90 days showed remarkable fluctuations in RBC count. The fishes showed a significant (P<0.05, 0.005) decrease in the value of RBC (3.25±0.35 (↓5.79)) during the first 15 days of exposure, which got drastically reduced to (2.80±0.22 (↓16.5)) in fishes exposed over a period of 90 days. However significant (P<0.05, 0.005) decrease was reported in RBC count in 15 days PE group protected with vitamin C (3.19±0.15 (↓7.53)), showing a covariance of 0.02

between the groups. A significant (P<0.05, 0.005) decrease was reported in RBC count in 90 days PE Vitamin C protected group (3.00 ± 0.14 (↑13.0)), as compared to the test group (2.80 ± 0.22 (↓16.5)), recording a covariance of 0.211. In the present test group, although there was a steep depression in RBC count in the treated group as compared to the control group, however, the protected group showed an increase from 8.11 to 13.0% as compared to the treated group.

More or less similar changes were reported in fishes exposed to 15 ppm (50.0% of 96 h LC50) lead nitrate. The fishes showed a significant (P<0.01, 0.005) decrease in the value of RBC (3.05±0.36 (↓11.6)) in the first 15 days of exposure, which got significantly (P<0.05, 0.005) reduced to (2.65±0.21 (↓23.2)) in fishes exposed to lead nitrate over a period of 90 days. However significant (P<0.05, 0.005) increase (8.11%) was reported in RBC count in the same test group 15 days PE group protected with vitamin C (3.17±0.37 (↑8.11)), showing a covariance of 0.08 between the groups. A significant (P<0.01, 0.005) increase (15.9%) was reported in RBC count in 90 days PE Vitamin C protected group (2.90± 0.22 (↑15.9)), as compared with the test group (showing a decline of 23.2%, as compared to the control group), recording a covariance of 0.32.

Fishes exposed to 20 ppm (66.6% of 96 h LC50) lead nitrate over the duration of 90 days exhibited significant depression in RBC count during the exposure time. The fishes showed a significant decrease (P<0.01, 0.001) in the value of RBC (2.85±0.36 (↓17.4)) in the first 15 days of exposure, which got significantly (P<0.01, 0.001) reduced to (2.21±0.14 (↓35.9)) in fishes exposed over a period of 90 days. However significant (P<0.05, 0.005) increase (11.6%) was reported (As compared to the control group) in RBC count in 15 days PE group protected with vitamin C (3.05±0.11 (↑11.6)), showing a covariance of 0.18 between the groups. A significant (P<0.005, 0.001) increase (22.3%) was reported in RBC count in 90 days PE Vitamin C protected group (2.68±0.14 (↑22.3)), as compared to the test group (2.21±0.14 (↓35.9)), recording a covariance of 0.768. There was a considerable decline in RBC count in lead-treated fish groups over a period of 90 days, however, the RBCs tended to increase in Vitamin C-treated groups, as compared to the test groups, but were considerably reduced as compared to the test group, thereby documenting the protective role of Vitamin C. The graphical representation showing the linearity in changes in RBC is depicted in figure 1. One-way ANOVA between the RBC counts in fishes exposed to various concentrations.

The practice and authentication of fish health monitoring tools have become progressively palpable due to the expansion of aquaculture [**32:16:8**]. The complete blood cell count (CBC) is an essential and authoritative diagnostic tool of a minimum database [**19**]. Exposure of fish to heavy metals can bring either increase or decrease in haematological parameters. Haematological indicators like haemoglobin (Hb) content, total red blood cell count (tRBC), total white blood cell (tWBC) count/leucocyte count (TLC), may fluctuate in fish after exposure to heavy metals. Anemia might be one of the earliest indications of metal toxicity. Christensen *et al*. (1972) reported haemoglobin as a useful index of fish health.

Conclusion

The present study reveals that ascorbic acid doses partly mitigate lead nitrate changes in RBCs of common carp, due to its antioxidant character. Lead toxicity is evident from the literature and there is almost no function in the body that is not affected by lead. Lead toxicity disrupts the functions of the digestive system, nervous system, respiratory system, reproductive system, etc. Studies revealed that trace metal mixtures can easily affect the life of freshwater fish Castaldo *et al* (2020). Lead increases WBC and significantly reduces RBC-related indices in the experimental group, while Vitamin C ameliorates lead effects on the RBC-related indices Aliyu et al (2017). Since fishes show remarkable fluctuations when exposed to different concentrations of lead nitrate initially the RBC count decreases which then increases by a considerate coefficient in the same test group protected with Vitamin C similar trend was observed in other groups which showed decreased value in RBC count and significantly level up when observed in the protection of Vitamin C.

Ascorbic acid (vitamin C) as a reducing agent has reduced oxidative stress induced by heavy metals; results of this research show that. Application of vitamin C has achieved a significant level to reduce the effects of heavy metals intoxication in all researched parameters.

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