

Protective role of ascorbic acid on the cadmium induced changes in hematology of the freshwater fish, *Channa orientalis* (Schneider)

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ABSTRACT

The study on fishes and their diseases has importance in life of human being because it has nutritive value. Fresh water fishes, *Channa orientalis* were exposed to chronic dose of CdCl_2 without and with ascorbic acid. Total leukocytes count and hemoglobin content were recorded. Remarkable increase in TLC and decrease in Hb content were observed in cadmium exposed fishes. Fishes were exposed to heavy metals with L-ascorbic acid (50mg/l.) showed less present variation on the TLC and Hb content. Pre-exposed fishes to heavy metal showed fast recovery with ascorbic acid as compared to these cured naturally. The role of ascorbic acid on exposure to cadmium of an experimental fish, *Channa orientalis* is discussed in the paper.

Keywords: Cadmium, Ascorbic acid, leucocytes (TLC), hemoglobin, *Channa orientalis*.

INTRODUCTION

Cadmium is hazardous; heavy metal is recognized to produce severe toxic effects in humans [2, 20]. Cadmium will invariably be present in our society, either in useful products in the form of nickel-cadmium batteries, dyes, plastics, electrochemistry and paint pigments or in controlled wastes as a major source of pollution in water and as constituent of food material [7]. In humans it has been found to produce wide range of biochemical and physiological dysfunctions as manifested in the forms of various diseases viz. Itai-itai, kidney and liver malfunction, inflammation, Parkinson's disorder [16]. The heavy metals are recognized as serious pollutants of the aquatic environment. Physico-morphological changes in blood indicate the changes in the quality of the environment for serving the purpose of such bio-indicators. Non-biodegradable metals such as lead, cadmium and mercury accumulate in living organism and cause various disease and disorders.

Cadmium in the form of cadmium chloride was toxic substances for fish. Hematological have been studied in various toxicant exposed fish, *Anabas testudineus* to cadmium [22]. Long term contamination are recorded when cadmium is deposited in bottom sedimentation it is highly toxic for aquatic organisms [13]. Leucocytes are one of the important components in blood. They exhibit phagocytic action increase in the leucocytes has an adaptive value to meet the stressful condition and defense mechanism. The decrease of leukocyte count and lymphopenia indicate a decrease of non-specific immunity in carp reported by [6]. The destruction of haemopoietic tissue in kidney and spleen result in decreased blood cell production and consequent reduction in erythrocyte count [9]. Antioxidant can play significant role in the treatment of metal induced oxidative stress as efficient chelators. Ascorbic acid being important constituent in cellular metabolism, the interactions of biomolecules gives proper idea of toxicant stress and its effect.

MATERIALS AND METHODS

Medium sized fresh water fishes, *Channa orientalis* (length 6-8 c.m. and wt. 30-35g.) were collected from Shivan River from Nandurbar district and acclimatized in the laboratory condition in well aerated dechlorinated for 8-10 days. Fishes were washed with 0.1% KMNO₄ solution to avoid dermal infection. The physico-chemical parameters as per the methods [1]. The fishes were divided into three groups A, B and C. Group A fishes was maintained as a control. The Group B fishes were exposed to LC_{50/10} dose of Cd⁺⁺ (1.248ppm) as CdCl₂ for 45 days, while group C fishes were exposed to respective chronic concentration of heavy metal with ascorbic acid (50mg/l.) for 45 days. Fishes from B groups were divided into two D & E groups after 45 days. D groups were allowed to cure naturally in normal water while E groups were exposed to ascorbic acid.

Blood was taken directly from fish for enumerating TLC and Hb content were recorded from A, B and C group fishes after 15, 30 and 45 days of exposure and from D and E groups after 50th and 55th days of recovery. Blood was obtained by cutting the caudal peduncle [17, 15], using heparin as an anticoagulant. First few drops were discarded and only the first 2ml. of blood was taken since the entry of lymph into the blood is reported [18] to affect haematocrit value. The blood was transferred to glass vials containing anticoagulant. TLC was counted by Neubauer haemocytometer expressed in per cu.mm and Hb was estimated by sahli's haemoglobinometer expressed in g/dl. were assessed.

RESULTS AND DISCUSSION

Fish blood parameters are important in diagnosing the structural, physiological and functional status of the fish exposed to toxicant. The physico-chemical properties is given in Table-1, while the TLC and Hb content after exposure to cadmium without and with ascorbic acid and during recovery are given in the Tables 1.1 to 1.2. After chronic exposure to CdCl₂ increases TLC and decreases Hb content as compared to control were found. The exposure of heavy metal with ascorbic acid the TLC decreases and Hb contents increase as compared to those of heavy metal intoxicated fishes.

The fishes pre-exposed to heavy metals salts showed fast recovery in TLC decrease and Hb counts increase more in the ascorbic acid as compare to cure naturally in normal water. Recovery in blood parameters was observed can be correlated to the biodegradation of accumulated heavy metal in the body the hematological parameters are quick to recover. Similar observation reported by [11, 21]. [3], blood parameters of *Cyprinus carpio* which were pre-exposed to cadmium and mercury. A decline in Hb in *Anguilla rostrata* after chronic cadmium exposure has been attributed to impaired erythropoiesis due to direct effect of metal on haematopoietic centers and defective Fe metabolism and or impaired intestinal uptake of Fe due to mucosal lesions [8]. The change in hematology may be due to the destructive action of cadmium on peripheral blood cells acts as a result of which the viability of the cells may affect. The WBC showed greatest sensitivity to changes in the environment. [14] the leucocytosis was the result of direct stimulation of the immunological defense due the presence of toxic substance or may be associated by induced tissue damage. [10], poisoning of fish with metals might cause not only alteration in count or share of particular WBC types, but it might also result in impairment of their function causing suppression of non-specific and specific immune response.

The increase in WBC count might be due to the increase in population of leucocytes which indicates an immune system and reduced Hb are positive correlation with the exposed periods that may be due to cumulative response of cadmium toxicity towards excessive red cell destruction there by lead to anemic to protect the fish against infections under cadmium stress [12]. Supplementations of AA to lead intoxicated rats reduce lead toxicity [19]. Vitamin C not only confers protection against heavy metal toxicity, but it can also perform therapeutic role against such toxicity in general. The vitamin C could provide a tangible solution to heavy metal toxicity against heavy metals [4]. Ascorbic acid also reported to reduce the levels of lead in blood, liver and kidney in rats. The decrease in Hb contents indicates the impact of heavy metals on the erythropoietic tissue. The cellular respiration in vertebrates depends on the availability of iron associated with Hb. The change in hemoglobin and leukocytes indicate the impact of lead and ascorbic acid [5].

The paper contributes to assessment of toxicity and effect of cadmium and ascorbic acid on toxicosis. The changes in TLC and Hb may be due to immunological reactions to produce antibodies to cope up with stress induced by cadmium and ascorbic acid. The aim was to asses the role of ascorbic acid on cadmium on the oxidative blood capacity and the non specific immune response by means of examination of WBC count and Hb content. Ascorbic

acid acts as a detoxifier reduces the toxicity of the heavy metals to protection to the cell from expansion or abnormalities in their structural features.

Table- 1:- Physico-chemical parameters of water used for experimentation

Temperature	25.1 ± 3.2^0
PH	7.60 ± 0.3
Conductivity	$140 \pm 15.7 \mu \text{ mho}^{-\text{cm}}$
Free Co ₂	$3.34 \pm 1.3 \text{ ml}^{-1}$
Dissolved O ₂	$6.3 \pm 1.1 \text{ ml}^{-1}$
Total Hardness	$204 \pm 12.0 \text{ mg}^{-1}$
Total Alkalinity	$585.6 \pm 32.8 \text{ mg}^{-1}$
Magnesium	$31.67 \pm 2.9 \text{ mg}^{-1}$
Calcium	$30.46 \pm 3.06 \text{ mg}^{-1}$
Chloride	$107.92 \pm 16.34 \text{ mg}^{-1}$

Table 1.1b:- Hb content in *Channa orientalis* after chronic exposure to CdCl₂; H₂O without and with ascorbic acid & during recovery (Values express as gr. /100ml of blood).

Group	Treatment	15d	30d	45d	50d	55d
A	Control	8.0±0.21	7.8±0.14	7.7±0.18	--	--
B	Cd ⁺⁺ (1.248ppm)	7.2±0.06* (-10)	6.9±0.16* (-11.53)	6.2±0.05** (-19.48)	--	--
C	Cd ⁺⁺ (1.248ppm) + AA	7.5±.12 ^{NS} (-6.25)	7.2±0.11* (-7.69)	6.9±0.16* (-10.38)	--	--
D	Recovery in Normal Water	--	--	--	6.5±0.016 ^{ΔΔ} (+4.83)	6.9±0.16 ^Δ (+11.29)
E	Recovery in AA	--	--	--	6.8±0.18 ^Δ (+9.67)	7.2±0.06 ^{ΔΔΔ} (+16.12)

Table1.2b:- WBC counts in *Channa orientalis* after chronic exposure to CdCl₂; H₂O without and with ascorbic acid & during recovery (Values express as 10³/mm³ of blood).

Group	Treatment	15d	30d	45d	50d	55
A	Control	21.26±0.81	22.33±0.650	23.66±0.576	--	--
B	Cd ⁺⁺ (1.248ppm)	30.15±1.70* (-41.81)	32.35±1.86** (-44.87)	34.70±0.57*** (-46.66)	--	--
C	Cd ⁺⁺ (1.248ppm) + AA	27.26±1.45* (-28.22)	29.76±1.59* (-33.27)	31.86±0.78** (-35.14)	--	--
D	Recovery in Normal water	--	--	--	31.60±1.33 ^{ANS} [+8.93]	29.50±0.73 ^{AA} [+14.98]
E	Recovery in AA	--	--	--	28.10±1.26 ^Δ [+19.02]	25.3±1.10 ^{AA} [+27.08]

AA = Ascorbic acid (50 mg/l., ± indicates S.D. of three observations.

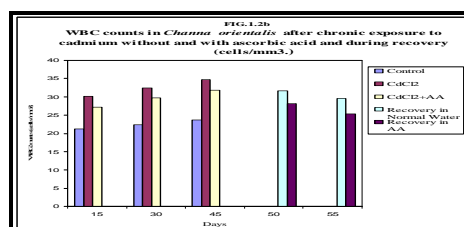
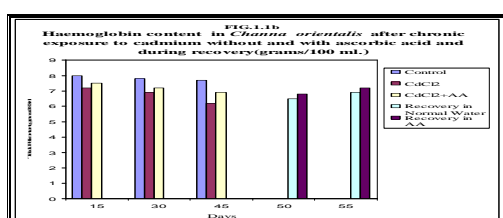
Values in () indicates percent change over respective control.

Values in [] indicates percent change over 45 days of respective B.

* indicates significance with the respective control.

^Δ indicates significance with 45 days of respective B.

$p < 0.05 = *$ & ^Δ, $p < 0.01 = **$ & ^{ΔΔ}, $p < 0.001 = ***$ & ^{ΔΔΔ},
^{NS} and ^{ΔNS} = Not significant



CONCLUSION

On the basis of this study, the following conclusions could be drawn.

1. The ascorbic acid act as antioxidant and efficient inhibitor against cadmium chloride.

2. Ascorbic acid acts as a detoxifier reduces the toxicity of the heavy metals to offer protection to the cell from expansion or abnormalities in their structural features.
3. After chronic exposure to CdCl₂ increases TLC and decreases Hb content as compared to control were found.
4. The exposure of heavy metal with ascorbic acid the TLC decreases and Hb contents increase as compared to those of heavy metal intoxicated fishes.
5. The fishes pre-exposed to heavy metals salts showed fast recovery in TLC decrease and Hb counts increase more in the ascorbic acid as compare to cure naturally in normal water.
6. Recovery in blood parameters was observed can be correlated to the biodegradation of accumulated heavy metal in the body the hematological parameters are quick to recover.

REFERENCES

- [1]APHA, AWWA and WPCF, Standard methods for the examination of water and waste water APHA (17'th) INC, New York, **1985.**]
- [2]ATSDR (Agency for Toxic Substances and Disease Registry). *Toxicological profile for Cadmium*. ATSDR / US department of Health and Human Services, Atlanta/, US, **1999**.
- [3]Beena S, and Viswaranjan S., *Environ Ecology*, **1987**, 5(4):726-737.
- [4]Bhattacharjee CR, Deys S, Goswami P, *Bulletin of Environmental Contamination Toxicology*, **2003**, 70: 1189 - 1196.
- [5]Borane VR, *Journal of Chemo and Biosphere*, **2010** p.29-32.
- [6]Drastichová J, SvobodováZ, Lusková V, Máchová J. *Bulletin of Environmental Contamination Toxicology*, **2004**, 72:725-732.
- [7]Friberg L, Kjellstrom T, Nordberg G and Piscator A., *Cadmium and Health: Toxicological and Epidemiological Appraisal*, US Environment Protection Agency, Washington DC, **1985a**.
- [8]Gill T S, Epple A, *Ecotoxicol Environmental Saf*, **1993**, 25: 227-235.
- [9]Iwama G.K., Greer G.L. and Randall D.J., *Journal of Fish Biology*.1986, 28:563-52.
- [10]Jezierska B, Witeska M, Metal toxicity to fish. Wydawnictwo ap, siedlce, poland **2001**.
- [11]Kaushik, N., A. study of the effects of certain toxicants in *Channa punctatus* (Bloch.). Ph.D. Thesis, University of Rajasthan, Jaipur, India, **2002**.
- [12]Karuppasamy R, Subathra S, Puvaneswari S., *Journal of Environmental Biology*., **2005**, 26(1):123-128.
- [13]Leontovičová D, Complex monitoring in selected profiles of state networks of water quality follow up in CHMU Periodicum fakulty ekologie an enviromentalistiky Technickej university Vozvolene Vol. 10 Suppl, **2003**.
- [14]Nair A, Vijayamohanan, Suryanarayanan, *Journal of Ecobiology*., **2000**, 9:243-246.
- [15]Reichenbach-Klinke HH, *Enfermedades de los Peces*. Ed Acribia,Zaragoza,España, **1982**, p507
- [16]Risk Assessment Information System (RAIS). *Toxicity summary for cadmium*. Chemical Hazard Evaluation and Communication Group, *Biomedical and Environmental Information Analysis Section, Health and Safety Research Division*. **1991**.
- [17]Roberts RJ, *Fish Pathology*. Bailliere Tindall. New York, USA, **1978**, p377.
- [18]Schermer S, Die Blutmorphologie der laboratorium-stiere Barth, Leipzig. *Experta Medica Foundation*, FADavisco. Philadelphia, **1954**.
- [19]Singh Sushitima, Chaturvedi Shelly, Gaur KK, Singh Ajay, *Nat. Journal of life Science*. 2 (Supp.), **2005**, 343-344.
- [20]Singh P, Chaudhary S, Patni A, Sankhla V, *Journal of Herbal Medicine and Toxicology*.1 (2): 67-71, **2007**.
- [21]Srivastava Neera, Agrawal Meena, *Journal of Ecobiology*, **2005** 17(4): 377-382.
- [22]Vijayram K, Geraldine, Varadarajan TS, Loganathan, *Journal of Ecobiology*, **1989**, 1:15-19.