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Research Article

ASCORBATE EFFECT ON THE LEAD INDUCED ALTERATIONS IN THE BEHAVIOR OF THE FRESH WATER FISH, *GARRA MULLYA*

Borane V. R*

Department of Zoology, Jijamata Arts, Science and Commerce College, Nandurbar (M.S.) India. 425 412

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ABSTRACT

Environmental contamination from the use of pesticides, metals ranges from water, air and soil pollution to alter the ecosystem resulting in detrimental effect to non-target organisms. Study assessed the behavioural dysfunctions in relation to the toxicity exposed to chronic dose of lead chloride without and with ascorbic acid in freshwater fish, *Garra mullya*. Behavioural dysfunctions have been documented as indicators of physiological stress. Hyperactivity, erratic, undirected jerky movement of fish exposed to lead chloride is associated with disruption of nervous and muscular coordination. Lethargy, loss of equilibrium and settling down at the bottom prior to death leading to hypoxia. The groups exposed to lead chloride along with ascorbic acid showed recovery than those exposed to only lead chloride. Pre-exposed fishes to lead chloride showed fast recovery than those, which were allowed to cure naturally.

INTRODUCTION

Environmental contamination from the use of pesticides, metals ranges from water, air and soil pollution to alter the ecosystem resulting in detrimental effect to non-target organisms. Heavy metals coming out from sources enter the fresh water habitats and bring about morphological and physiological changes in aquatic habitats. *Garra mullya* is local fresh water edible fish of great economic importance. Hence, it is prudent to study the chronic toxicity and the changes that occur in the behavior. Behavior allows an organism to adjust to external and internal stimuli in order to meet the challenges of survival in a changing environment while morphological changes are external changes occur due to the changing environment. Study of behavioral changes due to the effect of toxicants in fishes is the best suitable method to check the pollution in water resources. Thus, it is a promising tool in eco-toxicology and act as diagnostic endpoint for screening, differentiating the chemical effluents and their effect on aquatic organism.Lead is primary contaminants of natural environment use in batteries, alloys, paints, stabilizers etc. Human exposure to the metal occurs mainly through food intake or inhalation of soil or dust contaminated with heavy metals. The metal is highly mobile and is known to cause undesirable effects on metabolic processes of living organisms.

Fishes are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems. The abnormal behavioral changes exhibited by zebra fish on exposure to formalin such as erratic swimming behavior, increased secretion of mucous, reduced opercular movements have also been reported in fishes exposed to various toxicants like lead, cadmium, chromium and fertilizers, Tawari-Fufeyin *et al*.,(2008). Surfacing behavior and operculum movements are the indicators of changes in respiratory mechanisms while irregular, erratic and jerky movements indicate improper co-ordination between nervous system and muscular tissue. The heavy metals cause metabolic disorders, morphological changes, behavioral stress, immuno-suppression and toxicological manifestations among fishes by Pandey and Pandey (2004).

Ascorbic acid contents in the animal tissue increases in stress condition during metal toxicosis. Ascorbic acid protects extra cellular protein function through gene expression highlighted reported by Griffiths and Lunec, (2001). Antioxidant nutrients like ascorbic acid, vitamin E are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products reported by Anitra *et al.,* (2000).

Corresponding author:* **Borane V. R
Department of Zoology, Jijamata Arts, Science and Commerce College, Nandurbar (M.S.) India. 425 412

MATERIALS AND METHODS

Medium sized fresh water fishes, *Garra mullya* were collected from Shiven river area Nandurbar Dist. Nandurbar. *Garra mullya is* rich in protein has economically important edible fish. The physico-chemical parameters of the water used by the method APHA (2005).The fishes were divided in to three groups A, B and C. Group A fishes were maintained as a control. The group B fishes were exposed to $LC_{50/10}$ dose of Pb^{++} (2.867ppm) as lead chloride for 45 days, while group C fishes were exposed to chronic concentration of lead chloride with ascorbic acid for 45 days. Fishes from B groups were divided into two groups D &E after 45 days exposure to lead. Fishes of D groups were allowed to cure naturally while those of E groups were exposed to ascorbic acid. Behavioural aspects such as opercular movement, surfacing phenomenon and jerky movement were recorded from A, B and C group fishes after 15, 30 and 45 days of exposure and groups D and E after 50^{th} and 55^{th} days of recovery. Fishes were fed on earthworm.

RESULTS AND DISCUSSION

Aquatic organisms are continually being exposed to various pollutants in the environment. Pollutants toxicity to plants, animals, aquatic organisms and wildlife can be evaluated by exposing a group of organism under controlled conditions such as evacuation can be performed and is the indices of action. Pollutants can produce adverse effects in a biological system damaging structure and function of living system finally leads to death of organism. Lethal effect leads to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction and survival. Behavioral changes in *Garra mullya* after exposure to lead with and without ascorbic acid and during recovery are given in the Table 1.1 to 1.3. Behavioral changes in the *Garra mullya* were found more in lead chloride. In the presence of ascorbic acid the behavioral changes is less as compared to those of heavy metal stress fishes. The fishes pre-exposed to heavy metals salts showed fast recovery in the presence of ascorbic acid than those allowed to cure naturally.

Behavioral pattern of aquatic organism that have been tested or investigated during the last hundred years of aquatic toxicity testing deals with the avoidance of reaction, swimming and schooling behavior, level of swimming, predating behavior and respiratory behavior like rate of operculum movement etc. by Veeraiah and Durga Prasad,(2001). Behavior of the animal can serve as the link between physiological and ecological processes reported by Graham and Sloman, (2004). Increase in the frequency of surfacing phenomenon in the fish either due to hypoxia condition difficulty to respire in the media or for the protection of the gill epithelium. The increased opercular movement and gulping activity by the treated fish may be an attempt to extract more oxygen to meet the increased energy demand to withstand the Cadmium toxicity by Saxena and Parashari (1982). It might also be due to accumulation of mucous on the gill epithelium, Increased distance between gills and opercular movement producing hypoxic condition to the fish reported by Nagaraju *et.al*., (2011).

The heavy metals accumulate in the brain causes improper neural behavior due to pollutants induces jerky movements; the behavioral change shows the external manifestation of the abnormal physiological state of the animal is the marker of stress. Sluggishness observed at the end of exposure periods may be due to loss of energy as a result of erratic swimming movement and they appeared to be in distress, jumping, restlessness, hyper excitation, loss of equilibrium, flaring of gills, secretion of mucus from gills and swimming at upside down position. A film of mucus was observed all over the body and also gills. Loss of balance during swimming and jerky movements observed during this study, might be due to some neurological impairment in the central nervous system reported by Varusai Naina *et al*., (2012).Loss of equilibrium, erratic swimming and restlessness are common behavioral responses in several fishes exposed to a variety of toxicants as recorded in *Oreochromis niloticus* and *Clarias batrachus* exposed to copper sulphate Ezeonyejiaku, (2011). Behavioural responses and morphological anomalies observed in freshwater fish, Labeo rohita on being exposed to lead nitrate observed by Onkar Sing and Manjeet, (2015). Bhattacharjee *et al*., (2003) observed decreases in lead contents of blood of mice pretreated with ascorbic acid. Ascorbic acid play important role in the synthesis of collagen and thus can correct the altered situation due to heavy metals. The behavioral alterations in fish can be considered as biomarkers to access the health status of the fishes as well as aquatic bodies polluted by toxicants. Thus environmental protection is the major requirement of the society.

Table 1 Frequency of opercular movement in *Garra mullya* after chronic exposure to $PbCl_2$ without and with ascorbic acid (Counts/min.)

Group	Treatment	15d	30d	45d	50d	55d
A	Control	29±2.13	31±1.39	32±1.70	--	--
B	Pb^{++} (2.867ppm)	18±2.91* (-36.4)	15±2.06** (-45.02)	12±1.78** (-56.12)	--	--
C	Pb^{++} (2.867ppm)+AA	19±1.45 * (-27.10)	16±1.97** (-38.21)	17±2.17** (-42.85)	--	--
D	Recovery in Normal Water	--	--	--	$16±1.51^{\Delta NS}$ [+21]	$22±1.18^{\Delta}$ [+56]
E	Recovery in AA	--	--	--	$21±1.45^{\Delta}$ [+51.33]	$25±2.16^{\Delta}$ [+79]

Table 2 Frequency of surfacing phenomenon in *Garra mullya* after chronic exposure to $PbCl_2$ without and with ascorbic acid (Counts/hr.)

Group	Treatment	15d	30d	45d	50d	55
A	Control	100±4.54	96±4.08	93±5.95	--	--
B	Pb^{++} (2.867ppm)	135±5.34* (-31.31)	141±3.74** (-42.91)	150±2.51** (-58)	--	--
C	Pb^{++} (2.867ppm)+AA	122±4.35* (-21.52)	130±4.66** (-31.67)	135±4.30** (-42.30)	--	--
D	Recovery in Normal Water	--	--	--	$134±4.32^{\Delta}$ [+10.50]	$130±2.95^{\Delta\Delta}$ [+13.80]
E	Recovery in AA	--	--	--	$130±4.69^{\Delta}$ [+13.11]	$125±2.15^{\Delta\Delta}$ [+16.41]

Table 3 Frequency of jerky movement in *Garra mullya* after chronic exposure to $PbCl_2$ without and with ascorbic acid (Counts/hr.)

Group	Treatment	15d	30d	45d	50d	55
A	Control	3±0.412	4±0.211	5±1.10	--	--
B	Pb^{++} (2.867ppm)	7±1.53* (-300)	9±1.23** (-230.33)	10±1.41* (-221)	--	--
C	Pb^{++} (2.867ppm)+AA	5±1.07* (-200)	7±1.50* (-161.62)	10±1.90* (-174)	--	--
D	Recovery in Normal Water	--	--	--	$8±1.51^{\Delta NS}$ [+30.72]	$6±1.20^{\Delta}$ [+41.11]
E	Recovery in AA	--	--	--	$5±1.20^{\Delta}$ [+42.12]	$3±1.29^{\Delta}$ [+60.50]

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, $^{\Delta}$ indicates significance with 45 days of respective.

$p<0.05$ = * & $^{\Delta}$, $p<0.01$ = ** & $^{\Delta\Delta}$, $p<0.001$ = *** & $^{\Delta\Delta\Delta}$, ^{NS} and $^{\Delta NS}$ = Not significant.

CONCLUSION

The behavioral and morphological changes show direct response of the animals to the pollutants. These studies can be used as a sensitive model to monitor the aquatic pollution. The current study evidenced that lead is highly toxic and had a detrimental impact on the behavioral responses and morphology of the fish, *Garra mullya* at sub-lethal concentrations. It affects the ability of animals to adapt to its environment and affect the central and peripheral nervous system. These responses can be used as a tool in biomonitoring programme to monitor eco-toxicity risk of lead to the fish. The behavioral changes affecting the general health status of fish.

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