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Impact of Chelating agent (Zinc) on Heavy metal (Arsenic) caused variations of Hexokinase in different Brain regions of fresh water Teleosts

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Abstract

In the present study the author intend to study the sub-lethal and lethal effect of arsenic separately and in presence of chelating agent zinc on bio-chemical compartmentation of hexokinase, in various brain regions (cerebrum, diencephalon, cerebellum and medulla oblongata) in three phylogenetically and ontogenetically different fish species i.e. Labeo rohita (Ham) Clarias batrachus (Linn) and Channa punctatus (Bloch) under acute studies from a tropical environment.

Keyword: Chelating agent, heavy metal, fishspecies, enzyme hexokinase and detoxification.

Introduction

Water resources are being used by human being for various purpose like agriculture, industries, hydropower, fisheries recreational uses. At present the quality of water largely under threat due to release of municipal, industrial domestic and sewage wastes in the surface and groundwater[1,2].

Aquatic organisms have been reported to accumulate heavy metals in their tissues several times above ambient levels. Fishes have been used for many years to determine the pollution status of water and are thus regarded as excellent biological markers of metals in aquatic ecosystems.

Considerable interest has been shown in recent years in histopathological study while conducting sub-lethal tests in fish. Tissue changes in test organisms exposed to a sub-lethal concentration of toxicant are a functional response of organisms which provides information on the nature of the toxicant.

Among pollutants metals are of special concern because of their diversified effects and the range of concentrations that could cause toxic ill-effects to fish. Generally heavy metals exert their toxic effects in organisms by generating reactive oxygen species, causing oxidation stress. Therefore, most of the heavy metals are toxic carcinogenic in nature posing threats to the human health and the environment.

Metal accumulation in the environment continuously increases owing to the anthropogenic activities and they tend to concentrate in all the aquatic matrices. Heavy metals including lead are found in various tissues of fish and shrimps. High level of trace metals is found in liver, kidney, and muscles of Antarctic penguin *Pygoscelis adeliac*[1,2].

It is essential to find an appropriate approach to prevent and treat a lead-exposed person. The current approved treatment for lead poisoning is to administer chelating agents (thiol chelators and other complexions) that form an insoluble complex with lead and remove it from lead enriched tissue; but most of these chelating agents from many side effects (Flora et al., 1995) and are ineffective to reduce lead exposure. Free radical generation is referred to as the pathogenesis of lead, so supplementation of antioxidants could be considered as the alternative method for chelation therapy (Flora et al., 2003).

Material and Methods

Determination of safety, sub-lethal and lethal concentration: Safety, sub-lethal concentrations of arsenic were determined on *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* by the ***Probit Analysis Method (Finney, 1971)***. Higher concentration of arsenic were used and slowly reduced the amount of concentration to know the Lc 50/100 value for 96 hour exposure.

Results and Discussion

The sub-lethal arsenic concentrations induced hexokinase variations to a great extent in diencephalon at 08 hrs than in cerebrum (at 24 hrs), medulla oblongata (at 24 hrs) and cerebellum (at 16 hrs) in *L.rohita,* in comparison to *C.batrachus* (diencephalon at 08hrs, cerebrum at 24 hrs, medulla oblongata at 24 hrs and cerebellum at 24 hrs) and in *C.punctatus* (diencephalon at 08 hrs, cerebrum at 16 hrs, medulla oblongata at 24 hrs and cerebellum at 24 hrs).

The sub lethal arsenic exposure and hexokinase variations in presence of zinc as chelating agent were at low level then the hexokinase variations recorded alone with sub lethal arsenic concentrations and under these conditions the highest fall in hexokinase activity was recorded in diencephalon at 08 hrs accompanied by cerebrum at 24 hrs, medulla oblongata at 24 hrs and cerebellum at 24 hrs in *L.rohita,* in comparison to *C.batrachus* (diencephalon at 08 hrs, cerebrum at 24 hrs, medulla oblongata at 24 hrs and cerebellum at 24 hrs) and in *C.punctatus* (diencephalon at 08 hrs, cerebrum at 24 hrs, medulla oblongata at 24 hrs and cerebellum at 24 hrs) (table 1 and figure-1).

The lethal arsenic inhibited hexokinase in diencephalon at 08 hrs to a maximum extent in comparison to cerebrum (at 24 hrs), medulla oblongata (at 24 hrs) and cerebellum (at 24 hrs) in *L.rohita* than in *C.batrachus* (diencephalon at 08 hrs, cerebrum at 24 hrs, medulla oblongata at 24 hrs and cerebellum at 24 hrs) and in *C.punctatus* (diencephalon at 08 hrs, cerebrum at 24 hrs, medulla oblongata at 24 hrs and cerebellum at 24 hrs). The effect of lethal arsenic concentration on hexokinase in presence of zinc as chelating agent was also investigated and the influence of zinc as chelating agent was realised in different brain regions and the details are as follows.

Though the hexokinase fall was highest in diencephalon (in presence of zinc) but the fall in the hexokinase was significantly less than the fall in hexokinase directly exposed to lethal arsenic at 08 hrs followed by cerebrum (at 24 hrs), medulla oblongata (at 24 hrs) and cerebellum (at 24 hrs) in *L.rohita* in comparison to *C.batrachus* (diencephalon at 08 hrs, cerebrum at 24 hrs, medulla oblongata at 24 hrs and cerebellum at 16 hrs) and *C.punctatus* (diencephalon at 08 hrs, cerebrum at 24 hrs, medulla oblongata at 24 hrs and cerebellum at 16 hrs) .

Congenial atmosphere is a pre-condition for good physical and mental health. Stressful/anxiety/tension/contamination situation may spoil the balance between abiotic and biotic factors and these in turn may implant a number of disorders shortcomings and the former may affect the productivity and the affected productivity may lessen the yield of that water body directly and the revenue of the state/ country indirectly, thus contamination could be treated as contagious. At present the contamination is life threatening, as the accumulated contaminants causing histological, anatomical, hormonal, biochemical, genetic and embryological significant variations thus making body vulnerable for dysfunction/ disease prone and at last immunology too at stake. If improvised/ innovative/ practical devises are not developed to contain contamination each and every part of this nature including living organisms may start decline and may become extinct. Now it is the responsibility of all those aquatic biologist ichthyologist, aqua culturist, biochemist, physiologist and fishery scientist to innovate strategies to contain contamination and protect natural resources to enhance the yield and boost the revenue.

The zinc might have form multiple bonds with single metal ion like arsenic separately through their ligands and might have form stable ring structures. In chelate the chelating agent is an electron pair donor and metal ion is electron pair acceptor. Thus the bonding between arsenic and chelating agent i.e. zinc is a coordinate covalent bond.

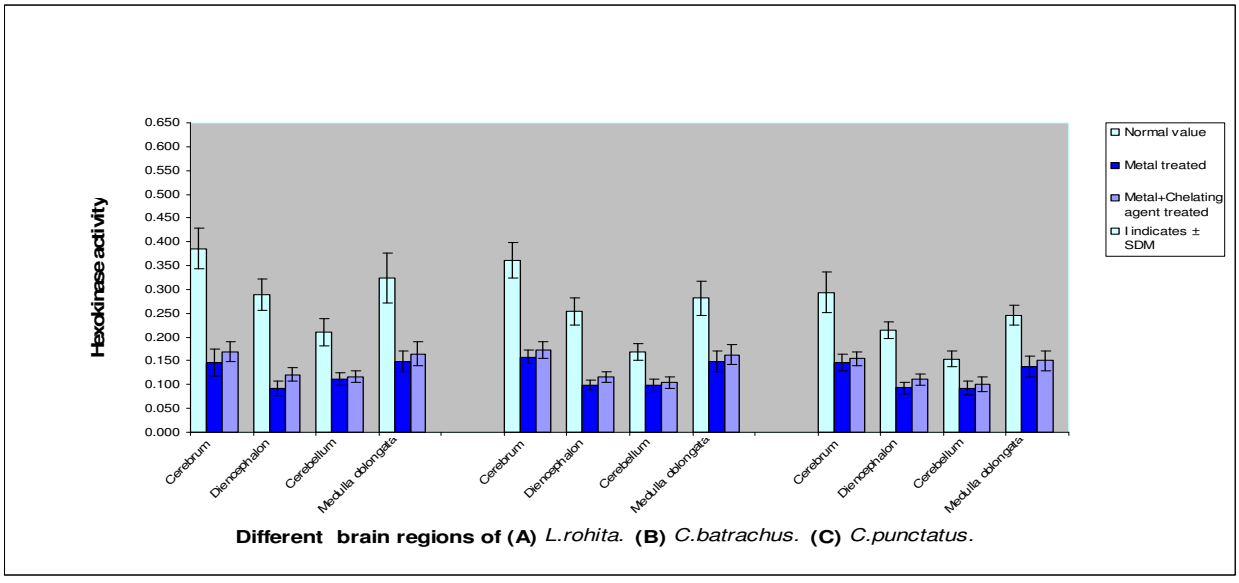

Figure-1
Different brain regions vs Hexokinase activity

Table-1
Influence of zinc on sub-lethal arsenic induced Hexokinase variations in various brain regions in three freshwater teleosts-acute studies

Name of Species	Regions of the brain	Sub lethal (Arsenic) exposure					Sub-lethal (Arsenic) exposure with zinc				
		Control	8hrs	16hrs	24hrs	% of F/R	Control	8hrs	16hrs	24hrs	% of F/R
***Labeo rohita* (Ham.)**	Cerebrum	0.386 ±0.042	0.336 ±0.048	0.296^{e} ±0.034	0.146^{b,c} ±0.028	**62**	0.386 ±0.088	0.329 ±0.032	0.296^{e} ±0.029	0.169^{b,c} ±0.021	**56**
	Diencephalon	0.289 ±0.032	0.176 ±0.016	0.149^{e} ±0.016	0.092^{b,e} ±0.016	**68**	0.289 ±0.056	0.162^{e} ±0.014	0.138^{d} ±0.016	0.121^{c} ±0.014	**58**
	Cerebellum	0.210 ±0.028	0.184 ±0.012	0.129^{e} ±0.016	0.111^{d,e} ±0.013	**47**	0.210 ±0.019	0.184 ±0.021	0.156 ±0.018	0.117^{d} ±0.012	**44**
	Medulla oblongata	0.324 ±0.052	0.279 ±0.032	0.216^{e} ±0.018	0.149^{b,e} ±0.022	**54**	0.324 ±0.038	0.296 ±0.032	0.246^{e} ±0.022	0.165^{b,e} ±0.026	**49**
***Clarias batrachus* (Linn.)**	Cerebrum	0.361 ±0.038	0.332 ±0.026	0.284^{e} ±0.022	0.158^{b,d} ±0.014	**56**	0.361 ±0.032	0.316 ±0.024	0.276^{e} ±0.017	0.173^{b,e} ±0.018	**52**
	Diencephalon	0.254 ±0.029	0.153^{e} ±0.013	0.136^{d} ±0.016	0.099^{c,d} ±0.010	**61**	0.254 ±0.026	0.149^{e} ±0.017	0.124^{d} ±0.012	0.116^{e} ±0.012	**54**
	Cerebellum	0.169 ±0.018	0.142 ±0.017	0.133 ±0.014	0.098^{e} ±0.013	**42**	0.169 ±0.016	0.148 ±0.014	0.132 ±0.010	0.104^{e} ±0.013	**38**
	Medulla oblongata	0.282 ±0.036	0.250 ±0.022	0.222^{e} ±0.019	0.149^{c,e} ±0.022	**47**	0.282 ±0.032	0.256 ±0.022	0.222^{e} ±0.031	0.163^{d} ±0.021	**42**
***Channa punctatus* (Bloch)**	Cerebrum	0.294 ±0.042	0.262 ±0.014	0.182^{e} ±0.024	0.147^{b,d} ±0.018	**50**	0.294 ±0.038	0.264 ±0.024	0.230^{e} ±0.029	0.155^{b,d} ±0.014	**47**
	Diencephalon	0.214 ±0.018	0.149^{e} ±0.022	0.122^{d} ±0.013	0.094^{c,e} ±0.012	**56**	0.214 ±0.014	0.154 ±0.019	0.138^{e} ±0.014	0.111^{c} ±0.012	**48**
	Cerebellum	0.154 ±0.016	0.136 ±0.014	0.122 ±0.010	0.093^{e} ±0.014	**39**	0.154 ±0.015	0.142 ±0.012	0.129 ±0.012	0.101^{e} ±0.016	**34**
	Medulla oblongata	0.246 ±0.021	0.216 ±0.022	0.184^{e} ±0.016	0.137^{d,e} ±0.022	**44**	0.246 ±0.019	0.226 ±0.016	0.194 ±0.024	0.150^{e} ±0.021	**39**

Values are mean ± SDM of 7 Replicates. The data was subjected to test of ANOVA and Superscripts a-e indicates that p> 0.01, 0.02, 0.03, 0.04 & 0.05. *F-Fall /R-Rise

Conclusion

In the present investigation too such a bonding might have taken place between arsenic as toxicants, zinc as chelators and the toxic impact of arsenic is reduced in presence of zinc and the recovery of enzyme activity during arsenic exposure in presence of zinc might be realised on the following school of thought.

The hexokinase, fall too was high in diencephalon than in cerebrum, medulla oblongata and cerebellum in *L.rohita,* in comparison to brain regions (cerebrum,diencephalon, cerebellum and medulla oblongata) in *C.batrachus* and *C.punctatus* at 08 to 24 hrs exposure. In presence of zinc the enzyme fall in various brain regions was comparatively less than the fall in the enzymes exposed directly to sub lethal and lethal arsenic.

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