EFFECT OF ALUMINIUM CHLORIDE ON THE TOTAL FREE AMINO ACIDS CONTENT IN DIFFERENT TISSUES OF FRESHWATER FISH *LABEO ROHITA*

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Abstract

The environmental pollutants, including metals can cause toxicological effects on aquatic animals especially fish species. The effect of aluminium chloride on the freshwater fish *Labeo rohita* was assessed. Sub-lethal concentration (LC_{50}) for 96 hours of exposure was found to be 32.5 ppm. The fishes were exposed to the experimental concentration $1/4^{th}$ of aluminium chloride for 96 hours. The estimated free amino acids concentration in the tissues (gills, liver, kidney and muscles) were found to be increased during the exposure periods. The present results showed that variation in biochemical parameters which is an adaptive response of vertebrates due to lower and higher sublethal concentrations exposure of aluminium chloride. Due to this reason different fish species showed different amount of metal accumulated in their bodies. This study also reveals that the metals, being conservative in nature have higher ability of biomagnifications.

Keywords - Heavy metal, Aluminium chloride ,Amino acids, Tissues and *Labeo rohita.*

I.Introduction

The aquatic ecosystem is threatened more and more by different sources of pollution that decrease its economic condition and produces deleterious effects to human health. Among all types of pollution, aquatic pollution is of greater concern as each and every kind of the life depends on water. Therefore management of aquatic environment in particular has become a major concern in recent years. Aluminium in river water is commonly associated with dissolved organic matter such as fulvic, humic and tannic acids and its solubility is highly pH dependent. Particularly, the aqueous aluminium is recognized as the principal toxicant to fishes (1),(2) .

Aquatic pollution is still a problem in many freshwater and marine environments; it causes negative effects for the health of the respective organisms (3). The number of studies dealing with effects of pollutants and concurrently occurring parasites is still relatively low (4). However the effect of environmental pollutants on fish parasites varies depending on the particular parasite and pollutant that interact (5). Pollutants may affect the immune system of the fish either directly or by change water quality; that in turn may reduce the fish immunity to parasites (6).

Heavy metals play an important role in various biological functioning of aquatic organisms and remain present in trace amount in the body (7). Aquatic system is an ultimate sink of heavy metal pollutants and since aquatic animals tend to accumulate heavy metals from various sources including sediments, soil erosion and runoff, air depositions of dust and aerosol, and

discharges of waste water (8), they provide the insights of toxicity mechanisms induced by these heavy metals. Those amino acids which exist in free from in tissues and not bound to proteins are called as free amino acids. Normally during diseased conditions in plants and animals there will be a change in the total free amino acids composition. Hence the estimation of total free amino acid gives as indication about the physiological and health condition of animal.

II.Material and Methods

The freshwater fish *Labeo rohita* (weight: 7±0.5 gm and length: 6±0.5cm) used in the present study were collected from the Maheshwari fish farm at Nalladai, Mayiladuthurai, Tamil Nadu, India. The fishes were acclimatized in the laboratory condition two weeks before experiment. Significant signs of unusual behavioural criteria were not observed in the control fishes throughout the acclimation and test period. During the acclimatization, the fishes were fed with pellet feed daily in the evening uneaten feed was removed the next day morning followed by 100% water exchange.

The biochemical constituents *viz*., free amino acids content was estimated as method described by (9), in four different tissues *viz*., gill, liver, kidney and muscle of the healthy fishes (control) and of those from the fishes exposed to sub-lethal concentration.

III.Results

The effect of aluminium chloride toxicity on free amino acid level of different tissues like gills, liver, kidney and muscles of freshwater fish, *Labeo rohita* is presented in the table 1.The free amino acid content of treated tissues exhibited significant variation ($P > 0.05$) against the control fish. Toxicity has been observed over the period of 28 days. It is found that the exposure concentration and time increases, the free amino acid content of the treated fish also found to be increases. In the gill of the control fish, the free amino acid level was determined as 4.10±0.23 mg/g of wet weight of tissue, which was found to be increased to 5.52 ± 0.22; 6.70 ± 0.41; 8.32±0.52 and 9.93±0.64 mg/g of wet weight of tissue at 7, 14, 21 and 28th days of exposure of aluminium chloride toxicity in lower concentration. Similarly, increasing trend of free amino acid level was observed in treated fish after the exposure of high concentrated aluminium chloride as 6.12±0.40; 7.62±0.43; 12.36±0.94 and 16.12±1.15 mg/g of wet weight of tissue at 7, 14, 21 and 28th days of exposures respectively.

Free amino acid content of toxicated liver tissue of *L. rohita* also showed an elevated level against the control and it was found to be directly proportional to concentration and time of exposure. In control fish the free amino acid level was estimated as 5.45±0.25 mg/g of wet weight of tissue. On the exposure of lower concentration of aluminium chloride, the free amino acid level was determined as 6.54±0.27; 7.67±0.21; 8.44±0.64 and 11.16±0.94 mg/g of wet weight of tissue at 7, 14, 21 and 28th days of exposures respectively. During the high concentration of exposure, free amino acid was observed to be increased as 7.14±0.22; 9.37±0.23; 13.36±0.84 and16.08±1.12 mg/g of wet weight of tissue at 7, 14, 21 and 28th days of exposures respectively. Changes in the free amino acid content of liver in treated fish against the control fish were statistically significant at $P> 0.05$. (Table .1)
Similar increase in free amino acid level of treated kidney tissue of *L. rohita* was observed for the exposure of aluminium chloride in both lower and higher concentrations in comparison with the control. The free amino acid level of control fish was found to be 3.06±0.16 mg/g of wet weight of tissue. After the exposure of lower concentration of aluminium chloride, the free amino acid level was recorded as 4.07±0.20; 4.89±0.46; 6.13±0.42 and 8.23±0.61 mg/g of wet weight of tissue at 7, 14, 21 and 28th days of exposure respectively. Similar increasing trend is observed for the exposure of high concentration and it was found to be 5.16±0.42; 6.86±0.37; 9.11±0.13 and 12.27±0.82 mg/g of wet weight of tissue at 7, 14, 21 and 28th days of exposures respectively (Table .1).

Muscle tissue of the treated freshwater fish was found to be contained the variation in the free amino acid level when compare with the control. In control fish the free amino acid level was found to be 2.12±0.65 mg/g of wet weight of tissue. After

the exposure of lower concentrated aluminium chloride, the free amino acid level was found to be 2.91±0.30; 3.67±0.08; 5.12±0.22 and 6.02±0.47 mg/g of wet weight of tissue at 7, 14, 21 and 28th days of exposures respectively .In Comparison against the control, it showed increase in its level. The level of free amino acid in muscle tissue of *L. rohita* exposed to high concentration of aluminium chloride was estimated 3.86±0.13; 5.68±0.65; 6.17±0.43 and 8.15±0.62 mg/g of wet weight of tissue at 7, 14, 21 and 28th days of exposure respectively.

In the control fish, the free amino acid content was in the order of liver > gill > kidney > muscle. During the exposure of sublethal concentration of aluminium chloride at lower and higher concentrations, the free amino acid level was found to be increased in the order of liver > gill > kidney > muscle. (Figure.1&2).

IV.Discussion

In the present analysis, free amino acid level in all the tissues of toxicant exposed fish was significantly increased. Amino acids are essential intermediates in the process of protein synthesis and its degradation products appear in the form of different nitrogenous substances. Amino acids and some nitrogenous compounds play an important part during osmotic stress hence increase or decrease in free amino acid content provide valuable information during stress phenomenon at the tissue level. The increased free amino acid level suggests tissue damage probably due to augmented proteolysis activity. Increment in free amino acid level was the result of breakdown of protein for energy requirement and imparted incorporation of amino acid is protein synthesis (10). (11*)* found that the increase in concentration of free amino acid attributed to stepped up proteolysis or increase synthesis of free amino acid by transaminase reaction. (12) observed free amino acids were not detected in the tissues of control as well as treated fish, free amino acid were detected in the muscle, liver and kidney tissues of the fish exposed to the lethal concentration and not in the gills. Thenmozhi (13) reported malathion causes increase in free amino acid in the various tissues of freshwater fish *Labeo rohita*. Bais and Lokhande (14) reported that cadmium chloride causes increase in free amino acids in the various tissues of freshwater fish *Ophicephalus striatus.* De Smet (15) has reported that proteolysis is intended to increase the role of proteins in the energy production during heavy metals stress. Stress condition induced elevation in the transamination pathway (16).

In present study levels of total free amino acid level increased significantly. They reported that the concentration of free amino acid and the activities of proteases were increased in gill, liver, kidney and muscles of carp *Labeo rohita* exposed to aluminium chloride also they observed an increase in the activities of GOT and GPT and they suggested that the observed proteolysis is intended to increase the role of protein in energy production during aluminium chloride stress.

Protein degradation and subsequent utilization of the released amino acids for anaplerotic reaction or energy production represent an important mechanism for changes in the total free amino acids concentration. In present investigation reduction in protein content in different tissues of aluminium chloride exposed fish is evident that proteolysis is main reason for increases in total free amino acids. Also in present study serum urea level is significantly increases for these reasons for detoxification of urea total free amino acids concentration is increases in aluminium chloride exposed fishes under stress condition. Under the increased energy demand associated with toxicant induced stress invertebrates may degrade proteins to augment the available energy supply, thus altering the free amino acids pool (17), (18).

Amino acids are essential intermediates in the process of protein synthesis and its degradation products appear in the form of different nitrogenous substances. Amino acids and some nitrogenous compounds play an important part during osmotic stress hence increase or decrease in free amino acid content provide valuable information during stress phenomenon at the tissue level. Similar result Increased free amino acid level suggests tissue damage probably due to augmented proteolysis activity (19). Increment in free amino acid level was the result of breakdown of protein for energy requirement and imparted incorporation of amino acid is protein synthesis (10). James (11) found that the increase in concentration of free amino acid attributed to stepped up proteolysis or increase synthesis of free amino acid by transaminase reaction. Shoba (12) observed free amino acids were not

detected in the tissues of control as well as treated fish, free amino acid were detected in the muscle, liver and kidney tissues of the fish exposed to the lethal concentration and not in the gills. Similar to present study Thenmozhi (13) have reported malathion causes increase in free amino acid in the various tissues of freshwater fish *Labeo rohita*. De Smet and Blust (19) have reported that proteolysis is intended to increase the role of proteins in the energy production during heavy metals stress. Stress condition induced elevation in the transamination pathway (16).

Increase in the free amino acid level due to heavy metal stress is mainly a consequence the higher catabolic activity of protein to meet the high energy demand by breaking down the protein into free amino acids. The incorporation of amino acid in the protein may also be suppressed by heavy metal exposure (20). The increase in amino acids level has been reported in different tissues of fingerlings when treated with nickel chloride (21). They have also suggested that the enhanced levels of total free amino acids are the result of an intensive proteolysis in the respective tissues. Sivaramakrishna and Radhakrishnaiah, (22) have also observed the increased free amino acids content in liver, kidney and muscle on mercury. *Cyprinus carpio* exposed to sublethal concentration of mercury the above results are consistent with present study. According to Sahib (*23*) an enhanced level of free amino acids were observed due to proteolysis and the derived amino acids were fed into the TCA cycle in the form of keto acid.

V.Conclusion

The present study inferred that different tissues of freshwater fish *Labeo rohita* biochemical changes in free amino acids levels increased. Variations of biochemical constituent are due to the adaptive response which is characteristic of vertebrates due to sub-lethal exposure of aluminium chloride. Fish with low protein value is not used for nutritional food purpose, so that cultured fish with high nutritive value could safely be utilized for human consumption.

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Table .1 Free amino acid level (mg/g wet weight of tissues) in *Labeo rohita* fish exposed to aluminium chloride

S.no	Tissues	Control	Experimental concentration	Duration (Days)			
				7th	14th	21st	28th
1.	**Gill**	4.10±0.23[a]	Lower	5.52±0.22 [b]	6.70±0.41 [c]	8.32±0.52 [d]	9.93±0.64 [e]
			Higher	6.12±0.40 [b]	7.62±0.43 [c]	12.36±0.94[d]	16.12±1.15 [e]
2.	**Liver**	5.45±0.25 [a]	Lower	6.54±0.27 [b]	7.67±0.21 [c]	8.44±0.64 [d]	11.16±0.94 [e]
			Higher	7.14±0.22 [b]	9.37±0.23 [c]	13.36±0.84 [d]	16.08±1.12 [e]
3.	**Kidney**	3.06±0.16 [a]	Lower	4.07±0.20[b]	4.89±0.46 [c]	6.13±0.42 [d]	8.23±0.61 [e]
			Higher	5.16±0.42 [b]	6.86±0.37 [c]	9.11±0.13 [d]	12.27±0.82 [e]
4.	**Muscle**	2.12±0.65 [a]	Lower	2.91±0.30 [b]	3.67±0.08 [c]	5.12±0.22 [d]	6.02±0.47 [e]
			Higher	3.86±0.13 [b]	5.68±0.65 [c]	6.17±0.43 [c]	8.15±0.62 [d]

The values are mean ± S.E of six replicates,
Significance at 5% (*p=0.05*)
One way ANOVA Based on DMRT.

Figure 1.Changes in the Free amino acid (mg/g wt weight of tissue) in different tissues of *Labeo rohita* on exposure to lower concentration of Aluminium chloride.

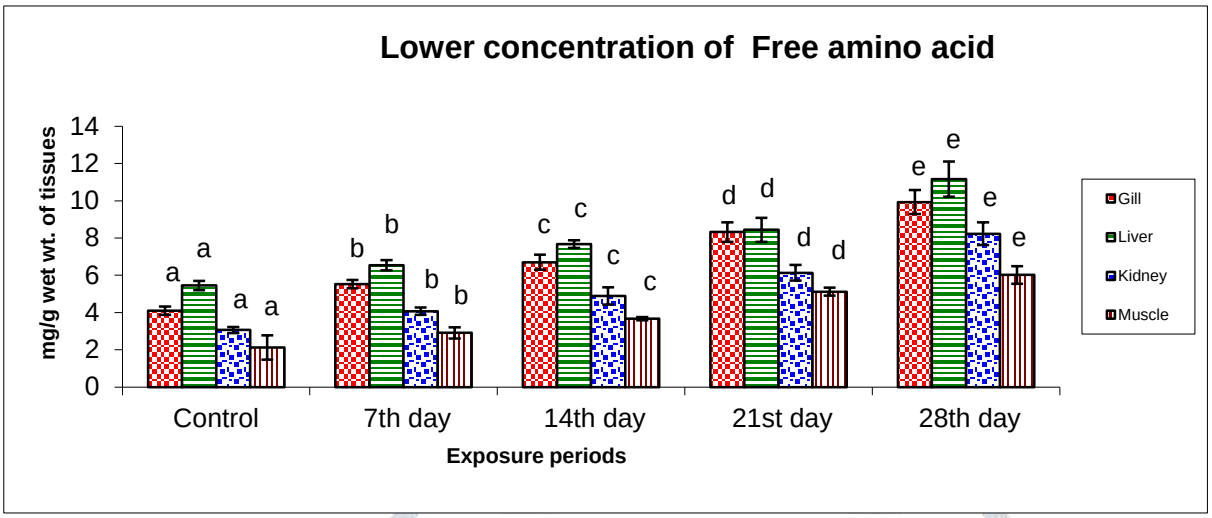
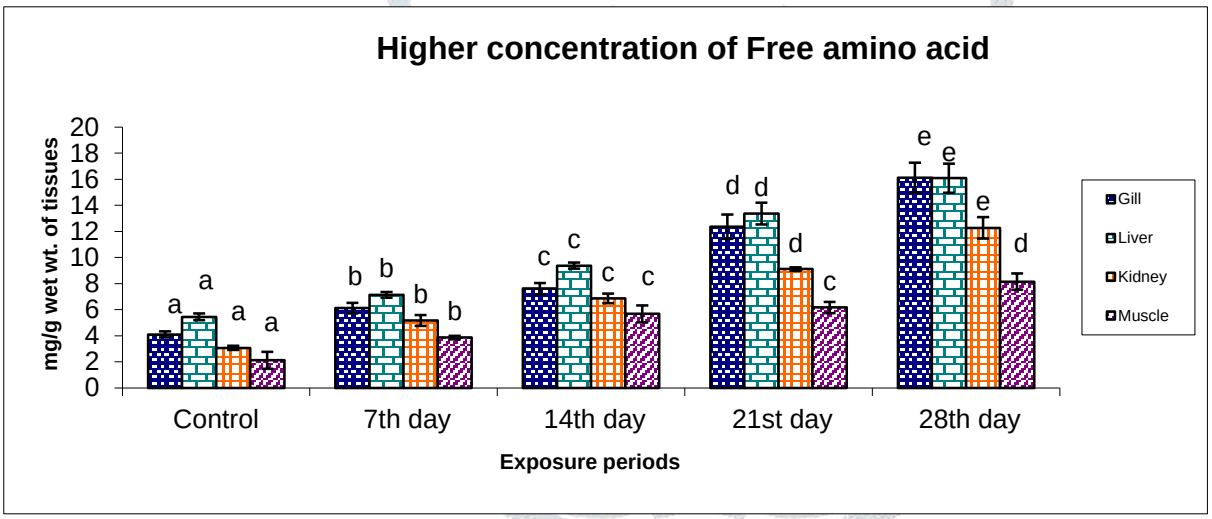


Figure 2.Changes in the Free amino acid(mg/g wt weight of tissue) in different tissues of *Labeo rohita* on exposure to higher concentration of Aluminium chloride.



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