Research Paper

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Ambient Ammonia Stress on Detoxification Enzymes In Brain Tissue of Fish Fingerlings of Cyprinus Carpio

Zoology

KEYWORDS : Ammonia, Enzymes, Cyprinus carpio, Brain tissue

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ABSTRACT

Ammonia is toxic to living animals and produces several biochemical and physiological changes at cellular level when present in higher concentration. Fingerlings of Cyprinus carpio weighing about 500±10mg and 4±0.5cm long were selected for the present study. Temperature and pH were maintained throughout the experimentation. Toxicity test were conducted using Ammonia solution. The LC50 was found to be 18.8 mg/L. 1/6th of the LC50 concentration i.e., 3.24 ppm was selected as sub lethal concentration and fishes were exposed to 14 days to this concentration. In order to understand the effect of ammonia stress on the detoxification aspect of the brain tissue, activity levels of Arginase, Xanthine oxidase (XOD), Superoxide dismutase(SOD), Catalase enzyme levels were estimated in the brain tissue of the animal.

INTRODUCTION

The aquatic environment was extensively contaminated with toxic chemicals released from domestic, industrial and other anthropogenic activities (Begum, 2004). The chemicals have contributed a lot to the "Green revolution", but their deleterious effect on various ecosystems cannot be ignored (Das, 1991; Comoglio *et al.,* 2005). Presently, aquatic pollution by ammonium ion due to their large scale production and the indiscriminate usage for the agriculture and aquaculture form. Industrial effluents and biodegradation of waste products also contribute for the formation of considerable quality of ammonia in the aquatic environment. When present in high concentrations, ammonia is a toxic. Ammonia, a chief constituent of fertilizers when present in high levels is quite toxic to most organisms and it must be either continuously eliminated or converted in to less toxic compounds to prevent a buildup to harmful concentration within the body (Randall and Tsui, 2002). Endogenous ammonia intoxication can occur when there is impaired capacity of the body to excrete nitrogenous waste, as seen with congenital enzymatic deficiencies. A variety of environmental causes and medications may also lead to ammonia toxicity (Ari Auron *et al.,* 2012).

In organisms, oxidative metabolism generates the continuous production of superoxide radicals and hydrogen peroxide, when reactive oxygen species (ROS) over whelms the cellular defenses, such as in ammonia stress conditions (Norenberg *et al.,* 2004). Recent reports have demonstrated enhanced free radical production in cultured astrocytes exposed to pathophysiological concentrations of ammonia (Murthy *et al.,* 2001), and increased superoxide production and reduced activity (Kosenko *et al.,* 1997).

The present study is aimed to understand the changes in detoxification metabolism in Fingerlings of *Cyprinus carpio* under ammonia stress. As the tissue of brain forms an important metabolic center for ammonia production and impact of ammonia, this tissue is selected for the study and some of the enzymes involved in detoxification have been selected for the study.

MATERIAL AND METHODS

Fingerlings of *Cyprinus carpio* weighing about 500±10mg and 40.5cm long were selected and maintained in the laboratory. Temperature and P^H were maintained throughout the experimentation. Toxicity test were conducted using Ammonia Solution. The lethal concentration was found to be 26 mg/L. The LC_{50} was selected, i.e., 18.8 mg/L. $1/6^{th}$ of the LC_{50} concentration namely 3.24 ppm were selected as sub lethal concentration and fishes were exposed to 14 days to this concentration. After 14 days exposure to the test chemical, the fishes were transferred to normal tap water for 14 days recovery for further study. The control, experimental and 14 days recovery brain tissue was collected and stored in deep freezer at -20^{0}C and used for biochemical analysis.

Arginase was assayed by the method of Campbell (1961), Xanthine oxidase (XOD) was estimated by Srikanthan and Krishnamoothy (1955), Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich(1972), Catalase (CAT) was assayed by the method of Aebi (1978), The proteins was assayed by the method of Lowry et.al (1951). The results were subjected to statistical analysis.

RESULTS

In the present study, in the brain tissue of fingerling of *Cyprinus carpio* Arginase, XOD, SOD and Catalase were estimated in control, ammonia exposure, and recovery for 14 days (Table 1).

Ambient ammonia exposure for 14 days has shown an increment in Arginase (+47.3%), XOD (+35.7%) and decrement of SOD (-22.04%), Catalase (-24.8%) activities in brain tissue when compared to control. In the 14 days recovery experimental, a decrement in Arginase (-0.77%), SOD (-0.87%) and Catalase (-0.37%) and increment in XOD (0.81%) over the control was observed.

"Table 1 about here"

Table 1: Changes in the enzyme levels of Arginase, Xanthine oxidase, Superoxide dismutase and Catalase in Brain tissue of fingerlings of *Cyprinus carpio* exposed to14 days ammonia and recovery period.

Name of parameters	Control	Ammonia	14 days recovery
Arginase Mean SD % Change over control	0.2058 ±0.0149	0.3033 ±0.0136 (47.3)	0.2042 ±0.0173 (-0.77)
XOD Mean SD % Change over control	0.2422 ±0.0148	0.3289 ±0.2380 (35.79)	0.2442 ±0.0102 (0.81)
SOD Mean SD % Change over control	4.1050 ±0.1102	3.2000 ±0.0894 (-22.04)	4.1017 ±0.1059 (-0.87)
Catalase Mean SD % Change over control	0.6123 ±0.0135	0.4600 ±0.0178 (-24.8)	0.6103 ±0.0109 (-0.37)

All values are Significant P<0.05 levels.

Units:
Arginase - (moles of urea formed/mg protein/hour)
XOD - (moles of formazon formed/mg protein/hour)
SOD - units of Superoxide anion reduced/ /mg protein/min
CAT - moles of H_2O_2 degraded/mg protein/min

DISCUSSION

Arginase plays an important role in the urea cycle in the breakdown of arginase. Increased arginase activity might be due to efficient ammonia detoxification. The detoxification of ammonia is achieved by synthesis of urea beside the production of arginine and ornithine (Smith, 1951; Hari *et al.,* 2012a). The presence of arginase in brain tissue was reported by several studies in different toxicities **(Wu, and Morris, 1998; Morris. SM., 2002).**

The elevated levels of Xanthine oxidase in the present investigation indicates the over production of Superoxide anion (O_2^-) in the brain regions of fingerlings of *Cyprinus carpio* in response to ammonia exposure. The increased activity of XOD in our study was in agreement with the finding of Munilashmi, 2010 who reported increased XOD activity in invivo and invitro conditions in adult fish under induced ammonia stress. Hari *et al.,* 2012b, was reported increased XOD levels in adult *Cyprinus carpio* exposed to ammonia.

Superoxide dismutase and Catalase are the antioxidant enzymes that provide cellular protection against the damage caused by free radicals and ROS (Ashraf *et al.,* 2007; Patra *et al.,* 2011). Present study reveals that exposure of ammonia causes significant decrease in activity levels of two enzymes (SOD, Catalase) in brain tissue of fingerlings of *Cyprinus crapio.* The production of radicals and ROS in the ammonia exposed fingerlings might have been reduced and probably is not removed by the reduced antioxidant enzymes and might have probably produced oxidative stress in the brain tissue of fingerlings of *Cyprinus crapio*. Similar results were reported in cadmium induced fish (Bhavani *et al.,* 2012).

In present investigation, results of the acute ammonia stress in fingerlings of *Cyprinus crapio* showed many changes. The ambient medium levels of Arginase, XOD levels were increased and SOD and Catalase were decreased during acute ammonia stress. The extent of changes was minimal and during recovery period suggesting that there is an improvement in the fingerlings. The values of the selected biochemical parameters are also nearer to the normal levels. During recovery period the reversal of stress conditions might have occurred and the fingerlings requires might have been metabolically recovered the stress conditions.

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