Research Paper

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Recovery Offish Fingerlings Stage of Cyprinus Carpio From Induced Ammonia Stress

ZOOLOGY

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ABSTRACT

Ammonia is a byproduct of fish metabolism, when present in high levels is quite toxic to most organisms has been attributed to the ammonia species.In the present study 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 LC50was 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 Glutamatedehydrogenase (GDH), glutamine synthetase(GS), Glutathione-S-transferases (GST)andGlutathione peroxidase(GPx)enzyme levels were estimated in the brain tissue of the animal.

INTRODUCTION

India is bestowed with nature's plentiful bounty with regard to fish germ plasm resources. But these are under stress owing to indiscriminate over exploitation, habitual destruction through water pollution, increased water abstraction etc. As a result, declining trend of fishes is observed. Aquatic pollution results mainly as a consequence of agricultural waste, surface run out, biodegradation of waste and discharge if industrial wastes products also contribute for the formation of considerable quality of ammonia in the aquatic environment (Singhtet al., 1991; Hari et al., 2012).

Aquatic animals, including invertebrates, fish and larval amphibians, excrete mostly ammonia. Ammonia is highly soluble in water and permeates cell membranes relatively easily. Despite its high solubility, an animal must use 400 ml of water to dilute every gram of ammonia to maintain ammonia concentrations below toxic levels. Only animals that respire in water, therefore, excrete ammonia as their major nitrogen waste product (Wright.,1995; LizanneRoxburgh et al., 2002).

Glutamine synthesis mediated by the astrocytic enzyme glutamine synthetase is the major pathway for ammonia detoxification in the brain and cerebrospinal fluid. In conditions in which excess ammonia exists, osmotically active glutamine concentrations in brain increase, leading to astrocytic damage and swelling. Consequently, the astrocyte promotes intercellular glutamate release, which decreases the glutamate intracellular pool and ultimately leads to cell death of the glutamatergic neurons (Connelly et al., 1993; Cooper, 2001; Braissant et al., 2010). Acute ammonia toxicity is also mediated by excessive activation of NADA receptors in brain (Monfort et al., 2002), leading to activation of neurotoxic signal transduction pathways that result in animal death.

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

MATERIAL AND METHODS

Fingerlings of Cyprinus carpio weighing about 500±10mg and 4.05cm long were selected and maintained in the laboratory. Temperature and PH were maintained throughout the experimentation. Toxicity test were conducted using Ammonia Solution. The lethal concentration was found to be 26 mg/L. The LC50 was selected, i.e., 18.8 mg/L. 1/6th of the LC50 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 -200C and used for biochemical analysis.

Glutamatedehydrogenase (GDH) activity was estimated by the method ofLee and Lardy (1965) with slight modification ofPrameelamma et al. (1975). Glutamine synthetase activity-was assayed by the method of Wu Chung (1963). Glutathione peroxidase(GPx)was estimated by Flohe and Gunzler (1984) method, Glutathione-S- transferases (GST)was estimated by Habig et al. (1974) method, 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 carpioGlutamate dehydrogenase (GDH), glutamine synthetase(GS), Glutathione-S-transferases (GST) and Glutathione peroxidase(GPx)were estimated in control, ammonia exposure and recovery for 14 days (Table 1).

Ambient ammonia exposure for 14 days has shown an increase in GDH (+37.5%), GS (+29.78%), GST (+27.20%) and decrease of GPx (-25.89%) activities in brain tissue when compared to control. In the 14 days recovery experimental, a decrement levels in GDH(-1.46%), GS (-2.31%), GST(-0.31%) and increment inGPx(+1.15%), activities in brain tissue over the control was observed.

Table 1: Changes in the enzyme levels of Glutamate dehydrogenase (GDH), glutamine synthetase(GS), Glutathione-S-transferases (GST) and Glutathione peroxidase(GPx)in brain tissue of fingerlings of Cyprinus carpio exposed to14 days ammonia and recovery period.

Name of parameters	Control	Ammonia	14 days recovery
GDH Mean SD % change over control	0.3145 ±0.0164	0.4327 ±0.0158 (37.5)	0.3099 ±0.0150 (-1.46)
GS Mean SD % change over control	0.5270 ±0.0146	0.7506 ±0.0120 (29.78)	0.5151 ±0.0219 (-2.31)

GST Mean SD % change over control	0.4811 ±0.0051	0.6120 ±0.0105 (27.20)	0.4796 ±0.0046 (-0.31)
GPx Mean SD % change over control	0.4325 ±0.0371	0.3205 ±0.0244 (-25.89)	0.4375 ±0.0214 (1.15)

All values are Significant P<0.05 levels.

Units: GDH-(μmoles of formazon formed/mg protein/hour)
GS- (μ moles of Glutamylhydroximate formed / mg of tissue / hour)
GST-(μmoles of thio ether formed/mg protein/hour)
GPx-(μ moles of NADPH Oxidized /mg protein/min)

DISCUSSION

GDH is a mitochondrial enzyme, catalyzes the oxidative deamination of glutamate generating α-ketoglutarate, an important intermediate of the Krebs cycle. The GDH activity in the present study exhibited enhancement in brain tissue fingerlings of Cyprinus carpio, suggesting a need for a α-ketoglutarate. The regulatory role of theenzymes observed in animal models in checking the deamination process was reported.(Reddy and Venugopal, 1990; Nagender Reddy etal, 1991 and David, 1995). Stimulated GDH activity under ammonia stress suggests the need for α- ketoglutarate in the TCA cycle for the liberation of energy (Nagender Reddy et al., 1991).

Glutamine synthetase is predominantly localized in astrocytes of intact brain physiology and plays a vital role in the W-amidation of glutamate to form glutamine. Elevation in the activity of glutamine synthetase in general depicts greater mobilization of glutamate for the synthesis of glutamine.In the present study glutamine synthetase activities was found to increase in the brain tissues of fingerlings of Cyprinus carpio exposed with ammonia. This indicates high mobilization of glutamate to form glutamine (Krebs, 1935). Similar results were observed in the fish walking cat fish Clariasbetrachusunder hyper ammonia stress (Nirmalenduet al., 2002; Zaiba Y Kharbaliet al., 2005).

Glutathione-S-Transferase is a group of multifunctional proteins involved in the detoxification of a wide spectrum of compounds (Jackogy, 1980). Glutathione-STransferases are involved in the initiation of repair of not only lipid peroxides to less reactive alcohols but also of direct damage since it substrates include DNA hydroperoxides (Tan et al.,1988). In the present study glutamine -S-Transferase activities was found to increase in the brain tissues of fingerlings of Cyprinus carpio exposed with ammonia.

Glutathione peroxidase (GPx) is located in both mitochondria and cytosol. Glutathione peroxidase system acts against oxidative stress in the tissues(Ji et al., 1998). It has a much affinity for H_2O_2 at low concentration compared with Catalase. Thus, when cellular levels of H2O2 are low Glutathione peroxidase is more active than CAT in converting H2O2 from the cell (Power et al., 1999).In the present study GPx activity has shown a significant decline in ammonia exposed fingerlings of Cyprinus carpio. The decreased GPx activity could be due to a decrease in reduced Glutathione (GSH) concentration as a result of excessive free radical production from ammonia intoxication which can leads to an increase the oxidative stress.

In present investigation, results of the acute ammonia stress in fingerlings of *Cyprinus carpio* showed many changes. The activity levels of GDH, GS, and GSTenzymes were found to increasewithGPXenzyme gave a decrease during acute ammonia stress and the extent of changes were minimal during recovery period suggesting that there is a 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 haveoccurred and the fingerlings requires might have been metabolically recovered from the stress conditions.

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