Research Paper

Volume : 3 | Issue : 6 | June 2014 • ISSN No 2277 - 8179

Ambient Ammonia Stress and Extent of Recovery in Fry of Fish *Cyprinus Carpio* Through Study of Certain Metabolic Aspects

Zoology

KEYWORDS : Ammonia, Enzymes, Fry, Cyprinus

K. RAMAKRISHNA REDDY Department of Zoology, Sri Venkateswara University, Tirupati-517 502, A.P

P.NEERAJA Department of Zoology, Sri Venkateswara University, Tirupati-517 502, A.P

ABSTRACT

Ammonia toxicity in lakes, ponds results due to draining of excess toxicants and pollutants from agriculture and industries. In the present study Fry of Cyprinus carpio weighing about 260±5mg and 1.8±0.5cm length were selected. Temperature and pH were maintained throughout the experimentation. Toxicity test were conducted using Ammonia solution. The LC50was found to be 13.8 mg/L. 1/6th of the LC50 concentration i.e., 2.3 ppm was selected as sub lethal concentration and fishes were exposed to 14 days to this concentration. Later they were kept in ammonia free water for 14 days to study the extent of recovery from ammonia stress by fish fry. In order to understand the effect of ammonia stress and impact of recovery on the detoxification aspect of the fry, activity levels of Glutamatedehydrogenase (GDH), Glutamine synthetase (GS), Glutathione-S-transferases (GST), Glutathione peroxidise (GPx) enzyme levels were estimated in the whole animal.

INTRODUCTION

The aquatic environment is very important because it is a store house and resource of variety of fishes. Presently aquatic pollution has because a series problem. It has been estimated that about 70,000 manmade chemicals are in our day to day use. The chemical have contributed a lot to the "Green revolution", but their deleterious effect on various ecosystems cannot be ignored (Das, 1991; Comoglio et al., 2005).

Ammonia occurs in natural water in un-ionized (NH_3) and ionized (NH_4^+) forms and can be a serious toxicant to fishes and other aquatic species. It enters water systems from several sources including industrial wastes, sewage effluent, agricultural input and animal feed lots. It is also a metabolic by product of fish. The accumulation of ammonia in water used for intensive fish culture is a potential problem because it is toxic to fish. Most nitrogen in feeds and fertilizers that is not converted to fish flesh enters the water as ammonia, either by direct excretion from fish or by bacterial action on wastes. Ammonia concentrations can increase rapidly when water exchange rates are low (Randall and Tsui, 2002; H.H. Abbas., 2006). The present study is to examine the relationship of the metabolites and enzymes involved in the mechanism of Glutamate in Fry on exposure to ambient ammonia solution.

MATERIAL AND METHODS

Fry of Cyprinus carpio weighing about 260±20mg and 1.8±0.5cm length were selected and maintained in the laboratory. Temperature and pH were maintained throughout the experimentation. Toxicity test were conducted using Ammonia Solution. The LC_{50} was selected, i.e., 13.8mg/L. $1/6^{th}$ of the LC_{50} concentration namely 2.3 ppm was 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 fry was collected and stored in deep freezer at -20^0C and used for biochemical analysis. The whole animal was used for experimentation due to its small size.

Glutamate dehydrogenase (GDH) activity was estimated by the method of Lee and Lardy (1965) with slight modification of Prameelamma 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 fry tissue of Cyprinus carpio Glutamate 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 (+43.78%), GS (+44.9%), GST (+39.4%) and decrease of GPx (-39.9%) activities in fry tissue when compared to control. In the 14 days recovery experimental, a decrement levels in GDH (-1.65%), GS (-1.80%), GST(-0.64%) and increment in GPx (+1.47%), activities in fry 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 fry of Cyprinus carpio exposed to14 days ammonia and recovery period.

Name of the parameters	Control	Ammonia	14 days recovery
GDH Mean SD % change over control	0.2898 ±0.0229	0.4167 ±0.0367 (43.78)	0.2850 ±0.02588 (-1.65)
GS Mean SD % change over control	0.4600 ±0.0178	0.6667 ±0.0216 (44.9)	0.4517 ±0.0299 (-1.80)
GST Mean SD % change over control	0.4183 ±0.0292	0.5833 ±0.0301 (39.44)	0.4150 ±0.0301 (-0.64)
GPx Mean SD % change over control	0.3383 ±0.0614	0.2033 ±0.03933 (-39.9)	0.3333 ±0.0427 (-1.47)

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 fry tissue of Cyprinus carpio, suggesting a need for α-ketoglutarate. The regulatory role of the enzyme 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 fry tissues 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 Clarias betrachus under 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-S-Transferases 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 fry tissues 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 H_2O_2 are low Glutathione peroxidase is more active than CAT in converting H_2O_2 from the cell (Power et al., 1999).In the present study GPx activity has shown a significant decline in ammonia exposed fry 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 fry of Cyprinus carpio showed many changes. The activity levels of GDH, GS, and GST enzymes were found to increase with GPx enzyme 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 fry. 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 fry requires might have been metabolically recovered from the stress conditions.

REFERENCE

1. Abbas. H.H (2006). Acute toxicity of ammonia to Common Carp Fingerlings (Cyprinus Carpio) at different PH levels. Pakistan journal of Biological Sciences, 9(2): 2215-2221. | 2. Comoglio, L., Amin, O., Roqe, A., Batan court –Lozano , M., Angas, D. and Haro, B.M. (2005). Ecotoxicology and environmental safety. 62(1): 66-74. | 3. Das, R.K. 1991. Environmental pollution and genetic hazards. J. Sci and Tech. Silver Jubliee(1967-19910, X: 1991, Sambalpur University , Orissa, India. | 4. David, M. (1995) Effect of fenvalerate on Behavioural, Physiological & Biochemical aspects of fresh water fish,Labeorohita. Ph.D. Thesis, S. K. University, Anantapur, Andhra Pradesh, India. | 5. Flohe, L and Gunzler, W.A. (1984). Glutathione Peroxidase. Methods Enzymol. 105: 115-121. | 6. Habig, W.H., Pabst, M.J and Jakpby, W.B., (1974). Glutathione-s-transferases. J. Biol. Chem., | 7. Jackoby, W.B, (1980). Detoxification enzymes. In enzymatic basis of detoxification. Academic Biol. Chem. Press, New York. | 8. Ji , L.L and Leeuwenburgh ,C., Leichtweiss, S., Gore , M., Fiebig, R., Hollander, J., and Bejma, J (1998). J Ann. Newyork. Acad. Sci. 854:102-117 | 9. Krebs, H.A (1935). Metabolism of amino acids. The synthesis of glutamine from glutamic acid and ammonia and the enzyme hydrolysis of glutamine in animal tissue. J.Biochem, 29: 1951-1969. | 10. Lee, Y.L., and Lardy, H.A. (1965) Influence of thyroidhormones on L-glycerophosphate dehydrogenase and other dehydrogenases in various organs of the rat. Journal of Biological Chemistry, 240: 1427-1430. | 11. Lowery D.H., N.J. Rosen brough, A.L.Farr and R.S. Randall (1951). Protein measurement with folin- phenol reagent. Journal of Biological chemistry, 193:265-273. | 12. Nagendra Reddy, A., NBRK Venugopal, and Reddy, S.L.N (1991) effects of endosulphan 35 EC on certain aspects of protein metabolism in various tissues of a fresh water field crab Berytelphusaquerini: Pesticide Biochemistry and Physiology, 39(2), 121-129. | 13. NirmalenduSaha, Zaiba Y. Kharbali, ArundhatiBhattarchajee, Carina Goswami and DreterHaussinger(2002).Effect of alkalinity (pH 10) on ureogenesis in the air-breathing walking catfish, Clariasbatrachus. Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology, 132(2):353-364. | 14. Power, S.K and Lennon, S.L (1999). Analyses of cellular responses to free radicals focus on exercise and skeletal muscleProce. J Nutria. Socie , 58 :1025-1033. | 15. Prameelamma, Y., Rao, K.V.K. and Swami, K.S. (1975) Regulation of succinate dehydrogenase activity in gastrocnemius muscle of frog. Ranahexadactyla. Indian J. Exp. Bio., 13: 177-179. | 16. Randall, D. J. and Tsui, T .K. N (2002). Ammonia toxicity in fish. Marine Pollution Bulletin.45(1): 17-23. | 17. Reddy, S.L.N and Venugopal, N.B.R.K. (1990). Flouride induced changes in protein metabolism in the tissues of fresh water crab, Varytelphusaquerini. Environmental Pollution, 67: 97-108. | 18. Tan, K.H., Mayer, K.J., Kettrer, B. and Gillies N, (1988).Irradiated DNA as a substrate for glutathione transferases. Int. J. Radiat. Biol.,53: 1003. | 19. Wu, C (1963). Glutamine synthetase-I: A comparative study of its inhibition by DL-amino hydroxyl-lysine. Comp. Biochem. Physiol. (8):335-351. | 20. Zaiba Y. Kharbali, ShritapaDatta, Kuhelibiswas, Debajit Sharma and Nirmalender-Saha, (2005) Expression of ornithineurea cycle enzymes in early life stages of air-breathing walking catfish Clariasbatrachusand induction of ureogenesis under hyper-ammonia stress. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. |