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AMBIENT AMMONIA STRESS ON CERTAIN DETOXIFYING ENZYMES IN KIDNEY TISSUES OF FISH, *CYPRINUS CARPIO*

P. HARI * AND P.NEERAJA

Department of zoology, Srivenkateswara University, Tirupathi – 517502 A.P INDIA

ABSTRACT

Presently, aquatic pollution by ammonium compounds, due to their large scale production and the indiscriminate usage for the agriculture and aquaculture farms, has earned an important place among the environmental pollutants. Industrial effluents, bio degradation of waste products and food remains also contribute for the formation of considerable quantity of ammonia in the environment. Ammonia when present in higher concentration is toxic to living animals and produces several biochemical and physiological changes at cellular level. In view of these some of the important physiological and biochemical events occurring under ammonia stress in the animal, Fish *Cyprinus carpio* is taken for the present study. Animal weighting 120±10 g and 17±2 cm length are exposed to 4 ppm of ammonia solution for 7days. In order to understand the effect of chronic ammonia stress on the detoxification aspect of the kidney tissue, activity levels of arginase,Glutathioneperaxidase(GPx),Glutathione–s-ransferase(GST),Glutaminesynthetase(GSH), Glutamate dehydrogenase (GDH),Xanthine Oxidase (XOD),Catalase (CAT), Superoxide dismutase(SOD) enzyme levels were estimated in the kidney tissue of the animal. Increased activity levels in Arginase, GPx, GST,GSH, GDH , XOD, CAT and SOD enzyme levels was observed on prolonged ammonia exposure.

KEY WORLDS: Ammonia stress, detoxification, *cyprinus carpio*

P. HARI
Department of zoology,Room no-205, Srivenkateswara University, Tirupathi – 517502 A.P

Corresponding author

INTRODUCTION

Industrial effluents, bio degradation of waste products and food remains also contribute for the formation of considerable quantity of ammonia in the environment. The ammonia so formed accumulates in soil and water and consequently contaminates the aquatic ecosystem which may endanger the life of aquatic fauna particularly fishes because of their high susceptibility to toxic ammonia. 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 into less toxic compounds to prevent a build up to harmful concentrations within the body (Randall and Tsui, 2002) [1] . The ammonia content depends upon the balance between its catabolism and detoxifying mechanisms. In biological systems, ammonia detoxification takes place mainly through conversion of NH_3 to glutamate by glutamate dehydrogenase activity [2] (Ip *et al*., 2008). Ammonia induced pathophysiological conditions are suspected to the involvement of free radical generation (Reactive oxygen species O_2^-, $H_2O_2^-$, OH^-) during oxidative stress in mammalian models [3] (Sarkar *et al*., 1997). Typically mammalian species have been used as models to study oxidative stress and to elucidate the mechanisms behind cellular damage and response largely because of the interest in human health issues. Fish exhibit many of the same defenses against oxidative stress, as do mammals, suggesting that fish and mammalian systems have similar cellular responses to oxidative stress. These defenses include enzymatic defenses such as SOD, GPX and Glutamine synthetase (GSH) besides low molecular weight free radical scavengers such as GSH, vitamin E and C. The antioxidant enzymes such as superoxide dismutase (SOD), Glutamine synthatase (GSH) and Glutathione peroxidase (GPX) are studied under this lethal ammonia stress in *Cyprinus carpio*.

MATERIALS AND METHODS

Cyprinus carpio weighting about 120±10g and 17±2 cm long were selected and maintained in the laboratory temperature and pH were maintained throughout experimentation. Toxicity test were conducted using ammonia solution. Lc50 is 24.04ppm for 24hours was determined to understand the impact of ammonia stress. 4ppm or 1/6 of Lc 50 was selected as experimental concentration to induce ammonia stress and prevent any mortality in fish. Steps were taken to maintain the experimental concentration constant throughout the experimentation. Kidney tissue was collected. Arginase was assayed by the method of Cambell [4] (1961) with slight modification, Glutathione peroxidase (GPx) was assayed by the method of Flohe and Gunzeer [5] (1984), Glutathione–s-Transferase (GST) was assayed by the method of Habing *et. al* [6] (1974), Glutamine Synthetase (GSH) was assayed by the Chung Wu [7] (1963), Glutamate dehydrogenase (GHD) was assayed by the method of Lee and Lardy [8] (1965), Xanthine Oxidase (XOD) activity was estimated by the dye reduction method of Srikanthan and Krishna moorthy [9](1955), Catalase (CAT) activity was measured by a slightly modified version of Aebi [10](1984), Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich [11] (1972), Total protein content was determined using Lowry *et al*., [12] (1951) method. The result was subjected to statistical treatment.

RESULTS

Ambient ammonia exposure for 7 days has shown an increment in activity levels of ginase,Glutathione peraxidase(GPx),Glutathione–s-ransferase(GST),GlutamineSynthetase (GSH),Glutamatedehydrogenase (GDH),Xanthine Oxidase(XOD),Catalase (CAT), Superoxide dismutase(SOD) enzymes when compared to control. The experiment tissue of fish has shown 7.52, 18.08, 36.69, 22.51, 22.10, 58.58, 59.05 and 26.23 percent of increment in Arginase, GPx, GST, GSH, GDH, XOD, CAT, and SOD respectively on prolonged exposure of ambient ammonia.

DISCUSSIONS

Arginase plays an important role in the urea cycle in the breakdown of arginine. Arginase activity was found to increase in experimental fish than control. Increased arginase activity might be due to efficient ammonia detoxification. Similar results were observed in both ammonium lactate administration and in denervated frogs [13] **(**Sreeramulu chetty, 1978). GSH provides cellular protection by assisting in the breakdown of peroxides and by reduction of disulfides[14] (Gallagher and Di Giutio, 1994). However, catalase is also involved in the reduction of hydrogenperoxide. Rana and Boora (1992) [15] suspect a functional competition between catalase and glutathione peroxidase (GPX) for the excess hydrogen peroxide. The enhanced GPX activity under liquor ammonia stress indicates the effective detoxification from inorganic and organic peroxides that were formed due to oxidative stress. Glutathione-S-Transferase is a group of multifunctional proteins involved in the detoxification of a wide spectrum of compounds [16] (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 [17] (Tan *et al* 1988). The glutamine sythetase activity in the kidney tissue was also found increase to compared to control animals. It converts ammonia into glutamine by the activity of glutamine synthetase. Similar results were observed in the fish walking cat fish *Clarias betrachus* under hyper ammonia stress by [18] Nirmalendu *et al*., (2002) and [19]Zaiba Y Kharbali *et al*., (2005). In the present study GDH activity gave an increase in the kidney tissue of fish exposed to sublethal concentration of liquor ammonia.GDH catalyzes the reversible reaction of oxidative deamination of glutamate to α-ketoglutarate and ammonia and plays an important role in the catabolism and biosynthesis of amino acid [20]**(**Muray *et al.,* 2007). The elevation observed in the GDH activity indicates its contribution to enhanced ammonia levels and glutamate oxidation during ammonia toxicity. The enhanced Xanthine oxidase levels in the present study under ammonia intoxication indicate effective detoxification of ammonia by this enzyme. Similar increased Xanthine oxidase activity of other animals exposed to heavy metals [21](Marry Chandravathy & Reddy, 1996) and cadmium [22](Haber and Weiss, 1984) was reported. Significant increase in the catalase activity in the selected tissue under liquor ammonia exposure. This increase might be due to its active involvement in decomposition of H_2O_2 generated during dissimulation of O_2 by SOD. [23]Mather Mihaich and Digivlio (1991**)** observed increased catalase activity in channel cat fish treated with bleached kraft mill effluents. [24]Iqbal Ahmad *et al*. (2006) reported increased catalase activity in different tissues of *Anguilla Anguilla L* exposed to chromium. Elevated SOD activity observed in this study might be to detoxify the superoxide anion radicals produced from xanthine oxidase reaction in order to arrest the radical damage to cellular organization; [25]Shiraz Bhasha (2002) reported increased SOD activity in Cadmium treated *Tilapia mossambica* in different tissues. [26]Haung *et al*. (2006) observed increased SOD activity in different tissues of *Cyprinus carpio* treated with mixed polluted yellow River of China. Thus ammonia stress seems to stimulate detoxifying and antioxidant enzymes to protect the animal from ammonia stress.

Table.

Changes in the enzyme levels of Arginase, Glutathione peraxidase (GPx), Glutathione–s-Transferase(GST),GlutamineSynthetase(GSH),Glutamatedehydrogenase(GDH),Xanthine Oxidase(XOD),Catalase (CAT), Superoxide dismutase(SOD) in kidney tissue of fish *Cyprinus carpio* exposed to sub lethal concentration of liquor ammonia for one week.

Parameter	Control	Experimental
Arginase (µ moles urea formed/ mg protein / hiour) **S.D , % Change**	±0.0075	±0.0057 (7.52)
GPX(µ moles of NADPH oxidized/min/g/wet wt) **S.D , % Change**	±0.0586	±0.1744 (18.08)
GST (µ moles of thio ether formed/mg protein/min) **S.D , % Change**	±00296	±0.0088 (36.69)
GSH (µ moles **γ** – glutamyl hydroxymate/mg protein/hour) **S.D , % Change**	±0.1289	±0.0606 (22.51)
GDH (µ moles of formazon/mg protein /hour) **S.D , % Change**	±0.0082	±0.0042 (22.10)
XOD (µ moles of formazon formed/mg protein/hour) **S.D , % Change**	±0.0426	±0.0307 (58.58)
CAT (µ moles of H_2O_2 metabolized /mg protein/min) **S.D , % Change**	±0.0425	±0.0096 (59.05)
SOD (units /mg protein**) S.D , % Change**	±1.2261	±0.9723 (26.23)

Each value is mean and ± S.D. of six individuals observations. All values are significant p<0.05 levels.

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