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AMBIENT AMMONIA STRESS ON CERTAIN METABOLIC ASPECTS IN LIVER TISSUE OF FISH, *CYPRINUS CARPIO*

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ABSTRACT
Ammonia has received increasing attention over the past few years as potentially important pollutant in aquatic system mainly due to the indiscriminate use of fertilizers and pesticides. Ammonia is toxic to living animals and produces several biochemical and physiological changes at cellular level when present in higher concentration. In view of these some of the important physiological and biochemical events occurring under ammonia in the fish animal were taken for the study. Fish *Cyprinus carpio* is taken for the present study. Animal weighting 120±10g 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 liver tissue, total protein, ammonia, urea content and activity levels of arginase, Glutathione peraxidase (GPx), Glutathione–s-Transferase (GST), Glutamate dehydrogenase (GDH), Superoxide dismutase (SOD) enzyme levels were estimated in the liver tissue of the animal. A decrement in total protein content along with increment in ammonia and urea and increased activity levels in GPx, GST, GDH and SOD enzyme levels was observed on prolonged ammonia exposure.

INTRODUCTION

The aquatic environment is very important because it is a store house of variety of fishery resource. 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. These 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). Presently, aquatic pollution by ammonium 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. 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 build up to harmful concentration within the body (Randall and Tsui, 2002). The ammonia content depends upon the balance between its catabolism and detoxifying mechanism. In biological system ammonia detoxification takes place mainly through conversion of ammonia to glutamine by detoxifying enzymes. Protein being involved in the architecture and physiology of the cell seem to occupy a key role in the cell metabolism (Murray *et al*., 2007). Catabolism of protein and amino acids make a major contribution to the total energy production in fish. Bradbury *et al*., 1987 pointed out that the decreased protein content might also be attribute to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery. Protein depletion in tissues may constitute a physiological mechanism and may play a role of compensatory mechanism under ammonia stress, to provide inter mediates to the Krebs cycle. The present study is to examine the relation ship of the metabolites and enzymes involved in the mechanism of protein in fish liver on exposure to ambient ammonia solution.

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. LC_{50} is 24.04ppm for 24h was determined to understand the impact of ammonia stress. 4ppm or 1/6 of LC_{50} was selected as experimental concentration. Steps were taken to maintain the experimental concentration constant throughout the experimentation. Liver tissue was collected. Total protein, ammonia and urea levels were estimated using the methods Lowry *et al*. (1951), Bergmeyer (1965) and Natelson (1971), Glutathione peroxidase (GPx) was assayed by the method of Flohe and Gunzler (1984), Glutathione–s-Transferase (GST) was assayed by the method of Habig *et al*. (1974), arginase was assayed by the method of Campbell (1961) with slight modification, Glutamate dehydrogenase (GHD) was assayed by the method of Lee and Lardy (1965), Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich (1972). The result was subjected to statistical treatment.

RESULTS

Ambient ammonia exposure for 7 days has shown a decrement

Table 1: Changes in the levels of total protein, ammonia and urea in liver tissue of fish *Cyprinus carpio* exposed to sub lethal concentration of liquor ammonia for one week

Parameters	Control	7 days
Total protein (mg/g wet wt.) S.D. % change	± 0.0607	± 0.1313(-68.33)
Ammonia (μ moles of ammonia/gm wet wt) S.D. % change	± 0.1782	± 0.0970 (+37.50)
Urea (μ moles of urea/gm wet wt) S.D. % change	± 0.3919	± 0.7012 (+35.70)

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

Table 2: Changes in the enzyme levels of arginase, Glutathione peraxidase (GPx), Glutathione–s-Transferase (GST), Glutamate dehydrogenase (GDH), Superoxide dismutase (SOD) in liver tissue of fish Cyprinus *carpio* exposed to sub lethal Concentration of liquor ammonia for one week

Parameters	Control	7 days
Arginase(μ moles urea formed/ mg protein/ hour) S.D.%change	± 0.0094	± 0.0073 (+44.58)
GPx (μ moles of NADPH oxidized/min/g/wet wt) S.D.%change	± 0.1429	± 0.0689(+27.28)
GST (μ moles of thio ether formed/mg protein/min) S.D.%change	± 0.0264	± 0.0558(+47.92)
GDH (μ moles of formazon/mg protein/hour) S.D.%change	± 0.0050	± 0.0085 (+27.70)
SOD(units/mg protein) S.D.%change		

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

in total protein content, while an increment was observed in ammonia, urea content and activity levels of arginase, GPx, GDH, GST and SOD in the experimental. The percent decrease in total protein content was -68.33 for one week period of liquor ammonia exposure. The experiment tissue of fish has shown an increase of 37.50 percent in ammonia levels and 35.70 per cent in urea contents. The Arginase enzyme activity levels were found to show increment of 44.58 per cent for one week ambient ammonia exposure. While 27.28, 47.92, 27.70 and 29.36 per cent of increment was observed in GPx, GST, GDH, and SOD respectively on prolonged ammonia exposure.

DISCUSSION

The levels of total protein content showed a decrease. The decrement in total protein levels suggests stimulation of protein break down on prolonged exposure of fish to ambient ammonia (Knapp and Weister, 1981). The liver is the primary organ for metabolism, biotransformation, and detoxification of xenobiotics and excretion of harmful substances. Since liver is the major functional and metabolic active centre to detoxify the ammonia, increased urea content in this tissue is quite reasonable. As has been observed through increased levels of ammonia and urea (Gregory, 1977;Colombo and Bachman, 1978). In the present investigation, the activity levels of arginase, GPx, GDH, GST and SOD found to increase under ambient ammonia stress. Arginase plays an important role in the urea cycle in the breakdown of arginine. Increased arginase activity might be due to efficient ammonia detoxification. The detoxification of ammonia is achieved by synthesis of urea besides the production of arginine and ornithine (Smith, 1951). GPx provides cellular protection by assisting in the breakdown of peroxides and by reduction of disulfides (Gallargher and DiGiulio, 1994). The enhanced GPx activity under liquor ammonia stress indicates the effective detoxification from inorganic and organic peroxides that were formed due to oxidative stress. The enhanced GST is a group of multi functional protein involved in the detoxification of a wide spectrum of compounds (Jackoby, 1980). GST involved in the initiation of repair of not only lipid peroxides to less reactive alcohols bur also of direct damage since it substrates induce DNA hydroperoxides (Tan *et al*., 1988). In the present study GDH activity increased in the liver tissue of fish exposed to sub lethal concentration of liquor ammonia. GDH catalyse the reversible reaction of oxidative deamination of glutamate to ± ketoglutamate and ammonia and play an important role in the catabolism and biosynthesis of amino acid (Murray *et al.,* 2007). The elevation of GDH activity indicates its contribution to enhanced ammonia levels and glutamate oxidation during ammonia toxicity. GDH helps in supplying keto acids to the TCA cycle inorder to compensate the energy crisis in liver tissue during ammonia toxicity. Elevation of SOD activity observed in the study might be to detoxify the super oxide anion radicals' inorder to arrest the radial damage to cellular organization. Similar response of SOD activity also reported by several authors Shiraz (2002) in Cadmium treated *Tilapia mossambica,* Haung *et al*. (2006) in *Cyprinus carpio* treated with mixed polluted yellow river of china. Thus fish seem to tolerate low levels of ammonia and were able to protect them selves by detoxifying it.

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