Asian Fisheries Science **25** (2012): 317-329

ISSN 0116-6514
E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2012.25.4.004

Asian Fisheries Science
The Journal of the Asian Fisheries Society

Ammonia-induced and Withdrawal-dependent Neurotoxic (Acetylcholinesterase) and Antioxidative (Catalase) Responses in Catfish, *Clarias batrachus* (Linnaeus, 1758)

A. SHIWANAND and G.TRIPATHI*
Department of Zoology, J.N.V. University, Jodhpur-342 033, India

Abstract

Ammonia-induced and its withdrawal-dependent changes in acetylcholinesterase (AChE) and catalase (CAT) of liver, gill and skeletal muscle of *Clarias batrachus* (Linnaeus, 1758) were investigated. AChE activity declined gradually in these tissues up to 35 days of ammonia exposure. In contrast, CAT activity increased up to 7 days and thereafter, it declined to some extent. Maximum effects of ammonia on CAT and AChE were on 7^{th} and 35^{th} day of treatment respectively. AChE activity declined maximally in gills (61%), but CAT activity enhanced maximally in liver (1.6 fold). Withdrawal of ammonia from water for 14 days after 35 days of exposure indicated incomplete recovery in AChE activity. However, withdrawal of ammonia showed complete reversal in CAT. Removal of ammonia from water may help in damage control. Since ammonia-induced neurotoxic (AChE) effect was more pronounced than antioxidative (CAT) response, AChE may be used as an indicator of ammonia toxicity in aquaculture.

Introduction

Freshwater bodies are polluted by extensive use of agrochemicals. Intense application of nitrogen fertilisers can increase ammonia concentration in aquatic ecosystem (Gangbazo et al. 1995; Palanivelu et al. 2005; Bobmanuel et al. 2006). High concentration of ammonia can reduce fish growth (EPA 1998; Wicks and Randall 2002).Ammonia can impair the tricarboxylic acid (TCA) cycle in fish, which is responsible for acetyl-CoA oxidation to CO_2, so increased NH_3 can lead to a build-up of acetyl-CoA (Kaizer et al. 2009). Acetylcholinesterase (AChE) is responsible for the hydrolysis of the acetylcholine to choline and acetate in cholinergic synapses. In fish, AChE is enriched in brain and muscle tissues and is considered as an indicator of aquatic pollution. Tham et al. (2009) assessed *C. batrachus* as a source of AChE for the detection of insecticides. AChE inhibition as a result of phosphorylation leads to accumulation of acetylcholine which triggers the release of catecholamines (Reddy et al. 1990). Torre et al. (2002) demonstrated the high sensitivity of AChE activity as an exposure biomarker. Low oxygen level increase ammonia toxicity in silver catfish, *Rhamdia quelen* (Quoy and Gaimard 1824) (Kaizer et al. 2009). Falfushynska and Stolyar (2009) showed that the AChE activity decrease in liver of *Cyprinus carpio* Linnaeus, 1758 in summer and it is the most sensitive biomarker of pollution. Bervoets et al. (2009) reported a significant relationship between metal load in tissues and AChE activity.

*Corresponding author. E-mail address: drgst@rediffmail.com

Catalase (CAT) is an antioxidant enzyme and also considered as an adaptation enzyme in fish (Orbea et al. 2000; Sole et al. 2004; Fernandez-Diaz et al. 2006). Ammonia has been shown to induce oxidative stress in gill and brain of the mudskipper *Boleophthalmus boddarti* (Pallas, 1770)(Ching et al. 2009). It also induces antioxidant defenses of juvenile crucian carp *Carassius auratus* (Linnaeus, 1758) (Yang et al. 2010). Hegazi et al. (2010) described ammonia associated increase in the activities of xanthine oxidase, aldehyde oxidase, superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase, γ-glutamyl cysteinyl synthetase and γ-glutamyl transpeptidase in liver and white muscle of Nile tilapia (*Oreochromis niloticus*) (Linnaeus, 1758). Long-term ammonia exposure significantly impairs the feeding and growth of juvenile crucian carp and also induces various antioxidant enzymes in liver of the fish (Yang et al. 2011). Chen et al. (2011) reported that oxidative stress and lipid peroxidation occur in the larvae of grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) exposed to a combination of ammonia and microcystin. Ammonia has been found to significantly increase body weight but affect antioxidant system in the larvae of bighead carp, *Hypophthalmythys nobilis* (Richardson, 1845) (Sun et al. 2012).

Ammonia is a potent neurotoxicant and may cause oxidative stress. AChE and CAT are the enzymes involved in nerve conduction and protection of cell from oxidative stress respectively. It is the reason why these two enzymes were chosen for study. The toxicological and withdrawal effects of ammonia on AChE and CAT of a freshwater fish were examined in this study. The hypothesis was to investigate whether enzymes can adapt to ammonia and whether its withdrawal from water can revert the neurotoxic and antioxidative functions in fish. The effects of ammonium chloride and its withdrawal-dependent changes in an economically important freshwater fish *C. batrachus* were investigated to test the proposed hypothesis.

Materials and Methods

Fish and chemicals

Adult and healthy specimens of the air-breathing walking catfish, *C. batrachus* weighing 184±6 g and with body length 23-29 cm were collected from local fish market of Jodhpur, India. This freshwater fish is commonly used for human consumption and is available in almost all fish markets of India. An experimental protocol for its collections was followed. Collections were done from November to February, 2010. This period is the reproductively regressed phase of the fish. The fish were maintained in tap water under laboratory conditions at least 1 week prior to experimentation. They were starved for 35 or 49 days depending on experiments. All chemicals used in this study were of analytical grade and procured from Sisco Research Laboratory (SRL), Mumbai, India. Double glass distilled water was used for preparation of chemicals.

Ammonia treatment and withdrawal experiments

Fish were exposed to sublethal concentration (60 $mg \cdot L^{-1}$) of ammonium chloride for 35 days. Water of container was changed on alternate days and a fresh concentration of ammonium chloride was maintained during ammonia exposure period. The pH of water varied from 7.2-7.6 during the experimentation period. Three separate experiments were carried out as follows:

(A) Ammonia treatment experiment for effects on AChE and CAT

Group 1: Normal individuals were kept untreated for 35 days (Control group).

Group 2: Individuals were exposed to sublethal concentration of ammonium chloride (60 $mg \cdot L^{-1}$) for 35 days (Treated group).

Liver, gills, and skeletal muscle were removed from untreated and ammonia treated individuals for assay and generating data on both AChE and CAT enzymes as shown in Fig. 1 and 2 respectively.

(B) Ammonia withdrawal experiment for AChE (Fig. 3)

Group 1: Normal individuals were kept untreated for 49 days (Normal/ Control 1 group)

Group 2: Individuals were exposed to ammonium chloride (60 $mg \cdot L^{-1}$) for 49 days (Treated/Control 2 group).

Group 3: Individuals were kept in freshwater for 14 days after 35 days of exposure of ammonium chloride (Withdrawal group).

(C) Ammonia withdrawal experiment for CAT (Fig. 4)

Group 1: Normal individuals were kept untreated for 35 days (Normal/ Control 1 group)

Group 2: Individuals were exposed to ammonium chloride (60 $mg \cdot L^{-1}$) for 21 days (Treated/Control 2 group).

Group 3: Individuals were kept in freshwater for 14 days after 7 days of exposure of ammonium chloride (Withdrawal group).

Samples (each comprising five individuals) were obtained on the day of completion of treatment mentioned against each group.

Tissue processing and enzyme preparation

Fish were killed by decapitation and liver, gill and skeletal muscle were removed immediately and rinsed in 0.6% saline**.** They were properly cleaned and weighed quickly. Tissues were stored at –40ºC in Remi Deep Freezer (BDI-8158) until used for analysis. Acetylcholinesterase (AChE, E.C. 3.1.1.7) and catalase (CAT, E.C. 1.11.1.6) activities remained almost unaltered during period of storage for several weeks. Tissues were homogenised (10% w/v) in sodium phosphate buffer (0.1M, pH 7.4) using Potter-Elvehjem homogeniser fitted with a Teflon Pestle (Remi RB1278). Homogenisation was done at about 4 ºC. Homogenates were centrifuged at 12000 g for 15 min in a high-speed refrigerated centrifuge (Remi Cooling Compufuge, CPR 24). The supernatant was collected and used for the assay of AChE and CAT activity. The subcellular fractionation and preparation of enzyme were done according to the method described by Tripathi and Shasmal (2010).

Enzyme assay

AChE activity was determined spectrophotometrically following the method of Ellman et al. (1961) using acetylthiocholine iodide as the substrate. Extinction coefficient was 13600 $M^{-1}{\cdot}cm^{-1}$. Volume of reaction mixture was 3 mL absorbance was recorded at 412 nm. Reading of blank without enzyme was substracted to yield the enzymatic activity. Reaction mixture contained 2.7 mL phosphate buffer (0.1 M, pH 7.4), 0.05 mL DTNB (5,5'- DithioBis (2- Nitrobenzoic acid) (0.18 mM), 0.10 mL enzyme sample and 0.15 mL acetylthiocholine iodide. Acetylthiocholine iodide is broken down to thiocholine and acetate by AChE and thiocholinereacted with DTNB to produce a yellow colour. Enzyme activity was expressed in $\mu mol{\cdot}min^{-1}{\cdot}g^{-1}$ wet mass. The procedure adopted for the assay of catalase was that of Aebi (1984). Catalase was assayed in a medium containing 50-150 mM H_2O_2 and 300 mM phosphate buffer (pH 7). Total volume of reaction mixture was 3 mL. The decrease in absorbance was noted at 240 nm using Cintra 5 UV-VIS spectrophotometer (GBC, Germany). The enzyme activity was expressed in mmol $min^{-1}g^{-1}$ wet mass.

Statistical analysis

A one-way analysis of variance (ANOVA) followed by Duncan's multiple rage test were used to analyse the data. The level of significance was $P<0.05$. Computations were done with the help of a computer and statistical package.

Results

ANOVA showed significant changes in the activities of AChE and CAT of liver, gill and skeletal muscle of *C. batrachus*. Higher values of SEM at certain places may be due to individual genetic differences in the expression of enzymes. The impacts of ammonium chloride and its withdrawal-dependent alterations in these enzyme activities were as follows:

Effects of ammonium chloride on AChE and CAT

Activity of AChE of liver, gill and skeletal muscle of *C. batrachus* varied significantly ($P<0.05$) with respect to changes in duration of exposure of ammonium chloride. Treatment of ammonium chloride for 35 days significantly ($P<0.05$) decreased the activity of AChE in all tissues (Fig. 1). Maximum decline in the enzyme activity was in gill (61.03%) followed by liver (55.11%) and skeletal muscle (51.03%). Activity of CAT from liver, gill and skeletal muscle also varied significantly ($P<0.001$) with increasing period of exposure of ammonium chloride (Fig. 2). In contrast to AChE, the CAT activity increased gradually and significantly in different tissues in response to exposure of ammonia. Maximum elevation in the enzyme activity was obtained on the 7^{th} day. It was maximally enhanced in liver (1.59 fold) followed by gill (1.52 fold) and skeletal muscle (1.36 fold). Thereafter, it declined gradually and showed incomplete recovery at 35 days. CAT activity did not differ significantly between the 28^{th} and 35^{th} day.

Effects of withdrawal of ammonium chloride on AChE and CAT

Withdrawal of ammonium chloride from water for 14 days after its exposure for 35 days showed a gradual recovery in AChE activity in liver, gill and skeletal muscle of the fish, but recovery was not complete (Fig. 3). The pattern of depurative response was similar in different tissues of catfish. Withdrawal of ammonium chloride for 14 days after 7 days of its exposure showed complete reversal in CAT activity of liver, gill and skeletal muscle of the fish (Fig. 4). The trend of depurative response was similar in different tissues in response to ammonia withdrawal after 7 days of exposure.

Discussion

AChE activity of liver, gill and skeletal muscle of *C. batrachus* declined gradually up to 35 days of ammonia exposure. Maximum decline was in gill followed by liver and skeletal muscle (Fig. 1). Decline in AChE activity can affect locomotion and equilibrium in aquatic organisms and may impair feeding, escape and reproductive behaviour (Habig and Di Gulio 1991; Saglio and Trijasse 1998; Bretaud et al. 2000). Activity of AChE has also been reported to decline in the brain of *Oreochromis mossambicus* (Peters, 1852) due to ammonia stress (Babu and Neeraja 2000). Modesto and Martinez (2010) studied AChE inhibition by subjecting it with anti-AChE action.

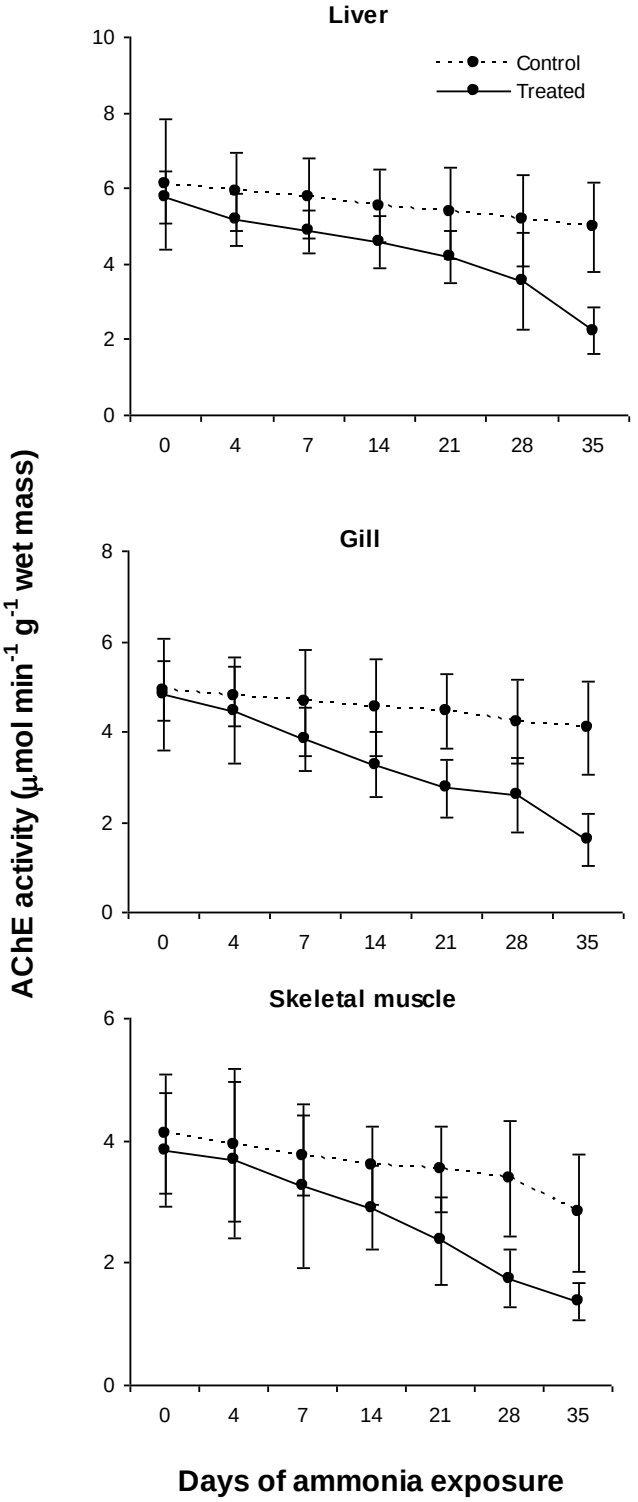

Fig. 1. Effects of exposure of ammonium chloride on activity (μmol min^{-1} g^{-1} wet mass) of acetylcholinesterase (AChE) from liver, gill and skeletal muscle of the freshwater catfish, *C. batrachus*. Each datum represents the mean ±SEM of five individuals (n=5).

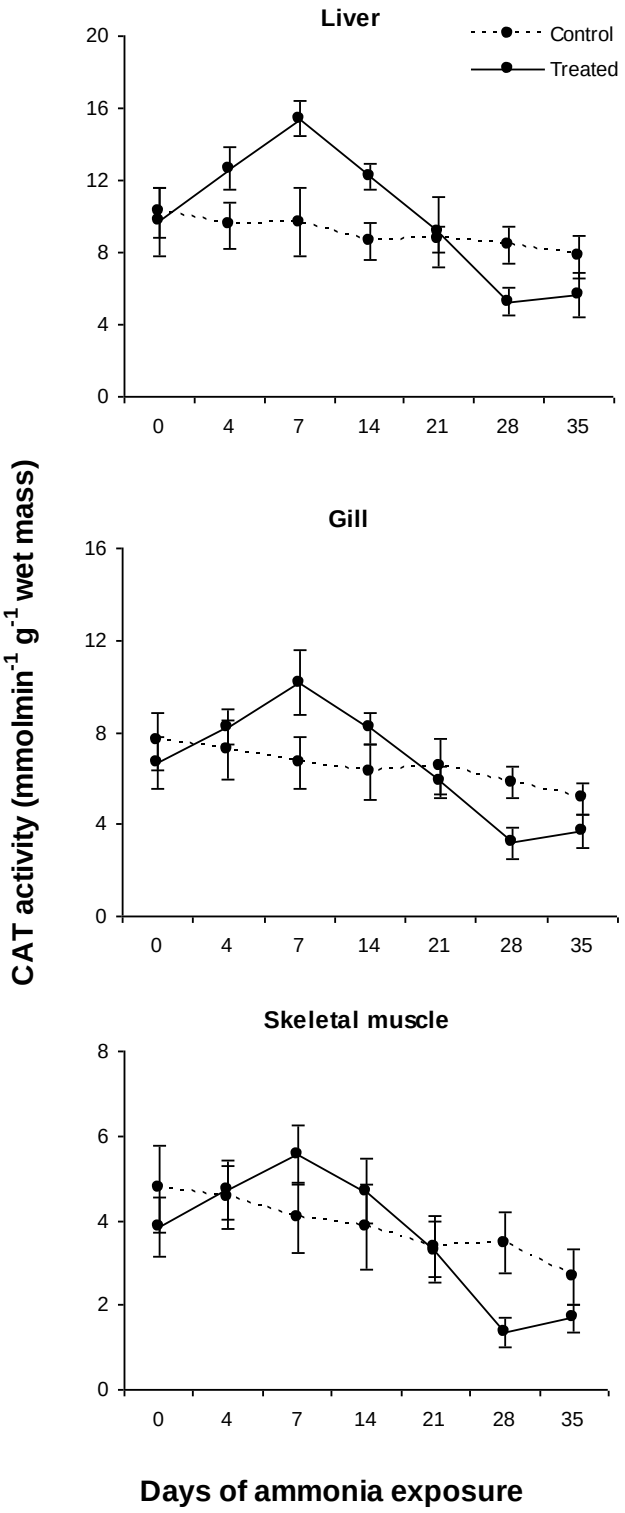

Fig. 2. Effects of exposure of ammonium chloride on activity (mmol min^{-1} g^{-1} wet mass) of catalase (CAT) from liver, gill and skeletal muscle of the freshwater catfish, *C. batrachus*. Each datum represents the mean ±SEM of five individuals (n=5).

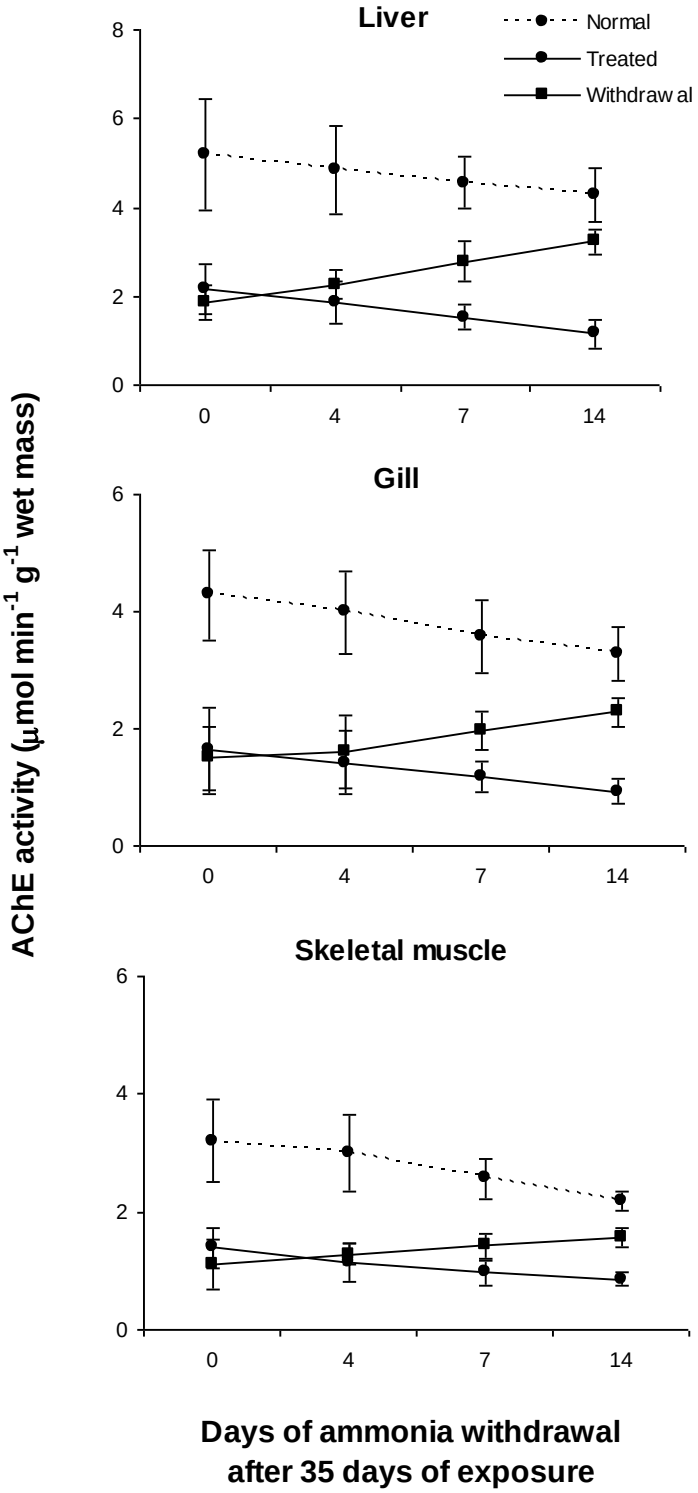

Fig. 3. Effects of withdrawal of ammonium chloride from water after 35 days of its exposure on activity (μmol min^{-1} g^{-1} wet mass) of acetylcholinesterase (AChE) from liver, gill and skeletal muscle of the freshwater catfish, *C. batrachus*. Each datum represents the mean ±SEM of five individuals (n=5).

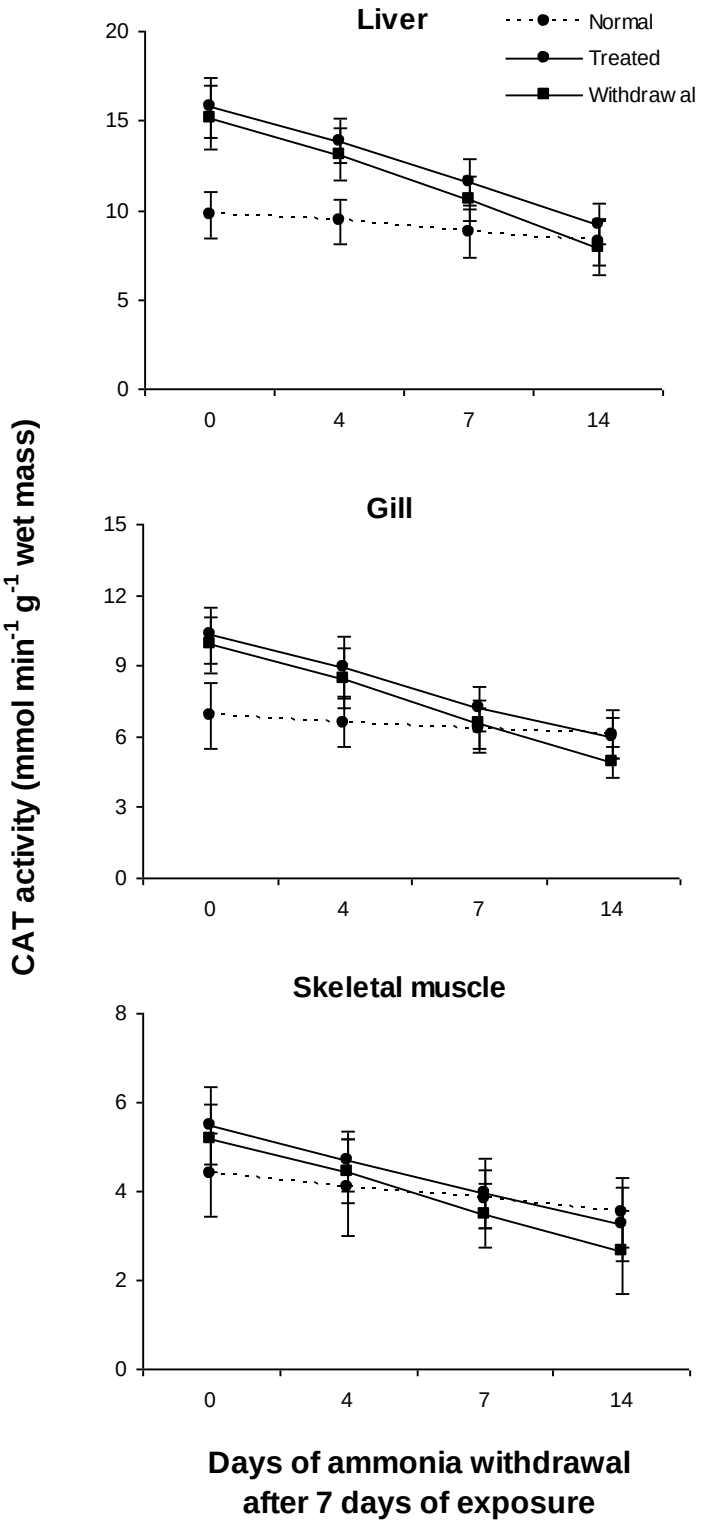

Fig. 4. Effects of withdrawal of ammonium chloride from water after 7 days of its exposure on activity (mmol min^{-1} g^{-1} wet mass) of catalase (CAT) from liver, gill and skeletal muscle of the freshwater catfish, *C. batrachus*. Each datum represents the mean ±SEM of five individuals (n=5).

When the AChE activity is inhibited, acetylcholine is not hydrolysed in nerve synapses and neuromuscular junctions, leading to overstimulation of brain and muscular tissue (Roex et al. 2003). In contrast to AChE, CAT activity initially enhanced up to 7 days and thereafter, declined gradually and reached below the control level at 35 days of ammonia treatment (Fig. 2). It means CAT tried to cope up with the oxidative stress initially but failed to do so later on. Ammonia associated changes in CAT activity may be due to allosteric modification in the enzyme molecule. Though the exact mechanism is not known, elevated activities of antioxidant and associated enzymes in fish in response to environmental stress have been observed by many workers (Winston 1991; Valavanidis et al. 2006; Craig et al. 2007). Activities of antioxidant enzymes are often enhanced in situations of high oxidative stress as an adaptive response to help detoxify oxygen free radicals and prevent cellular damages. Ching et al. (2009) demonstrated ammonia induced decrease in catalase from gills and brain of *B. boddarti.* Like the present observations, ammonia associated fluctuation in activity of CAT in liver of *C. auratus* with time has been reported (Yang et al. 2010). Ammonia significantly affects the antioxidant system (Sun et al. 2012) and the CAT of fish is highly sensitive to ammonia intoxication (Chen et al. 2011). Faria et al. (2010) studied the responses of biomarkers to pollutant concentrations and differences were reflected in high activities and levels of antioxidant enzymes. Hegazi et al. (2010) suggested that the levels of oxidative stress biomarkers and the activities of the enzymes significantly increased in liver and white muscle of fish exposed to ammoniacal nitrogen.

The withdrawal of ammonia from water for 14 days after 35 days of its exposure showed incomplete recovery in AChE activities of liver, gill and skeletal muscle (Fig. 3). Lack of recovery in activity of AChE might be due to ammonia-induced irreversible changes in the enzyme molecules and damages in the tissues. Therefore, AChE may be accepted as a potent indicator of ammonia toxicity in the catfish, *C. batrachus.* Withdrawal of ammonium chloride from water for 14 days after 7 days of exposure presented complete recovery in CAT activity of *C. batrachus* (Fig. 4). It showed reversible effect of ammonia on catalase of the catfish. The present investigations demonstrated inhibitory effect of ammonia on AChE and CAT reflecting neurotoxic and antioxidative responses in fish. Withdrawal of ammonia from water showed complete recovery in CAT but not in AChE. Lack of ammonia withdrawal-dependent complete recovery in AChE activity disclosed the possibility for this enzyme being an indicator of ammonia toxicity in fish. Therefore, it can be suggested that ammonia is irreversibly neurotoxic but reversibly antioxidative in the freshwater fish. The findings may have some ecotoxicological and pisciculture relevance in aquatic biology.

Conclusion

AChE activity declined gradually in liver, gill and skeletal muscle of *C. batrachus* after ammonia exposure. In contrast, CAT activity increased up to 7 days and then declined. Withdrawal of ammonia from water exhibited incomplete/complete recovery in AChE and CAT activity respectively. Removal of ammonia from water may help in damage control. Since ammonia-induced

neurotoxic effect was more pronounced than antioxidative response, AChE may be used as biomarker of ammonia toxicity in fish.

Acknowledgements

The authors gratefully acknowledge the University Grants Commission (UGC), New Delhi for providing financial support in the form of UGC research project (No. 36-104/2008(SR). One of us, A. Shiwanand is thankful to UGC for providing a project fellowship.

References

Aebi, H. 1984. Catalase in vitro. In: Methods in Enzymology (ed. L. Packer) pp. 121-126.105. Academic Press, New York,

Babu, C.S. and P. Neeraja. 2000. Acetylcholine esterase and monoamine oxidase activity levels in brain regions of *Oreochromis mossambicus* exposed to chronic ammonia stress. Journal of Nature Conservation 12:191-194.

Bervoets, L., K.V. Campenhout, H. Reynders, D. Knapen, A. Covaci and R. Blust. 2009. Bioaccumulation of micropollutants and biomarker responses in caged carp *Cyprinus carpio*. Ecotoxicology and Environmental Safety 72:720-728.

Bobmanuel, N.O.K., U.U. Gabriel and I.K.E. Ekweozor. , 2006. Direct toxic assessment of treated fertilizer effluents to *Oreochromis niloticus*, *Clarias gariepinus* and catfish hybrid. African Journal of Biotechnology 5:635-642.

Bretaud, S., J.P. Toutant and P. Saglio. 2000. Effects of carbofuran, diuron and nicosulfuron on acetylcholinesterase activity in goldfish (*Carassius auratus*). Ecotoxicology and Environmental Safety 47:117-124.

Chen, Y., H. Sun, W.Wang and Z.Yang, ,2011. Oxidative stress responses of grass carp *Ctenopharyngodon idella* larvae exposed to purified microcystin under different ammonia concentrations. Fresenius Environmental Bulletin 20:2869-2874.

Ching, B., S.F. Chew, W.P. Wong and Y.K. Ip. 2009. Environmental ammonia exposure induces oxidative stress in gills and brain of *Boleophthalmus boddarti* (mudskipper). Aquatic Toxicology 9:203-212.

Craig, P.M., C.M. Wood and G.B. Mclelland. 2007. Oxidative stress response and gene expression with acute copper exposure in zebrafish (*Danio rerio*). American Journal of Physiology 29:R1882-R1892.

Ellman, G.L. D.K. Courtney and R.M. Andres. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7: 88-95.

EPA (United States Environmental Protection Agency) 1998. Update of ambient water quality criteria for ammonia. USA. United States Environmental Protection Agency. Washington D.C. 20460. 822-R-98-008. 1-160.

Falfushynska, H. I. and O.B. Stolyar. 2009. Responses of biochemical markers in carp *Cyprinus carpio* from two field sites in western ukraine. Ecotoxicology and Environmental Safety 72:729-736.

Faria, M., D. Huertas, D.X. Soto, J.O. Grimalt, Catalan, M.C. Riva and C. Barata. 2010. Contaminant accumulation and multi-biomarker responses in field collected zebra mussels (*Driessena polymorpha*) and crayfish (*Procambarus clarkii*), to evaluate toxicological effects of industrial hazardous dumps in the Ebro river (NE spain). Chemosphere 78:232-240.

Fernandez-Diaz, C., J. Kopecka, J.P. Canavate, C. Sarasquete and M. Sole. 2006. Variations on development and stress defences in *Solea senegalensis* larvae fed on live and microencapsulated diets. Aquaculture 25:573-584.

Gangbazo, G., A.R. Pesant, G.M. Barnett, J.P. Charuest and C.C. Cluis. 1995. Water contamination by ammonium nitrogen following the spreading of hog manure and mineral fertilizers. Journal of Environmental Quality 2:420-425.

Habig, C. and R.T. Di Gulio. 1991. Biochemical characteristics of cholinesterases in aquatic organisms. In : Cholinesterase-Inhibiting Insecticides. Their Impact on Wildlife and the Environment (ed. P. Mimeau), pp. 19-34. Elsevier, Amsterdam.

Hegazi, M.M., Z.I. Attia, and O.A. Ashour. 2010. Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure. Aquatic Toxicology 99:118-125.

Kaizer, R.R., V.L. Loro, M.R.C. Schetinger, V.M. Morsch, L.A. Tabaldi, C.S. Rosa, L.O. Garcia, A.G. Becker and B. Baldiserotto. 2009. NTPDase and acetylcholinesterase activities in silver catfish, *Rhamdia quelen* (Quoy & Gaimard, 1824) (Heptapteridae) exposed to interaction of oxygen and ammonia levels. Neotropical Ichthyology 7:635-640.

Modesto, K.A. and C.B.R. Martinez. 2010. Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. Chemosphere 78:294-299.

Orbea, A., D.H. Fahini and P.M. Cajaraville. 2000. Immunolocalization of four antioxidant enzymes in digestive glands of mollusks and crustaceans and fish liver. Histochemistry and Cell Biology 114:393-404.

Palanivelu, V., K. Vijayavel, S. Ezhilarasibalasubramanian and M.P. Balasubramanian. 2005. Impact of fertilizer (urea) on oxygen consumption and feeding the freshwater fish *Oreochromis mossambicus.* Environmental Toxicology and Pharmacology 19:351-355.

Reddy, M.S., P. Jayaprada, K.V.R. Rao and K.V. Raman Rao. 1990. Impact of methyl parathion and malathion on non-cholinergic enzyme systems of penaeid prawn *Metapeneus monoceros*. Biochemistry International 22:769-779.

Roex, E.W.M., R. Keizers and C.A.M. Van Gestel. 2003. Acetylcholinesterase inhibition and increased food consumption rate in the zebrafish, *Danio rerio*, after chronic exposure to parathion. Aquatic Toxicology 64:451-460.

Saglio, P. and S. Trijasse. 1998. Behavioural responses to atrazine and diuron in goldfish. Archives of Environmental Contamination and Toxicology 35:484-491.

Sole, M., J. Potrykus, C. Fernanadez-Diaz and J. Blasco. 2004. Variations on stress defences and metallothionein levels in the senegal sole, *Solea senegalensis*, during early larval stages. Fish Physiology and Biochemistry 3 :57-66.

Sun, H., K. Lü, E.J.A. Minter, Y. Chen, Z.Yang and D.J.S. Montagnes. 2012. Combined effects of ammonia and microcystin on survival, growth, antioxidant responses and lipid peroxidation of bighead carp *Hypophthalmythys nobilis* larvae. Journal of Hazardous Materials 221-222 : 213-219.

Tham, L.G., N. Perumal, M.A. Syed, N.A. Shamaan and M.Y. Shukor. 2009. Assessment of *Clarias batrachus* as a source of acetylcholinestearse (AChE) for the detection of insecticides. Journal of Environmental Biology 30:135-138.

Torre, F.R., L. Ferrari and A. Salibiajn. 2002. Freshwater pollution biomarker: response of brain acetylcholinesterase activity in two fish species. Comparative Biochemistry and Physiology 131 C:271-280.

Tripathi, G. and J. Shasmal. 2010. Reparation of chlorpyrifos-induced impairment by thyroxine and vitamin C in fish. Ecotoxicology and Environmental Safety 73: 1397-1401.

Valavanidis, A., T. Vlahogianni, M. Dassenakis and M. Scoullos. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicology and Environmental Safety 64:178-189.

Wicks, D.J.and D.J. Randall. 2002. The effect of sub-lethal ammonia exposure on fed and unfed rainbow trout : the role of glutamine in regulation of ammonia. Comparative Biochemistry and Physiology 132 A:275-285.

Winston, G.W. 1991. Oxidants and antioxidants in aquatic animals. Comparative Biochemistry and Physiology 100 C:173-176.

Yang, W., F. Xiang, L. Liang and Z. Yang. 2010. Toxicity of ammonia and its effects on oxidative stress mechanisms of juvenile crucian carp (*Carassius auratus*). Journal of Freshwater Ecology 25:297-302.

Yang, W., H. Sun, F. Xiang, Z. Yang and Y. Chen. 2011. Responses of juvenile crucian carp (*Carassius auratus*) to long-term ammonia exposure: Feeding, growth and antioxidant defenses. Journal of Freshwater Ecology 26 : 563-570.

Received: 22/04/2012; Accepted: 10/10/2012 (MS12-29)