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Assessment of Biochemical Markers of Carbofuran Toxicity and Recovery Response in Tissues of the Freshwater Teleost, *Clarias Batrachus* (Linn)

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Abstract The effects of a sublethal concentration (7.66 mg/L) of carbofuran, were assessed on *Clarias batrachus*. The fish were exposed to 7.66 mg of carbofuran/L for 6 days. After 6 days, fish were released into carbofuran-free water in order to study the recovery pattern. Proteins were decreased in gill and kidney and recovery was greater in gill than in kidney. Total amino acids were increased in both tissues. Ammonia level declined in gill and enhanced in kidney throughout the study period. The activities of all enzymes measured were induced in both tissues, except for aspartate aminotransaminase, which was inhibited in gill tissues.

Keywords *Clarias batrachus* · Tissues · Carbofuran toxicity and recovery

Pesticides are widely used substances in current agricultural practices. Owing to their toxic effects on non-target organisms, many pesticides may produce serious detrimental effects on the ecosystem (Bretaud et al. 2000). Aquatic habitats are particularly subject to contamination by pesticides, due to leaching and runoff water from treated areas. In natural waters, seasonal agricultural practices and weathering processes (rainfall, photo degradation, and volatilization) contribute to spatial and temporal uneven concentrations of pesticides in the water mass. Short-term exposures of aquatic animals to environmentally non-persistent pesticides are therefore more common than sustained exposure situations.

Among carbamate pesticides, carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-methyl carbamate) is a broad non-persistent spectrum systemic insecticide, nematicide and acaricide commonly used throughout the world. Reports are available on the effect of carbofuran on biochemistry and behavior (Bretaud et al. 2002) and on brain and muscle acetylcholinesterase enzyme of gold fish, *Carassius auratus* (Bretaud et al. 2000) and *Gambusia yucatana* (Osten et al. 2005). There is a dearth of information on the comprehensive effects of carbofuran in physiologically important tissues of fish during exposure and post exposure. Previously, the author reported the sublethal effects of carbofuran on lipids and free fatty acids in different tissues of *C. batrachus* (Begum and Vijayaraghavan 2001) and on certain biochemical parameters of liver and muscle tissue of fish, *C. batrachus* (Begum 2004). Gills are the major respiratory and osmoregulatory organs and are responsible for acid-base regulation and gas exchange. Kidneys are the chief nitrogenous waste excretory organs. The present study evaluated the impact of carbofuran (3%G) exposure and post exposure on gill and kidney of a freshwater air breathing teleost fish, *C. batrachus* (Linn). The parameters analyzed were total protein, total amino acids, ammonia, transaminases (alanine and aspartate) and glutamate dehydrogenase, as biomarkers of carbofuran stress and recovery pattern.

Materials and Methods

The fish *C. batrachus,* 30 ± 2 g and length 22 ± 2 cm were purchased from the local market and transferred to

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the laboratory. They were acclimatized to laboratory conditions for 2 weeks in glass aquaria at 26°C ± 2 with 12 h:12 h light and dark period. The fish were fed chopped boiled beef liver and commercial fish feed ad libitum during acclimation and experimentation. Fish were acclimated for 2 weeks and 24 h starved fish were used for the experiment. A group of 36 fish were exposed to 7.66 mg commercial carbofuran/L for 6 days. The exposure concentration is one-third of the 96 h LC 50 value for commercial carbofuran (23 mg/L), determined by the method of Finney (1964). After 6 days of exposure, 18 fish were released into carbofuran-free water and held for 6 days to study the recovery pattern. A group of 18 naive fish were kept in fresh water throughout one experiment and used as controls. Six fish each from exposed, recovery, and control were killed at room temperature by severe blow to the head after 1, 3, and 6 days. Tissues were immediately removed, frozen and used within an hour for the assay of metabolites and enzymes.

Gill (branchial) and kidney (renal) tissues were immediately removed and frozen and used within an hour for the assay of metabolites and enzymes. Total proteins were estimated by the method of Lowry et al. (1951), total amino acids by the method of Moore and Stein (1954). The ammonia content was estimated by Nessler reagent as described by Bergmeyer (1965). The enzymes ALAT (alanine aminotransaminase E.C.2.6.1.2) and AAT (aspartate aminotransaminase E.C. 2.6.1.1) were assayed by the method of Reitman and Frankel as described by Bergmeyer (1965). Glutamate dehydrogenase (glutamate-NAD(p)-oxidoreductase E.C.1.4.1.3) was estimated by the method of Lee and Lardy (1965).

For the assay of enzyme activities, 10% (w/v) homogenates of gill and kidney tissues were prepared in 0.25 M ice-cold sucrose solution in an ice-jacketed homogenizer with a motor driven Teflon-coated pestle. The homogenates were centrifuged in a cold (4°C) centrifuge machine at 3,000g for 20 min to remove nuclei and cell debris. Clear cell-free extracts were used for the estimation of enzyme activities. Measurements of enzyme activities were performed spectrophotometrically at 37°C with appropriate enzyme and reagent blanks. For the three individual enzymatic assays which include ALAT, AAT, and GDH the same assay conditions were followed as outlined in detail (Begum 2004, 2005a, b). Six individual observations of each metabolite and enzyme were made and mean and standard error was calculated. The difference between the mean values of control and exposed and also between control and recovery group was evaluated statistically by Students' t-test. Differences were considered statistically significant at $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, NS, non significant.

Results and Discussion

The results of total protein, total amino acids ammonia, ALAT, AAT, and GDH are shown in Figs. 1 and 2. Total protein content in gill tissue (Fig. 1) declined significantly after 1 and 3 days of carbofuran exposure. There was an increase in protein concentration after 6 days of exposure and the increase continued for 1 and 3 days in clean water. Thereafter there was a declined. Decrease in total proteins was observed in kidney throughout the experimental period, and the recovery response was less than in the branchial tissue. An increase in total amino acid levels were observed in gills and kidneys on all days of exposure period. When the fish were released into carbofuran-free water, recovery in total amino acids was greater in kidney tissue. Renal ammonia levels were enhanced significantly during carbofuran exposure and after 6 days of recovery period these levels were still elevated compared to control. Ammonia levels of gill tissue were not significantly different from control after 6 days of exposure, and stayed that way thereafter.

The activity level of alanine aminotransaminase was induced in branchial and renal tissues on exposure to carbofuran. AAT recovery to control levels was almost complete in kidney tissue. There was an inconsistent inhibition of aspartate aminotransaminase in gill tissue, the difference being significant only at day 3. At the end of the recovery period the difference in AAT activity of control and recovered gill tissue was +3.12 percent. Kidney AAT activity was elevated during carbofuran exposure. When the fish were transferred to freshwater for recovery, a considerable recovery in AAT activity was observed. The enzyme, glutamate dehydrogenase was induced significantly in branchial tissue on day 1, 3, and 6 of carbofuran exposure. On transfer of the fish to clean water a decline in percent increase was observed. At the end of the recovery period (6 days) the change in GDH was +39.28% but not significant (Fig. 1). GDH activity of renal tissue was significantly different from control only at day 1 of exposure group (Fig. 2). At the end of the recovery period the difference in the levels of GDH enzyme was −6.00%.

The results reveal significant effects of carbofuran on teleost food fish, *C. batrachus* total protein, total amino acids, ammonia and enzymes viz: transaminases, and glutamate dehydrogenase. The total protein in the gill and kidney of test fish was found to be reduced compared to those in controls. A similar decrease in the protein content of gills in *Anguilla anguilla* fish exposed to thiobencarb was reported by Vega et al. (2002). A possible explanation for these findings was that proteolytic activity was induced in these organs due to the carbofuran exposure. Another reason for the decrease in proteins could be due to the activation of physiological compensatory mechanisms to

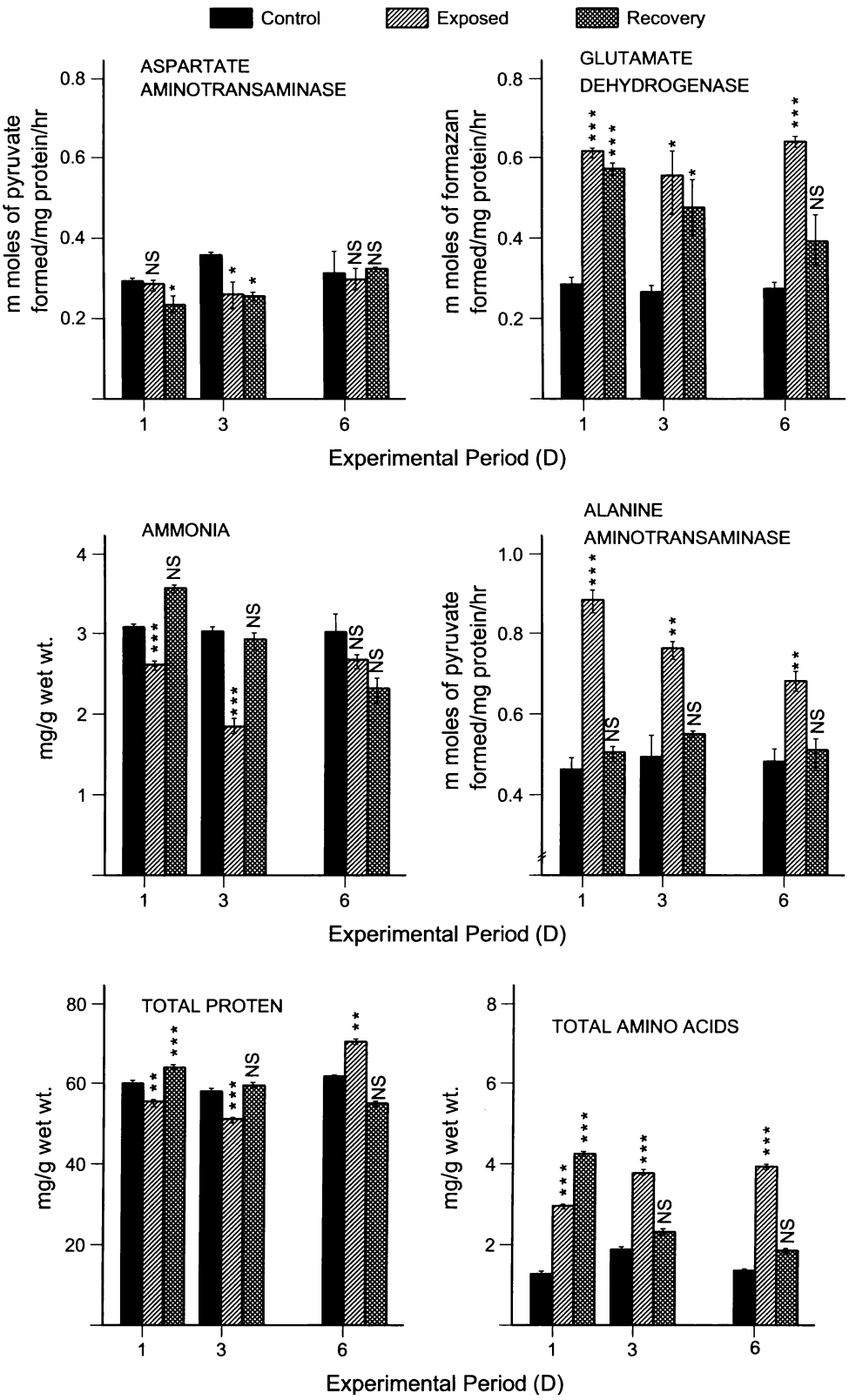

Fig. 1 Proteins, amino acids, ammonia content (mg/g wet wt.) alanine aminotransaminase, aspartate aminotransaminase (m moles of pyruvate formed/mg protein/h) and glutamate dehydrogenase (m moles of formazan/mg protein/h) in *Clarias batrachus* gill as response to carbofuran exposure and recovery period. Values are expressed as means ± SE (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$,$^{***}p < 0.001$, NS: not significant

provide intermediates for deriving energy through the Kreb's cycle. When the fish, *C. batrachus*, were transferred to clean water the protein content in gill tissue showed recovery. Total amino acids were increased in both the tissues during carbofuran exposure period. The enhanced amino acids might have been channelized for energy

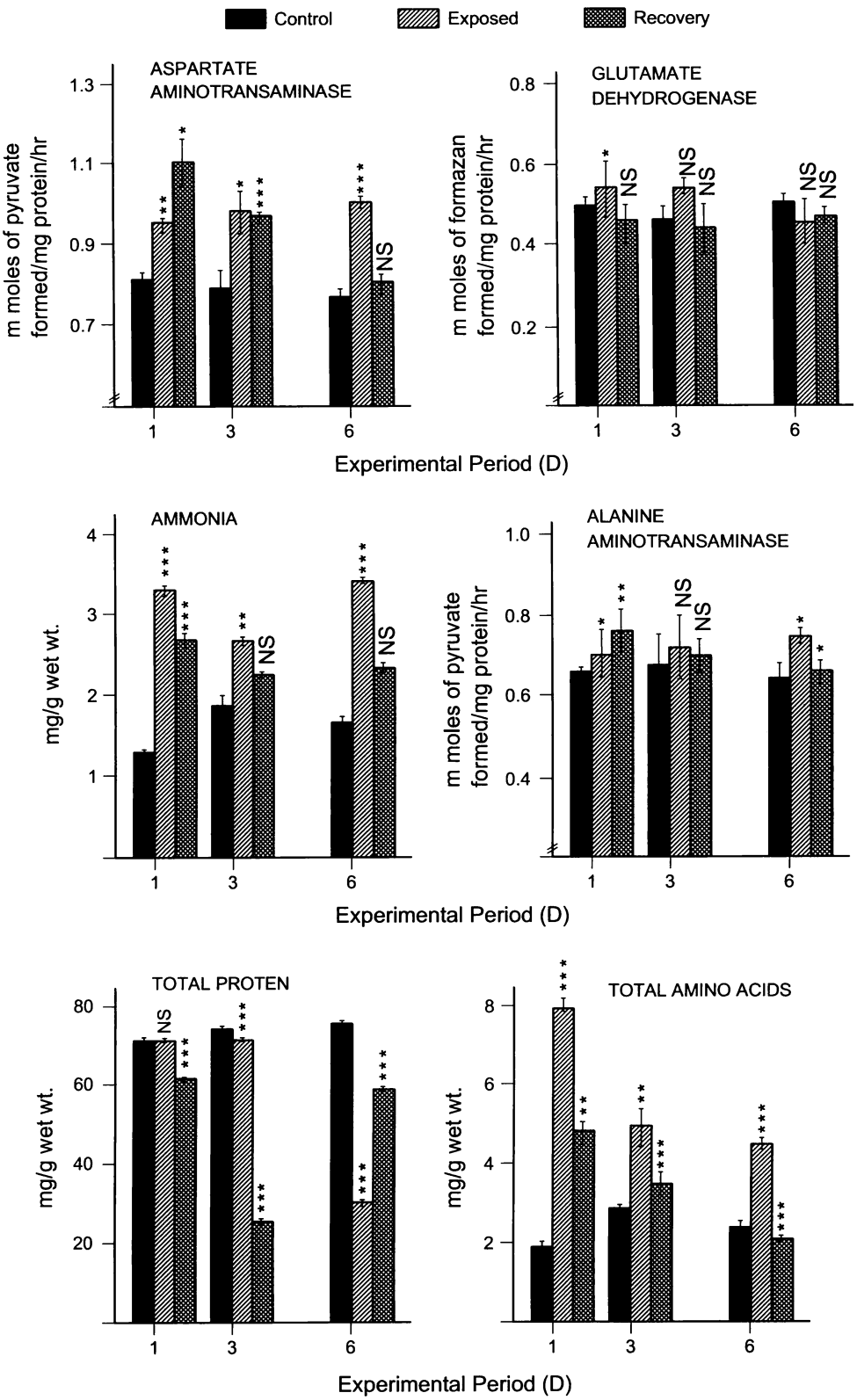

Fig. 2 Proteins, amino acids and ammonia content (mg/g wet wt.) alanine aminotransaminase aspartate aminotransaminase (m moles of pyruvate formed/mg protein/h) and glutamate dehydrogenase (m moles of formazan/mg protein/h) in *Clarias batrachus* kidney as response to carbofuran exposure and recovery period. Values are expressed as means ± SE (n = 6). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS: not significant

synthesis and other metabolic reactions (Shobha Rani and Janaiah 1991). The increased amino acids in kidney recovered to some extent after transfer of fish to carbofuran-free water. The ammonia level in the kidneys of exposed fish exceeded those in the controls on all sampling days. This could be due to increased ammoniagenesis or decreased excretion of ammonia into the ambient medium. Carbofuran toxicity resulted in depletion of gill ammonia,

suggesting the removal and excretion of ammonia from gills by the process of diffusion. However, the current findings are in good agreement with previous studies which showed decreases in gill ammonia concentration in the fish *C. batrachus* exposed to trichlorfon insecticide (Shobha Rani and Janaiah 1991).

Induction of alanine and aspartate activities occurred in the kidney for 6 days of the exposure period. Enhanced activity of the transaminases provides the oxaloacetic acid and pyruvate, α-ketoglutarate and glutaric acid to meet the increase energy demand during carbofuran toxicity. The oxaloacetic acid, pyruvate and α-ketoglutarate might have been channeled into the citric acid cycle. The glutamic acid formed from transamination may be subsequently deaminated leading to the formation of ammonia (Bidigare and King 1981). This is consistent with the increase observed in ammonia concentration of kidney. At the end of the recovery period, kidney transaminases were near control values. Exposure to carbofuran resulted in marginal inhibition of aspartate activity but alanine activity was increased in gills throughout the 6 day exposure period. Alanine aminotransaminase catalyses the transfer of the amino group from alanine to α-ketoglutarate to form glutamate and pyruvate (Vander Oost et al. 2003). It is clear from the results that in gill tissues transfer of the amino group from alanine were induced due to carbofuran stress. After 6 days recovery, the percent change in AAT in gill tissue was only +3.12. The GDH activity was found to increase in gill and kidney tissues of exposed fish, which indicates higher oxidation of amino acids to combat the toxic effect of carbofuran. Similar results were observed in gill tissue of *C. batrachus* exposed to trichlorfon (Shobha Rani and Janaiah 1991) and support the present findings. After 6 days of maintaining the fish in clean water, (recovery period) kidney GDH was near control values whereas gill GDH was significantly elevated (+39.28%). From these results, it is clear that carbofuran toxicity impairs certain metabolites and enzymes of protein metabolism in gill and kidney of freshwater food fish, *C. batrachus*.

The results of the present study are useful for assessing early warning signs of pesticide poisoning in food fishes. Determination of the significance of carbofuran pollution on the biochemical responses of food fishes in carbofuran polluted aquatic ecosystems is needed. The parameters of the present study could be used as suitable biomarkers of carbofuran stress to aquatic animals. The data from the recovery period is helpful in suggesting remedial measures for the treatment of toxicant affected fish. Routine testing of fish samples, before marketing is advisable in order to protect fish consumers from ill effects of pesticide contaminated fish.

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