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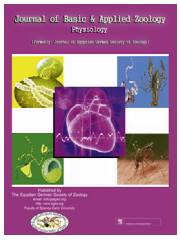

Responses of metabolic and antioxidant enzymatic activities in gill, liver and plasma of *Catla catla* during methyl parathion exposure

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Abstract *Background:* Use of pesticides in agricultural fields has a series of toxicological and environmental problems. Methyl parathion (MP), an organophosphorus (OP) insecticide is a widely used pesticide and is highly toxic to non-target organisms. Fish has been used as indicator species for monitoring of pollution in the aquatic environment. *Catla catla* an Indian major carp is an edible fish and is highly sensitive to slight stress. Recently, fish biomarkers are widely used to determine the internal and external health status caused by chemicals.

Results: *C. catla* were exposed to acute (0.09 ppm) and sublethal (0.009 ppm) concentrations of MP to determine the alterations in antioxidant and metabolic enzyme activities in blood plasma and tissues (gill and liver). Intoxication with MP resulted in induction of oxidative stress which implies that fish utilizes enzymatic mechanisms to tolerate the effects caused by generated ROS due to MP accumulation. Significant alterations in GOT and GPT activity in plasma and tissues during acute as well as sublethal exposure might have resulted from the organal damage. The significant increase of LDH activity indicates severe cellular damage in organ/tissues of MP treated fish.

Conclusion: The results of the present investigation suggest that gill is the most sensitive organ to MP toxicity. The alterations of the enzymatic parameters can be effectively used as potential biomarkers for monitoring of the organophosphorus pesticides in aquatic environment. Further, MP should be used with caution in order to protect natural waters and aquatic organisms.

Introduction

Extensive use of pesticides for the control of agricultural pests finds their way to various segments of the environment and becomes a serious problem throughout the world (Pimentel, 1995; Tripathi and Shasmal, 2011). The contamination of

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aquatic environment by pesticides can affect the health and survival of non target organisms such as fish (Oruç and Üner, 1999; De Menezes et al., 2011; Saravanan et al., 2011). Among the pesticides, organophosphorus (OP) is widely used worldwide to control a variety of sucking, chewing and boring insects (Abhilash and Singh, 2009; Barr et al., 2004; Celik and Suzek, 2008). OP pesticides are highly toxic to fish even at recommend levels due to their persistence in the environment and bioaccumulation in the various organs of fish (WHO, 1984; Fulton and Key, 2001; Rahman et al., 2002). The toxicity of these pesticides mainly includes inhibition of acetylcholinesterase (AChE) by their active metabolites or oxygen analogs generated during OP metabolism (Jokanovic, 2009). They may also cause lipid peroxidation in vertebrates (Gultekin and Akdogan, 2000).

Methyl parathion o,o-dimethyl o-4-nitrophenyl phosphorothioate is widely applied as an insecticide in agriculture, food storage shelters and pest control programs due to its efficiency against broad spectrum of insect pests (Aguiar et al., 2004; Celik and Suzek, 2008). However, the uncontrolled application of MP may cause potential risk to the aquatic organisms and interfere with the general health, reproductive and developmental process (US EPA, 2003; Rico et al., 2010). The accumulation of MP and its residues in different components of aquatic environment including fish has been reported (Huang et al., 2011). Based on its toxicity and residue level, MP has been classified as extremely hazardous and is listed in the HazDat Database of chemicals detected in surface or ground water at National Priorities List (NPL) sites (WHO, 2004). Thus prompting several countries to ban or restrict its use. However, it is still misused in many developing countries (Ghosh et al., 2010).

Analysis of biomarkers in aquatic organisms particularly in fish is a validated approach for early warning of chemical exposure (Van der Oost et al., 2003; Osman et al., 2010). During stress conditions fish change and adapt their metabolic functions (Malarvizhi et al., 2012) and the inhibition or induction of the enzymes such as glutamate oxalacetate transaminases (GOT), glutamate pyruvate transaminase (GPT), lactate dehydrogenase (LDH), acid phosphatase (ACP), alkaline phosphates (ALP) etc., can be used to indicate tissue damage (Nemcsok and Boross, 1982; Webb et al., 2005). GOT, GPT and LDH not only function as link enzymes between the protein and carbohydrate metabolism, but also serve as an indicator of chemical stress. Moreover, pesticides may cause an increase in the production of reactive oxygen species (ROS) in aquatic organisms which may lead to impairment of many cellular functions (Uner et al., 2006; Monteiro et al., 2009; Modesto and Martinez, 2010; De Menezes et al., 2011). The deleterious effect of free radicals can be prevented or counterbalanced by antioxidant systems (Lushchak, 2011; Singh et al., 2011). Fish possess both enzymatic (mitochondrial and cytosolic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase) and non-enzymatic (reduced glutathione and vitamin E) antioxidants as in mammals (Droge, 2002) for generation and detoxification of free radicals (Lushchak, 2011). The enzyme SOD (detoxifies toxic superoxide anion radicals) and CAT (primary antioxidant defense component) can be used as biomarkers of pollutant induced oxidative stress in aquatic organisms (Borkovi et al., 2005). These antioxidants are necessary to maintain the redox status of fish cells. Likewise, alterations in lipid peroxidation (LPO) concentration are also used to express severe oxidative damage and are used as a biomarker of effect (Lackner, 1998; Van der Oost et al., 2003). Malondialdehyde (MDA) is a naturally occurring product of LPO which acts as its indicator (Charissou et al., 2004; Pampanin et al., 2005).

India is a predominantly agrarian country where pesticides have been constantly used for advanced farming and increasing crop yields. In India about 464 tones of MP was produced during the year 2006 (AGROW 508, 2006). Pesticides such as DDT, malathion, methyl parathion and dimethoate were detected in many water samples of India (Ray, 1992; Leena et al., 2012). High accumulation of MP (10,250–10,850 ppb) was detected in fishes collected from many pond and wet lands (Chowdhury et al., 1994). Previous literature have reported the changes in AChE activity, hematological and biochemical parameters of fish exposed to MP (Uzunhisarcikli et al., 2007; Duquesne and Kuester, 2010) but to our best knowledge, there is paucity of information on effect of MP on metabolic and oxidative stress parameters on Indian major carps. Therefore, this study was designed to evaluate the acute and sublethal concentrations of MP on certain antioxidant (SOD, CAT and LPO) and metabolic enzymatic (GOT, GPT and LDH) parameters in different tissues and plasma of *Catla catla*. Further, an attempt was made to use the alterations of these parameters as possible biomarkers for monitoring of pesticides in the aquatic environment. In this study we used *C. catla*, a common inhabitant of freshwater bodies and a common food for many people in India to assesses MP toxicity (Cengiz et al., 2010; Swain et al., 2012).

Materials and methods

Collection and maintenance of animals

The Indian major carp, *C. catla* (length 5–7 cm and weight 8–10 g) were procured from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Aliyar, Tamil Nadu, India and acclimatized to laboratory condition for a period of one month in a large cement tank (1000 L capacity). Fish were fed *ad libitum* with rice bran and groundnut oil cake daily once. Three fourth of the water was renewed daily to avoid accumulation of excretory materials. In the present study chlorine free tap water was used which had the following physicochemical characteristics (APHA, 2005); temperature 26 ± 1.0 °C, pH 7.4 ± 0.06, salinity 0.26 ± 0.1 ppt, dissolved oxygen 6.6 ± 0.4 mg/L, total hardness 18 ± 0.4 mg/L and alkalinity 36.0 ± 0.2 mg/L.

Toxicant used

Commercial methyl parathion (o,o-dimethyl o-4-nitrophenyl phosphorothioate) 50 I.C. (R. Meth; 50:50 w/w) was purchased from Sera Ramicide Chemicals Pvt. Ltd., Chennai, India. Stock solution of MP was prepared by dissolving 1 ml of MP in appropriate amount of normal tap water.

Acute toxicity test

The median lethal concentration (LC 50) of MP to the fish *C. catla* was found to be 0.09 ppm for 96 h (Abhijith et al., 2012). For the determination of acute toxicity test, five glass aquaria

each of 55 L capacity were taken and filled with 50 L of water and 96 h LC50 concentration (0.09 ppm) was added after removal of same quantity of water. The experiment was initiated by introducing 20 fish in glass aquaria. Simultaneously a common control was also maintained in five different glass aquaria. The alterations in metabolic (GOT, GPT, and LDH) and antioxidant (SOD, CAT and LPO) enzymatic parameters were assayed at the end of 96 h.

Sublethal studies

One-tenth value (0.009 ppm) of the 96 h LC 50 was taken as the sublethal concentration (Sprague, 1971). For sublethal toxicity tests 600 fingerlings were selected and divided into six groups (three controls and three experimental) with 100 fish in each glass aquaria. The desired concentration of the toxicant was added directly into each glass aquarium after removal of the same volume of water and renewed daily in order to maintain a constant concentration. At the end of the stipulated period (7, 14, 21, 28 and 35 days) of exposure, fish were randomly selected (15 fish per glass aquaria) and sacrificed for further analysis. No mortality was observed during the above treatment period.

Collection of blood and tissues for the assay

Blood samples were collected in small vials by heart puncture using plastic disposable syringes fitted with prechilled and heparinized 26 gauge needle. The blood sample was centrifuged at 10,000 rpm for 20 min to separate the plasma. Simultaneously, liver and gills were excised and homogenized in ice-cold 0.25 M sucrose buffer, pH 7.4. The homogenate was centrifuged at 5000 rpm for 15 min at 4 °C. The tissue supernatant as well as plasma were further used for measurement of metabolic (GOT, GPT and LDH) and antioxidant parameters (SOD, CAT and LPO).

Determination of GOT, GPT and LDH activity

GOT and GPT were measured following the method of Reitman and Franckel (1957). The enzyme activities were calculated using the standard curve and expressed in IU/L of sample. LDH activity was measured according to the method of Anon (1984). Enzyme activity was converted to LDH units, by standard curve and expressed in IU/L.

Determination of SOD, CAT and LPO activity

Total protein was quantified according to the method developed by Lowry et al. (1951) using Folin's reagent, and BSA as a standard. The SOD activity in the tissue extract and the plasma was assayed by the method of pyrogallol auto oxidation by superoxide radicals and expressed as U/mg protein (Marklund and Marklund, 1974). Catalase activity was determined by monitoring the decrease in absorbance of H_2O_2 at 240 nm and expressed as μmol/mg protein/min (Aebi, 1984). LPO was assayed by measuring MDA formation as described by Devasagayam and Tarachand (1987) and was expressed nmoles of MDA formed/mg protein.

Statistical analysis

The significance of sample mean between control and MP treated fish was tested using Student's "*t*" test.

Results

The acute effect of MP on metabolic enzyme (GPT, GOT and LDH) activities in gill, liver and plasma of fish *C. catla* is presented in Table 1. GPT and GOT activity in gill, liver and plasma significantly ($p < 0.05$) increased in MP exposed fish whereas LDH activity significantly ($p < 0.05$) decreased when compared with the control group. Results of the antioxidant enzyme (SOD, catalase and LPO) activities in gill, liver and plasma of control and MP exposed fish are given in Table 2. A significant ($p < 0.05$) increase over controls was observed in SOD activity in gill and liver at the end of 96 h. However, no significant differences in SOD activity were observed in plasma between control and MP treated group. MP exposure caused a significant ($p < 0.05$) increase in catalase activity in tissues whereas a significant decrease was observed in plasma when compared with the control group. LPO level was found to be increased in gill and plasma whereas a significant decrease was observed in liver tissue when compared with the control group.

The effect of sublethal concentration (0.009 ppm) of MP on metabolic enzyme activities in gill, liver and plasma of *C. catla* is represented in Fig. 1a–i. A biphasic response in GOT activity was observed both in gill and liver (Fig. 1a and b). The data revealed that the changes in GOT activities were statistically significant ($p < 0.05$) in relation to respective controls. In contrast, plasma GOT activity was increased significantly ($p < 0.05$) in MP treated fish throughout the study period (Fig. 1c). In MP treated fish a significant ($p < 0.05$) decrease in GPT activity was noted in gill up to 35th day (Fig. 1d). The GPT activity in the liver was significantly increased throughout the study period (except at the end of 35th day) (Fig. 1e). However the activity of GPT in plasma was found to be increased in MP treated fish throughout the study period of 35 days (Fig. 1f). LDH activity in gill of MP treated fish was found to be decreased up to 21st day and underwent significant increase during subsequent exposure period (Fig. 1g). LDH activity in liver was increased (except at the end of 7th day) significantly ($p < 0.05$) in MP treated fish throughout the study period (Fig. 1h). A time significant ($p < 0.05$) and time dependant decrease in LDH activity was noted in plasma of fish exposed to MP (Fig. 1i).

The antioxidant enzyme activities viz., SOD, CAT and LPO in fish *C. catla* exposed to sublethal concentration of MP for 35 days showed significant alterations when compared to control groups (Fig. 2a–i). A significant ($p < 0.05$) increase in SOD activity was observed in gill of *C. catla* throughout the study period when compared to the control group (Fig. 2a). However, the increase in SOD activity in the liver was not significant on day 21 and 28 (Fig. 2b). In contrast, a significant ($p < 0.05$) decrease in SOD activity was observed in blood plasma (Fig. 2c). The catalase activity in gill and plasma of MP treated fish showed a significant ($p < 0.05$) decrease throughout the study period (except on day 7 in gill) (Fig. 2d and f). In contrast, the catalase activity in the liver was found to be increased throughout the study period when

Table 1 Enzymatic alterations in *C. catla* during acute treatment of methyl parathion.

	Gill		Liver		Plasma	
	Control	Experiment	Control	Experiment	Control	Experiment
GPT (IU/L)	82.60 ± 1.200	50.30 ± 2.600*	319.7 ± 0.900	396.2 ± 5.600*	75.30 ± 1.500	89.20 ± 3.100*
GOT (IU/L)	90.50 ± 1.300	82.80 ± 3.500*	132.1 ± 1.700	350.7 ± 2.100*	65.80 ± 1.400	79.30 ± 2.600*
LDH (IU/L)	1195.8 ± 5.600	113.2 ± 9.100*	1152.2 ± 9.100	929.3 ± 12.40*	1328.3 ± 8.200	1138.6 ± 11.30*

Values are mean ± SE of five individual observation. Comparisons of means (control and treated fish) were done by Student's *t*-test.
* Significant at 5% level ($p < 0.05$).

compared to control groups (Fig. 2e). However, the increase in catalase activity was not significant on days 7, 28 and 35. The LPO activity in gill and blood plasma showed a significant increase ($p < 0.05$) throughout the study period (Fig. 2g and i). However, a non-significant difference was observed in the liver throughout the study period except for a significant increase at the end of 21st day (Fig. 2h).

Discussion

Organophosphorus pesticides due to their low cumulative ability, rapid breakdown in water and short-term persistence in the environment, have fully replaced the persistent chlorinated pesticides in the beginning of 1980s (Videira et al., 2001). Thus, they are recognized and marked as one of the major pollutants of aquatic ecosystems (Ren et al., 2007). MP in water bodies can easily be absorbed through gills and accumulates in the organs like liver, gut and brain (De la Vega Salazar et al., 1997) causing the inhibition of acetylcholinesterase (AChE) activity (Muttraya et al., 2005). In the present study the fish showed symptoms of hyper excitability, loss of equilibrium, faster opercular activity and erratic swimming which indicates the neurotoxic effect of MP. Transaminases like GOT and GPT are usually present in various tissues and injuries to the cells of these tissues may result in the release of enzymes into plasma. Moreover, the response of these enzyme activities to stressors may occur via. direct enzyme inhibition or induction and changes in metabolic pathways or fluxes. In the present study, the significant increase in GOT and GPT activity in plasma during acute as well as sublethal exposure might have resulted from the organal damage due to MP accumulation. The observed increase in gill and liver indicates an increase in biotransformation and detoxification process. Murugesan et al. (1999) observed similar results and suggested that the energy demand is met by gluconeogenesis. Therefore, the elevation in transaminases activity can be effectively used as biomarkers of MP toxicity in fish.

The observed decrease in enzyme activity in the liver during sublethal exposure of MP may be due to damaged hepatocytes that are no longer capable of synthesizing GOT protein. Rao (2006) reported that the decrease in activity of aminotransferases in the liver of fish *Oreochromis mossambicus* exposed to organophosphorus pesticide (RPR – II) may be due to liver damage. The decrease in GOT and GPT in gill, liver and muscle of fish *Cyprinus carpio* during carbamazepine (CBZ) exposure indicates that detoxification mechanism may not be sufficiently effective to prevent the toxicity and effect of drug on the system (Malarvizhi et al., 2012). Furthermore, the decrease in the activities of the metabolic enzymes during organo phosphorus pesticides exposure indicates the direct action of these pesticides on enzymes (Tripathi and Shasmal, 2011).

LDH is a tetrameric glycolytic enzyme and recognized as a potential marker of tissue damage (Diamantino et al., 2001). In the present study the decrease in LDH activity in gill and liver indicate the higher glycolysis rate under pesticide stress. MP may inhibit the aerobic and anaerobic metabolism of fish resulting in a decrease of LDH activity. The reduction in LDH activity in gill, liver, brain and muscle of fish *Heteropneustes fossilis* exposed to chlorpyriphos may be due to binding of the pesticides or its metabolites with the enzyme molecule (Sastry et al., 1982; Tripathi and Shasmal, 2011). Moreover, pesticide induced decrease in glycolytic process may also result in its decrease due to low metabolic rate. Prevalence of anoxia during stress conditions may lead to an increase in LDH activity in tissues (Das et al., 2004). The observed elevation of LDH activity in gill and liver during sublethal treatment might have resulted from damage of these tissues. The increase in plasma LDH activity in benomyl treated fish *Oreochromis niloticus* indicate that the toxicity may be produced through anaerobic mechanism (Min and Ju-Chan, 2008).

Natural or manmade chemicals may stimulate ROS production by a variety of mechanisms such as redox cycling (Livingstone, 2001) resulting oxidative damage which may lead to mortality of fish. Generation of ROS due to biotransformation reactions of xenobiotics may significantly contribute to their toxicity (Chovanec et al., 2003). The general mechanisms of oxidative toxicity both in mammalian and piscine systems

Table 2 Antioxidant enzyme alterations in *C. catla* during acute treatment of methyl parathion.

	Gill		Liver		Plasma	
	Control	Experiment	Control	Experiment	Control	Experiment
SOD (U/mg protein)	0.033 ± 0.004	0.052 ± 0.009*	0.021 ± 0.009	0.048 ± 0.006*	5.200 ± 0.400	4.400 ± 0.800
Catalase (μmol/mg protein/min)	22.00 ± 1.300	38.00 ± 2.900*	26.00 ± 1.100	35.00 ± 2.400*	0.010 ± 0.005	0.007 ± 0.003*
LPO (nmol of MDA formed/mg protein)	1.390 ± 0.070	2.870 ± 0.140*	1.450 ± 0.030	1.160 ± 0.060*	8.200 ± 0.050	10.80 ± 0.700*

Values are mean ± SE of five individual observation. Comparisons of means (control and treated fish) were done by Student's *t*-test.
* Significant at 5% level ($p < 0.05$).

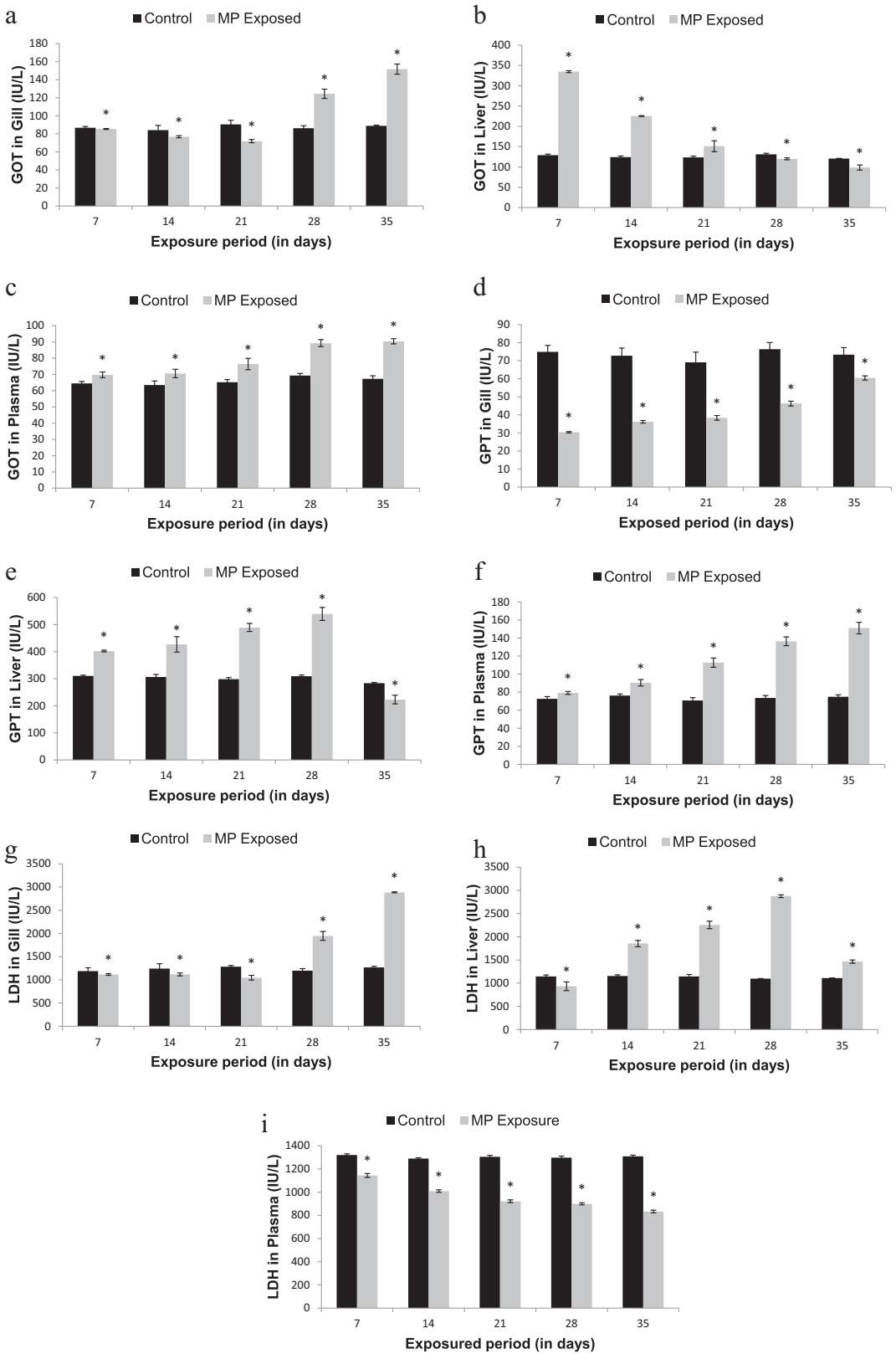

Figure 1 (a–i) Enzymatic alterations (a – GOT in gill; b – GOT in liver; c – GOT in blood plasma; d – GPT in gill; e – GPT in liver; f – GPT in blood plasma; g – LDH in gill; h – LDH in liver; i – LDH in blood plasma) in a fresh water fish *C. catla* exposed to methyl parathion for 35 days. Bar represents SE of the mean. Comparisons of means (control and treated fish) were done by Student's *t*-test. * Significant at 5% level ($p < 0.05$).

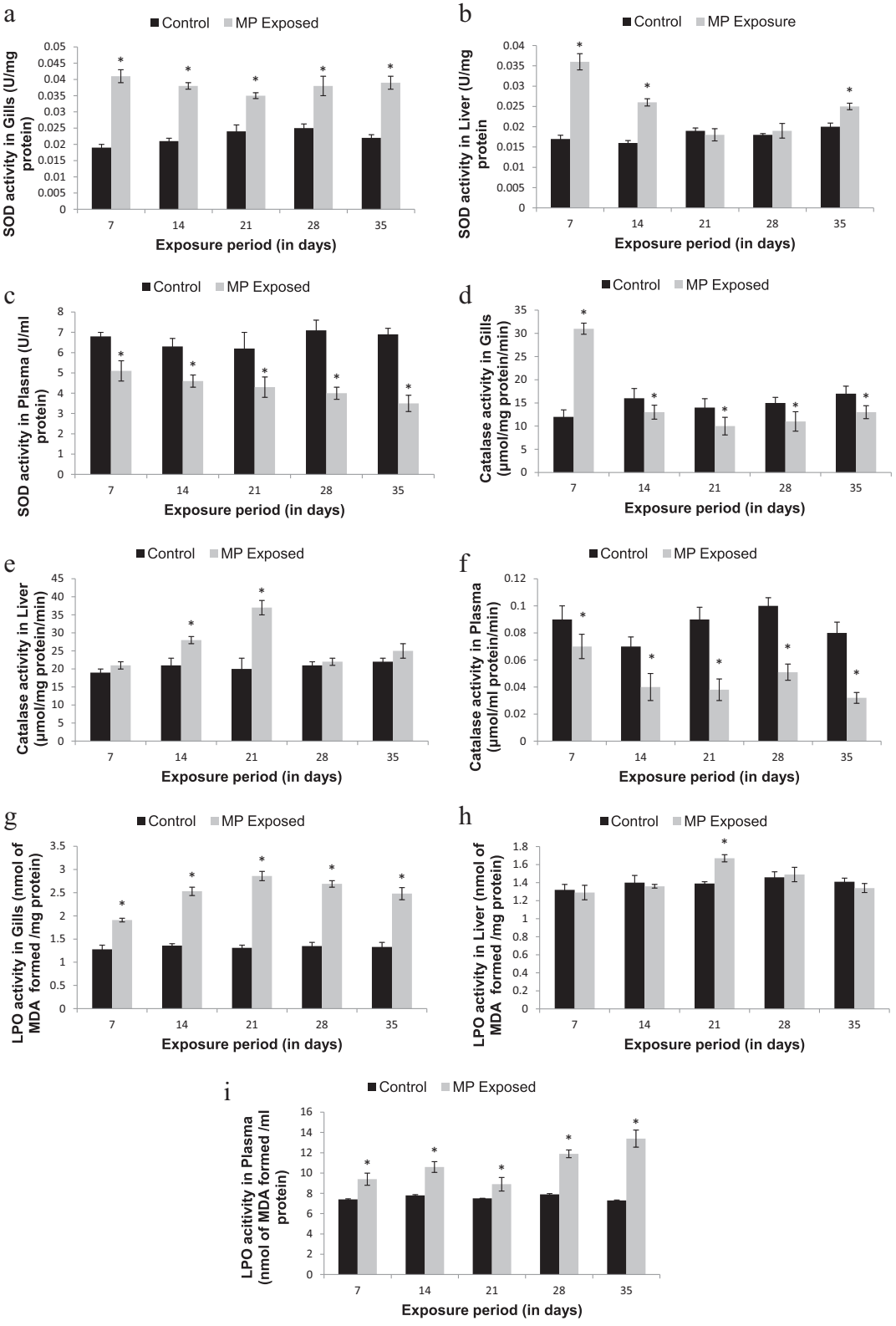

Figure 2 (a–i) Alterations in the antioxidant enzymes (a – SOD activity in gill; b – SOD activity in liver; c – SOD activity in blood plasma; d – catalase activity in gill; e – catalase activity in liver; f – catalase activity in blood plasma; g – LPO activity in gill; h – LPO activity in liver; i – LPO activity in blood plasma) in a fresh water fish *C. catla* exposed to methyl parathion for 35 days. Bar represents SE of the mean. Comparisons of means (control and treated fish) were done by Student's *t*-test. * Significant at 5% level ($p < 0.05$).

appear to be more or less similar with comparable lesions and its responses serve as markers of oxidative stress (Vutukuru et al., 2006). The primary mechanism of toxicity by OP pesticide is dependent on binding capacity at the cholinesterase active site and accumulation of acetylcholine at the synaptic cleft (Dutta et al., 1995). Breakage of neuronal signaling leads to intracellular influx of Ca^{2+}, triggering the activation of enzymes and generation of free radicals resulting in oxidative stress (Beal, 1995). Recently in toxicological research OP induced oxidative stress has widely been used to demonstrate the mechanism of OP toxicity (Sharma et al., 2005; Ambali et al., 2007; Thomaz et al., 2009).

Livingstone (2001) has stated that the oxidative stress caused by pesticides in aquatic organisms may lead to ROS production and alterations in antioxidants enzymes. The production of ROS may attack nearby molecules resulting in damage of the molecular structure and function or dysfunction of many organs and systems (Lukaszewicz-Hussain, 2010). The excessive production of ROS production and their damaging effects can be minimized by the cellular antioxidant systems (Sureda et al., 2004; Dorts et al., 2012). Generally, organisms under stress conditions use the antioxidant enzymes to adapt to environmental stress and altered activities depend on the dose, species and route of exposure (Gravato et al., 2006). The enzymes such as SOD and catalase play a major role in eliminating the ROS produced during bioactivation of xenobiotics and the induction of SOD/CAT system may be the first defense mechanism against ROS (Nwani et al., 2010). Moreover, SOD and CAT are highly sensitive and respond more quickly thereby protecting organisms from oxidative stress (Dewez et al., 2005; Rao, 2006).

SOD is the first enzyme that responds to oxidative stress during any stress condition in animals (McCord and Fridovich, 1969; Winston and Di Giulio, 1991). The observed increase in SOD and catalase levels in gill and liver in the MP treated fish indicates a detoxifying mechanism against the toxicity. A similar observation was also noted in tissues of *Brycon cephalus* exposed to MP (Monteiro et al., 2009; Modesto and Martinez, 2010). The increase in SOD activity in male rats after exposure to atrazin indicates an adaptive response to increased generation of ROS (Singh et al., 2011). Sharbidre et al. (2011) have suggested that the alterations in the activity of antioxidant enzymes in *Poecilia reticulata* exposed to sublethal concentrations of MP and chlorpyrifos indicate the adaptive response to ROS. The highest SOD and CAT activity in the liver of *Acipenser naccarii* and trout *Oncorhynchus mykiss* indicate that both enzymes have an important role in fighting the generation of superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) from the intense metabolic activity characteristic of liver (Trenzado et al., 2006). The generated ROS on MP exposure might have caused damage to gills and liver resulting in leakage of these enzymes into plasma as noted in the present study. Plasma SOD activities of fish are not much referred to in the literature, but the values observed here are consistent with the presence of very low levels and/or its absence in human plasma (Halliwell and Gutteridge, 1990; Kocak-Tokey et al., 1993). In general, the elevation of antioxidant activities indicates adaptive responses of organisms to counteract the oxidative effect of generated ROS (Hegazi et al., 2010).

In contrast to above increase, both SOD and catalase levels decreased in plasma of fish. Min and Ju-Chan (2008) observed similar results and suggested that the O_2 radicals or their transformation to H_2O_2 may cause oxidation of cysteine in the enzyme which results in decrease of SOD activity. Excess production of ROS may also inhibit the SOD activity (Manduzio et al., 2004; Min and Ju-Chan, 2008). The fluctuations (decreased and increased) of SOD activities in various tissues of rats exposed to MP may indicate a cellular oxidative stress due to MP exposure (Celik and Suzek, 2008). The inhibition of catalase activity may be due to binding of toxicants –SH groups of enzymes, increased H_2O_2 and/or superoxide radical (Ruas et al., 2008). The significant decrease in catalase activity in gill might have resulted from its inactivation by the superoxide radical triggered by MP exposure (Vutukuru et al., 2006). Gradual increase in catalase activity in the liver to sublethal exposure observed in the present study may be an adaptive response to H_2O_2 produced by SOD activity since catalase is responsible for its detoxification to water (Elia et al., 2002; Nwani et al., 2010).

The variation in the activity of antioxidant enzymes may be used as indicators of pollutant mediated oxidative stress (Sayeed et al., 2003). Li et al. (2009) suggested that the biphasic alterations in antioxidative enzymes in *Onchidium struma* during copper stress indicate that different tissues may have qualitatively different response patterns. In the present study also the variation in SOD and CAT activity during the exposure period might have resulted from the different response pattern of the organs such as sensitivity, accumulation and detoxification processes. Results from the present investigation imply that the fish utilizes enzymatic mechanisms to tolerate the effects caused by generated ROS due to MP accumulation.

Lipid peroxidation has been reported as a major contributor to the loss of cell function under oxidative stress (Huang et al., 2003). Moreover, organophosphorus pesticides may increase lipid peroxidation by direct interaction with the cell membrane (Hazarika et al., 2003). Avellini et al. (1993) considered LPO as the first step signifying the cellular damage caused by OP pesticides. MDA is the final product of lipid peroxidation and their concentration gives direct evidence of toxic process caused by free radicals (Sieja and Talerczyk, 2004). In the present study the level of LPO in gill and plasma of MP treated fish was found to be increased whereas in the liver its level decreased during acute exposure. A similar increase in plasma LPO level was noted in cypermethrin treated freshwater mussel *Unio elongatulus eucirrus* by Koprucu et al. (2008). Similar results were also observed by Hai et al. (1997) in *C. carpio* and *Ictalurus nebulosus* following exposures to dichlorvos. The elevation of LPO content in gill indicates an increase in free radicals due to MP toxicity or metabolism. Organophosphorus pesticides may cause an increase of lipid peroxidation level and an increase in MDA content in tissues can be used as an indicator of lipid peroxidation (Freeman and Crapo, 1981; Gultekin and Akdogan, 2000). However, the responses of LPO may vary with the concentration of chemicals, exposure period and functions of species (Parvez et al., 2006; Ruas et al., 2008).

In the present investigation tissue specific responses in the activities of antioxidant enzymes such as SOD, catalase and LPO were observed during acute and sublethal MP exposure which may indicate the different rates of free radical generation and different antioxidant potentials of these tissues and also the varied concentration of MP in these tissues as reported by Monteiro et al. (2006) The present study also demonstrated that methyl parathion has a high oxidative-stress-inducing

potential in *C. catla* and gill is the most sensitive organ in both acute as well as sublethal concentration.

Conclusion

The results of the present investigation indicate that acute and sublethal exposure of methyl parathion induces significant changes in the enzymatic profiles in *C. catla*. The presence of such level of methyl parathion in the natural environment is dangerous to the ecosystem and will definitely affect the survival of fish. Gills, due to their large surface area and permeability, are the primary sites for absorption. The experimental data obtained with *C. catla* can be considered as a useful reference for comparisons with biomarker responses of organisms living in polluted environments. These parameters can be used as biomarkers in assessing the pesticide toxicity in aquatic ecosystem. Further studies have to be carried out to evaluate the residual effects of this pesticide in different body tissues of fish in order to provide a better understanding of the physiological significance of the methyl parathion status in natural populations.

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