Chemosphere 132 (2015) 172–178

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The role of vitamin C as antioxidant in protection of biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus*

Madhusudan Reddy Narra [a], Kodimyala Rajender [a], R. Rudra Reddy [a], J. Venkateswara Rao [b], Ghousia Begum [b,*]

[a] Department of Zoology, College of Science, Osmania University, Hyderabad 500 007, India
[b] Toxicology Unit, Biology Division, CSIR – Indian Institute of Chemical Technology, Hyderabad 500 007, India

HIGHLIGHTS

- Modulatory and protective role of vitamin C on the toxicity of chlorpyrifos.
- Experiments include control group, E_1 and E_2 groups.
- The E_1 group showed less weight gain, survival rate and changes in other parameters.
- Potential impacts of Vitamin C on improvement of toxic stress are discussed.

ARTICLE INFO

Article history:
Received 26 June 2014
Received in revised form 2 March 2015
Accepted 3 March 2015

Handling Editor: David Volz

Keywords:
Clarias batrachus
Pesticide
Vitamin C
Haematology
Biochemistry

ABSTRACT

The study was conducted to explore the modulatory effects of chlorpyrifos and protective role of vitamin C in tissues of *Clarias batrachus*. Treatments include E_1 group (basal diet plus 1.65 mg L^{-1} CPF) and E_2 group (basal diet + 200 mg kg body weight vitamin C and 1.65 mg L^{-1} CPF) along with a control group of fishes (fed on basal diet only). After 1, 7, 15, and 30 d of treatment, fish tissues (brain, blood and liver) were used for the estimation of growth, biochemical and haematological parameters. The results of E_1 group indicated significantly lower weight gain and survival rate. Brain AChE activity was inhibited. The RBC, Hb, respiratory burst activity, total protein and HSI were also reduced whereas WBC count, plasma glucose and haematocrit were elevated. In contrast, liver glycogen content, lactate dehydrogenase, alkaline and acid phosphatase activities were inhibited and malate dehydrogenase, aspartate, alanine amino transferase were enhanced. The E_2 group of fish exhibited significant improvement in growth, survival, haematological indices, brain AChE, liver glycogen and oxidative enzyme activity. The findings support that dietary vitamin C supplementation might be helpful in abrogation of chlorpyrifos toxicity and improves growth, survival, biochemical and haematological conditions in fishes.

1. Introduction

Aquatic ecosystems are becoming more vulnerable to pesticide contamination, which is debilitating the sustenance of life. Pimentel (1995) reported that often less than 0.1% of an applied pesticide reaches the target pest, leaving 99.9% as an unintended pollutant in the environment. Presently, pesticides are found all over the aquatic habitats and even found their way into subsurface groundwater resources at varying concentrations due to direct overspray, drift, atmospheric transport, agricultural and residential runoff, individual misuse, and improper disposal (Gilliom and Hamilton, 2006; Singh and Singh, 2008).

Organophosphate chemicals are widely used as pesticides in residential settings and in agricultural practice to increase crop yields. The use of organophosphate pesticides have been remain pervasive in both developed and developing nations, concerns are increasing regarding the relative safety of these chemicals to the environment, wildlife and fish. One such organophosphate, is chlorpyrifos [*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothionate] which is widely used throughout the world under the registered trademark (LORSBAN and DURSBAN). It has been detected in air, rain, and fog (Majewski and Capel, 1995), and very highly toxic to fish and has caused fish kills in waterways near treated fields (USEPA, 2000). Sun and Chen (2008) reported CPF

* Corresponding author. Tel.: +91 (40) 27191377; fax: +91 (40) 27193227.
E-mail addresses: ghousia@iict.res.in, ghousia786@yahoo.com (G. Begum).

http://dx.doi.org/10.1016/j.chemosphere.2015.03.006
0045-6535/

effects in controlling arthropods in soil and foliage. Santerre et al. (2000) reported 11% of farm raised *Ictalurus punctatus* samples contained CPF residues ranging from 0.01 to 0.32 mg kg^{-1}.

Vitamins and minerals are included in fish food to promote optimal growth and health. Dietary vitamin C significantly enhanced growth, non-specific immunity and protection against infections (Zhou et al., 2012); however, high level was required to improve stress resistance of fish (Garcia et al., 2007). Vitamin C served as an antitoxic agent against pesticide stress (Vani et al., 2011) and enhanced nonspecific immune responses and disease resistance (Lin and Shi-Yen, 2005). It has been observed that increased tissue reserves of AA through dietary supplement helped *Heteropneustes fossilis* to counter stress induced by cypermethrin (Saha and Kaviraj, 2009). A low level (50 mg kg^{-1} bw) in the diet did not, but a high level of AA (100 mg kg^{-1} bw) removed stress induced by the pesticide fenvalerate (Datta and Kaviraj, 2003). Misra et al. (2007) reported 100 mg kg^{-1} body weight vitamin C stimulated immune response and growth in *Labeo rohita*.

Therefore, the present study evaluated (i) weight gain and survival performance of fish, (ii) hepatosomatic index (HSI), Ascorbic acid (AA) levels of liver and plasma, (iii) haematological parameters such as red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), hematocrit (Hct) levels, plasma proteins, glucose and respiratory burst activity (NBT), (iv) assay of liver enzymes; such as malate dehydrogenase (MDH), lactate dehydrogenase (LDH), acid and alkaline phosphatase (ACP, ALP) and aspartate and alanine aminotransferase (AAT, AlAT) and (v) and assay of brain acetylcholinesterase enzyme.

2. Materials and methods

2.1. Pesticide and chemicals

All the chemicals used in the present study were purchased from Sigma–Aldrich chemical company (Saint Louis, USA). The test compound chlorpyrifos (20% EC) was purchased from local market (NOCIL Bombay-India).

2.2. Animal maintenance

The freshwater catfish, *Clarias batrachus*, were obtained from a local supplier and were transported to the laboratory in large aerated drums. They were first given prophylactic dip in 2% salt solution for 1 min followed by oxytetracycline treatment (15 mg L^{-1}) for the first three days, and were then acclimatized to laboratory conditions for 4 weeks prior to the experiment, during which they were fed basal diet.

2.3. Experimental diet

Two types of feed was prepared, one is basal diet and the other is an extra vitamin C (200 mg kg^{-1} bw) (Table 1A). The source of vitamin C is L-ascorbyl 2-polyphosphate (Sisco Research Labs Pvt., Ltd, Mumbai-India). All ingredients were mixed thoroughly, and dough was prepared with required amount of water. The dough was kept for half an hour for proper conditioning followed by steaming for 20 min in a pressure cooker. After cooling, vitamin and mineral mix (Agrimin; Glaxo India Ltd, Mumbai-India) were added to the dough and pellets were made to feed E_2 group of fish.

Table 1A
Composition of the basal diet.

Ingredients (% dry weight)			
Rice bran	18.43	Fish meal	40.71
Wheat flour	18.43	Vitamin mix[a]	02.00
Mustard oil cake	18.43	Mineral mix[b]	02.00
Proximate composition (%)			
Dry matter	94.30	Crude protein	31.08
Lipid	15.00	Ash	11.32

[a] Vit mix (%): B_1, 7.14; B_2, 2.55; B_4, 10.2; B_6, 1.02; B_{12}, 0.012; Biotin, 0.025; Calcium Pantothenate, 2.55; Niacin, 76.3; Vit A, 0.10; Vit C, 0.103.
[b] Mineral mix (%): Cu, 3.12; Co, 0.45; Mg, 21.48; Fe, 10.8; I, 1.6; Zn, 21.30; Ca, 30.0; P, 8.25; Mn, 3.00.

2.4. Pesticide preparation and experimental design

The LC_{50} (96 h) value of CPF was determined in the laboratory using the semi-static method of Finney (1971) and 1/10th of LC_{50} (1.65 mg L^{-1}) was selected for the study. 72 healthy fishes (average weight 40 ± 5 g and 22 ± 2 cm length) were distributed in three different groups (Control, E_1 and E_2), of 24 fish per tank. Each group was maintained in 140 L of water (40 × 70 × 50). Control group was fed with basal diet and kept in pesticide free water, E_1 was fed with basal diet and exposed to 1.65 mg L^{-1} chlorpyrifos pesticide whereas E_2 was fed with basal diet and vitamin C (200 mg kg^{-1} body weight) and exposed to 1.65 mg L^{-1} pesticide. Fish were fed twice a day to a level of 2.5% body weight. Siphoning of uneaten feed and fecal matter was done daily evening.

The ambient condition of natural photoperiod was maintained (light:12, dark:12) throughout the experiment period. The average mean values of water quality during investigation are; temperature 25 ± 3 °C, pH 7.4 ± 0.4, dissolved oxygen 8.24 ± 0.22 mg L^{-1}, total hardness 415 ± 1.2 mg L^{-1} as $CaCo_3$, alkalinity 348 ± 1.6 mg L^{-1} as $CaCo_3$, and total chloride 245.57 ± 1.44 mg L^{-1}.

2.5. Sample preparation

At the end of the experimental period (interval of 1, 7, 15 and 30 days), six fish per group were sampled, weighed and anaesthetized on ice for 10 min and dissected, liver tissue was used for the estimation of different parameters. Brain was used for the assay of AChE enzyme. Blood was collected from the caudal vasculature by syringe from fish selected randomly and divided into two aliquots. The blood samples were transferred to tubes (contained 1.0% (v/v) of 15% EDTA), centrifuged (9000*g* for 10 min at 4 °C) and plasma was collected for haematological assay.

2.6. Survival and growth performance

$$\text{Weight gain}\,(\%) = \text{Final weight} - \text{initial weight}/\text{initial weight} \times 100$$

$$\text{Survival}\,(\%) = \text{Number of fish survived after 30 d}/\text{initial number of fish stocked} \times 100$$

2.7. Hepatosomatic index (HSI)

Whole liver was removed, weighed to calculate hepatosomatic index (HSI) according to equation of Adams and McLean (1985) (without gall bladder): Liver weight (g)/whole body weight (g) × 100.

2.8. Ascorbic acid analysis

The levels of AA in liver and blood were determined by the method of Roe and Keuther (1943) by using UV spectrophotometer (E-Merck, Germany) at 540 nm (using 2, 4-dinitrophenylhydrazine).