Modification of saltwater stress response in *Cyprinus carpio* (Linnaeus, 1758) pre-exposed to pesticide indoxacarb

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ABSTRACT

To evaluate the effects of indoxacarb on saltwater stress response in *Cyprinus carpio*, the fish were pre-exposed to indoxacarb (0, 0.75, 1.5 and 3 mg/L denoted as CP, 0.75IT, 1.5IT and 3IT, respectively) for 21 days and then released to saltwater. A negative control (CN) group was included (the fish were held in indoxacarb-free water for the entire experiment). The fish were sampled immediately (0 h) and 24, 48 and 72 h after the salinity exposure for the analysis of plasma cortisol, glucose and sodium, chloride, potassium and calcium levels. All fish pre-exposed to 3 mg/L indoxacarb, died after the first day of salinity challenge. CP showed typical cortisol response after the salinity challenge, but, cortisol response of the fish pre-exposed to indoxacarb (0.75IT and 1.5IT) was blocked. Plasma glucose increased significantly in all groups compared to the CN; however, this elevation had no consistent trend in 0.75IT and 1.5IT which indicated interference in glucose response due to indoxacarb exposure. Plasma sodium increased (compared to CN) in all groups after the salinity challenge. However, elevation in plasma chloride and potassium was significantly different among the groups and the indoxacarb-treated fish showed slightly sooner ionic disturbance. The results clearly indicate that indoxacarb impairs stress response of *C. carpio* and the fish may not be able to respond normally to additional stressors, which threatens their survival.

1. Introduction

Water pollution is a widespread problem in many aquatic environments (Hori et al., 2008). Xenobiotics enter water bodies due to agricultural, industrial and municipal activities. Upon entrance, xenobiotics cause several physiological and pathological alterations in fish which may weaken the animal and decrease its survivorship (Hedayati et al., 2014, 2016; Hoseini et al., 2014, 2016b; Hoseini and Tarkhani, 2013).

Aquatic animals are exposed to environmental stressors and react to these stressors with a variety of physiological responses (Barton, 2002). These physiological responses restore the fish body homeostasis and guarantee the fish survivorship. Osmotic stress is one of the common stressors in aquatic environment which threatens fish life (Assem and Hanke, 1981). It causes hydromineral imbalance and increases energy demand by elevation in cortisol secretion and glucose mobilization (Hoseini and Hosseini, 2010). Cortisol is the main stress hormone in fish which has important role in hyperosmotic stress tolerance. Cortisol provides demanded energy for osmoregulation, increases chloride cell number and size, and stimulates Na^+,K^+-ATPase activity; all these

changes help the fish to restore hydromineral balance (Hoseini and Hosseini, 2010; Madsen, 1990; McCormick, 1990). Xenobiotics may deteriorate fish stress responses (Barton, 2002; Cericato et al., 2009). Pacheco and Santos (1996) and Nascimento et al. (2012) suggested that exposure to xenobiotics cause fish lost their capacity to elevate cortisol in response to additional stressors. Several field studies have confirmed the adverse effect of prolonged exposure to agrichemicals on wildlife hypothalamus–pituitary–interrenal axis (HPI) (Hontela, 1998).

Indoxacarb is an applicable pesticide, organophosphate alternative, to manage some crops such as tobacco and cotton in the north of Iran (Malekzadeh and Javadzadeh, 2002; Mohaghegh Naishabouri et al., 2009) and also is used to control lepidopteran larvae in tomato, lettuce, bean and some other crops (Ghelichpour et al., 2017; Mirghaed and Ghelichpour, 2015). Although, indoxacarb is moderately hydrophobic with a low water solubility and short aqueous photolysis half-life it is classified as "moderately to very highly toxic" to freshwater and estuarine fish (Moncada, 2003). Little is known about this pesticide effects on fish species. Indoxacarb is used in the north of Iran for pest control, near the Caspian Sea, where common carp inhabits; thus it is

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necessary to investigate toxic effects of indoxacarb on common carp. Previous studies showed that indoxacarb is toxic in common carp and causes histopathological damages, thyroid hormones' interference and suppressed protein synthesis (Ghelichpour et al., 2017; Mirghaed and Ghelichpour, 2015). However, there is no study about indoxacarb effects on osmotic stress tolerance in fish. It is important to investigate indoxacarb effects on common carp osmotic stress responses, because this species may be exposed to both osmotic stress and indoxacarb toxicity. In the present study, the effects of long term indoxacarb exposure were investigated on osmotic stress response in common carp.

2. Materials and methods

2.1. Fish acclimation and experimental design

Common carp, weighing 45.2 ± 5.81 g were obtained from the Sijawal Fish Reproduction Center (Golestan province, Northeast Iran). The fish were randomly assigned to fifteen 300 L tanks with 28 fish per tank and acclimated during 7 days. The physicochemical parameters of the water were constantly monitored in the acclimation period and the experiments (temperature = 23 ± 1.27 °C; pH = 8.07 ± 0.25; dissolved oxygen = 7.14 ± 0.84 mg/L conductivity = 4630 ± 55 μs cm^{-1}; salinity = 2.63 ± 0.15 g L^{-1}; hardness = 300 ± 17.5 mg/L (as CaCO$_3$); alkalinity = 350 ± 20.3 mg/L (as CaCO$_3$), and calcium = 110 ± 11.7 mg/L). During the acclimation, the fish were fed 1.5% body weight once a day with commercial carp feed (Mazandaran Animal & Aquatic Feed Co., Sari, Mazandaran, Iran) under continuous aeration condition and 75% water exchange daily.

After the acclimation week, the tanks were divided into three main groups: 1) indoxacarb treatment group = nine tanks (3 replicate tanks per treatment) were exposed to three different indoxacarb (Hef Iran Co. Tehran, Iran) concentrations [0.75 mg/L (0.75IT), 1.5 mg/L (1.5IT) and 3 mg/L (3IT)] followed by a salinity challenge, 2) positive control (CP) group = three tanks were held in indoxacarb-free water followed by the salinity challenge. A negative control (CN) was assigned too. Experimental design is shown in Fig. 1. The indoxacarb concentrations were chosen according to 96-h-LC$_{50}$ (0.75 mg/L as 5% of the LC$_{50}$, 1.5 mg/L as 10% of the LC$_{50}$ and 3 mg/L as 20% of the LC$_{50}$). The indoxacarb exposure period was 21 days. During this period, the tanks' water was daily exchanged (75%) by clean-water. Appropriate indoxacarb amount was added to each tank to maintain the pesticide levels. Feeding was conducted same as the acclimation period.

At the end of the indoxacarb exposure period, feeding was ceased and all groups except CN group were subjected to saltwater (addition of 10 g NaCl to one litter of water) over 72 h. This salinity was chosen after a preliminary experiment to determine tolerable salinity for the experimental fish. Blood samples of all groups were taken 0, 24, 48, and 72 h after the salinity challenge from caudal vein, using heparinized syringes (six samples at each point). Two fish per tank were sampled using a dip net and immediately anesthetized with 100 ppm eugenol within 60 s. Blood samples (2 mL) were taken using heparinized syringes and poured into plastic tubes. Plasma was separated after 10 min centrifugation ($1000 \times g$), and maintained at -80 °C until analysis. All experiments were conducted under a protocol approved by the committee of ethics of the faculty of sciences of the University of Tehran (357; 8 November 2000).

2.2. Analysis

Water temperature, dissolved oxygen, electro-conductivity, salinity, and pH were measured daily during the experiment by Hach HQ40d portable apparatus (Loveland, Colorado, USA). Total hardness, alkalinity, and calcium were determined using photometer (Wagtech 7100, Berkshire, UK). Plasma glucose levels were determined spectrophotometrically by glucose-oxidase method using Pars Azmun kits (Tehran, Iran). The kit detection limit is 5–400 mg/dL. Calcium levels were determined spectrophotometrically by ARSENAZO III method using Pars Azmun kits (Tehran, Iran). This kit detection range is 1–20 mg/dL. Plasma chloride levels were determined spectrophotometrically by mercuric cyanate method using Zist Shimi kit (Tehran, Iran). This kit is suitable for detection in 2–200 mEq/L. Appropriate dilution was performed by distilled water when necessary. Plasma cortisol was determined by solid phase ELISA method based on competition principle using a commercial kit (IBL, Gesellschaft für Immunchemieund Immunbiologie, Hamburg, Germany). The test performed on microplate reader and detection limit was 0.05–800 ng/mL. cortisol was determined in duplicate and mean was used for statistical analysis. Plasma samples were assayed for sodium and potassium using a flame photometer (SEAC, Florence, Italy).

2.3. Statistical analysis

Data had normal distribution (Shapiro-Wilk test). Data were analyzed with a one-way ANOVA and Tukey test. $P < 0.05$ was considered as significance. Data were presented as mean ± SD. All analyses were performed using SPSS software (v.22).
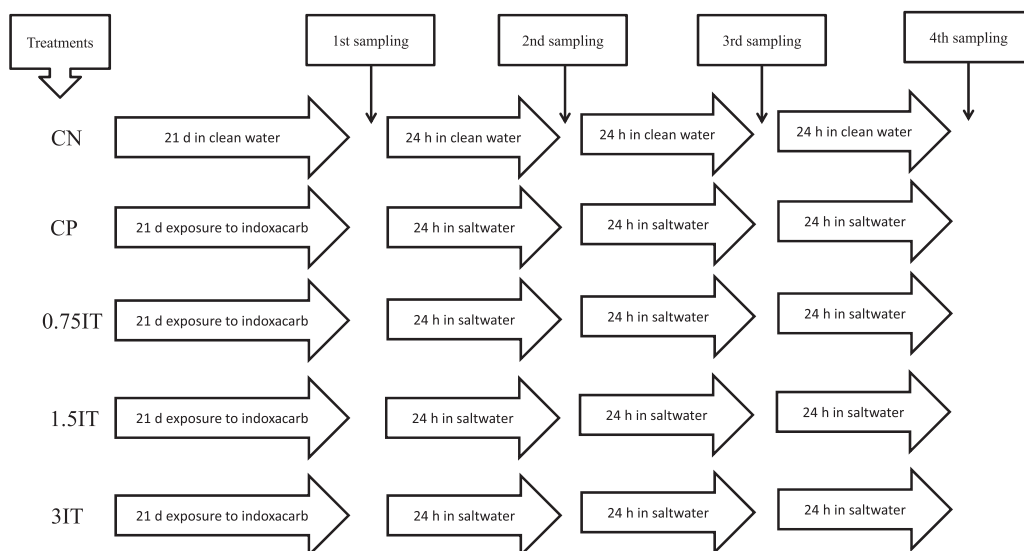

Fig. 1. Schematic of experimental design.