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Cypermethrin toxication leads to histopathological lesions and induces inflammation and apoptosis in common carp (*Cyprinus carpio* L.)

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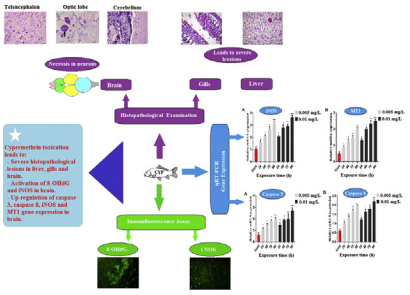
HIGHLIGHTS

- Cypermethrin toxication caused histopathological changes in brain, gills and liver.
- Immunopositivity of iNOS and 8-OHdG were observed in cyp exposed to brain.
- Up-regulation of caspase 3, caspase 8, iNOS and MT1 genes were indicated in cypermethrin exposed to brain.
- Cypermethrin toxication induced inflammation, oxidative stress and apoptosis in brain of common carp.

GRAPHICAL ABSTRACT



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ABSTRACT

Cypermethrin (Cyp), a known neurotoxic pesticide, is widely used in agricultural applications. In the present study, the aim was to determine the histopathological effects of Cyp toxication and evaluate the activation of inducible nitric oxide synthetase (iNOS) and 8-hydroxy-2-deoxyguanosine (8-OHdG) using an immunofluorescence assay. Thereafter, we identified the expressions of caspase 3, capsase 8, iNOS, and metallothionein 1 (MT1) genes in common carp using quantitative reverse transcription polymerase chain reaction (qRT-PCR). High and low doses of Cyp were administered to experimental groups for 24, 48, 72, and 96 h. As a result, necrotic neurons in different stages and desquamation of ependymal cells due to necrosis were detected in the brain. Histopathological changes, including hyperplasia of lamellar cells, telangiectasia of lamellae and thickening due to cellular infiltration in gills, hemorrhage, diffuse hydropic degeneration, and focal necrosis in the liver were observed in the experimental groups. Immunopositive reactions of 8-OHdG were clearly observed in the nuclei and cytoplasm of neurons, and positive reactions for iNOS were detected in the cytoplasm of neurons and in the glial cells of the experimental groups. Furthermore, we found that caspase 3, capsase 8, iNOS, and MT1 genes were up-regulated in the brain when exposed to both high and low doses of Cyp. In conclusion, our findings revealed that Cyp toxication harms the organs of common carp, particularly the brain, and also gives rise to inflammation, DNA damage, and apoptosis. Therefore, the use of Cyp should be restricted to protect the health of aquatic animals.

Abbreviations: Cyp, cypermethrin; cas-3, caspase 3; cas-8, caspase 8; iNOS, inducible nitric oxide synthetase; 8-OHdG, 8-hydroxy-2-deoxyguanosine; MT1, metallothionein 1; IF, immunofluorescence.

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1. Introduction

People have taken the precaution of using yield enhancers in certain areas to boost agricultural production because of the possibility of the global population outstripping food production. Xenobiotics have been extensively used by farmers to increase yield and protect against harmful organisms in agricultural areas (Lamberth et al., 2013; Kohler and Triebskorn, 2013; Qureshi et al., 2016). As a result of repeated and uncontrolled applications, not only target species acquire resistance and develop a high survival rate against these chemicals but non-target useful species are also affected (Damalas and Eleftherohorinos, 2011). These chemicals can reach water resources and aquatic environments as a result of rainfall and agricultural runoff, which may lead to toxic effects for both human and aquatic life. This chemical contamination causes the death of aquatic animals and enters the food chain (Xiao et al., 2009; Murthy et al., 2013; Gibbons et al., 2015). Although the effects of xenobiotics on target organisms is clearly known, their effects on non-target organisms and their threat rate are not fully understood (Kohler and Triebskorn, 2013).

Cypermethrin (Cyp), a type II synthetic pyrethroid, is a commonly used environmental and animal health agent because it is highly toxic to insects. It has also been used to control ectoparasites in veterinary medicine (Atamanalp and Cengiz, 2002; Anadon et al., 2009; Yang and Suh, 2015). In addition, it is used to protect against agricultural pests (Palmquist et al., 2012). Cyp is reported to accumulate in tissue, particularly in the central nervous system (CNS), due to its lipophilic feature (Starr et al., 2012). A high concentration of Cyp in the brain leads to symptoms of neurobehavioral toxicity (Ray and Fry, 2006). There are only a few reports about its harmful effects on non-target animals, including honey bees, dog, and particularly fish in the existing literature (Karise et al., 2007; Rodriguez-Vivas et al., 2017). Common carps are an important bioindicator species for toxication studies. This species of fish is also hunted and cultivated as a food source worldwide (Huang et al., 2007). Therefore, it can potentially be exposed to Cyp toxication. However, the adverse effects of Cyp on common carp are not clearly known, and research about Cyp toxication in relation to common carp is therefore important to ensure the health of these aquatic animals and of aquatic animals in general.

The number of studies on the effects of xenobiotic toxication via biochemical methods has significantly increased (Chupani et al., 2014; Ullah et al., 2015; Velisek et al., 2006). In addition to blood and biochemical parameters in these reports, immunohistopathological and molecular evaluations have recently emerged as efficient assessment methods (Macirella et al., 2016; Liu et al., 2015; Khoshnood et al., 2015). In the present study, the adverse effects of Cyp toxication were assessed using an immunofluorescence (IF) assay and quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Apoptosis is an essential pathway for the elimination of damaged and infected cells that may turn into cancer cells and disrupt the normal functioning of multicellular organisms (Portt et al., 2011). It is regulated by several modulators, including genes, proteins (caspases), and organelles (Ulukaya et al., 2011). The members of the caspase family of aspartic acid-directed cysteine proteases induce disruption of cellular structure and function, ultimately resulting in apoptosis (Walsh et al., 2008; Gonzalez et al., 2010). There are two groups of caspase proteins, initiator and effector caspases, that act upon the apoptosis pathway in eukaryotic cells (Kuranaga, 2011). Initiator caspases are caspase 2, 8, 9, and 10. They activate the effector caspases, including caspase 3, 6, and 7 (Kumar, 2007). Caspase 3 plays a pivotal role in both the extrinsic pathway (death receptor pathway) and the intrinsic pathway (mitochondrial pathway) of apoptosis (Martinez et al., 2010; Wong, 2011). Ultraviolet (UV) irradiation (Bivik et al., 2007), chemotherapy agents (Seitz et al., 2010), infection by pathogens (Zhang et al., 2011), polychlorinated biphenyls (PCBs) (Zhang et al., 2009), polycyclic aromatic hydrocarbons (PAHs) (Solhaug et al., 2004), pesticides (Li et al., 2007), and heavy metals (Pathak et al., 2013) can cause apoptosis. Caspase 3 has been identified and characterized in common carp (Gao et al., 2013a), but there are no studies about caspase 3 activation and Cyp toxication in common carp. It is therefore necessary to investigate the immunological mechanisms of Cyp toxicity.

Metallothioneins (MTs) are low-molecular mass (approximately 6 kDa) cysteine-rich proteins (Vasak and Meloni, 2008) and are important for controlling zinc and copper. They also eliminate the harmful effects of exposure to toxic elements, such as heavy metals and pesticides, and protect organisms from several stress conditions (Kagi and Schaffer, 1988; Ali et al., 2009; Ferencz and Hermesz, 2015). Pesticides activate MT synthesis in fish (Linde-Arias et al., 2008). Nitric oxide (NO) is an important signaling molecule produced by the nitric oxide synthase enzyme (NOS). This enzyme is encoded by different genes, including neuronal NOS, endothelial NOS (eNOS), and inducible NOS (iNOS). iNOS can be induced by the toxicity of pesticides and heavy metals (Ortiz Ortiz et al., 2009; Pi et al., 2003) and has been found in goldfish and trout (Virgili et al., 2001). 8-OHdG is known as a biomarker of oxidative stress and DNA damage. 8-OHdG activation is a risk factor for a variety of diseases, including cancer, and is also associated with elevated 8-OHdG (Villaño et al., 2015; Hintsala et al., 2016; Ye et al., 2016). However, there are no reports about whether Cyp exposure activates iNOS and 8-OHdG in common carp. The investigation of these markers in tissues exposed to Cyp is important to gain a new perspective about the molecular effect mechanism in aquatic animals.

The aim of our study was to determine the harmful effects of Cyp exposure on common carp. First, we evaluated the histopathological changes in gill, liver, and brain tissue exposed to Cyp. iNOS and 8-OHdG activation were assessed using an IF assay, and caspase 3, caspase 8, iNOS, and MT1 mRNA expression levels were measured in brain tissue exposed to Cyp using qRT-PCR.

2. Material and methods

2.1. Experimental design

In present study, cypermethrin, $C_{22}H_{19}C_{l2}NO_3$ (α- Cyano- (3-phenoxy- phenyl)- methyl 3- (2,2- dichloro-vinyl)- 2,2- dimethyl-cyclo- pro-pane- carboxylate) (CAS Number 52315-07-8, ≥98%, Molecular Weight 416.30) was purchased from Sigma-Aldrich (Germany). The molecular structure of Cyp was shown in Fig. 1. Common carps were obtained from Atatürk University, Faculty of Fisheries and the Inland Water Fish Breeding and Research Center.

The experiments were performed in accordance with the approved ethical rules of Atatürk University. Fish were fed for 45 days in a stock pond to provide their acclimatization to the environmental conditions; measured water quality parameters were O_2 = 8.3 ppm, pH = 7.6, Cl = 6.78 ppm, SO_4^{-2} = 10.32 ppm, CO_3^{-2} = 124 ppm, HCO_3^- = 145.6 ppm, NO_3^- = 3.45 ppm, NO_2^- = trace,

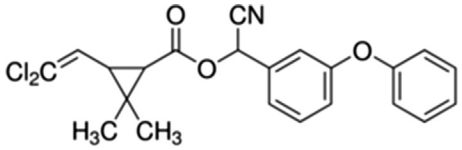

Fig. 1. Molecular structure of Cypermethrin.