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CYP1A gene expression as a basic factor for fipronil toxicity in Caspian kutum fish

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Chemical compounds studied in this article:
Fipronil (PubChem CID: 15278226)
Phenoxyethanol (PubChem CID: 17848643)
Haematoxylin (PubChem CID: 442514)
Eosin (PubChem CID: 11048)
Picric acid (PubChem CID: 6954)
Formaldehyde (PubChem CID: 712)
Acetic acid (PubChem CID: 176)
Ethanol (PubChem CID: 702)
M-xylene (PubChem CID: 7929)
Periodic acid (PubChem: CID 65185)
Cresyl violet (PubChem: 44134641)
NaCl (PubChem: 5234)
EDTA (PubChem: 6049)
Tris base (PubChem: 6503) and NaOH (PubChem:14798)
Triton X100 (PubChem: 5590)
DMSO (PubChem: 679)
Agarose (PubChem:11966311)
Giemsa (PubChem:13735)
Beta-mercaptoethanol (PubChem:1567)
Bromophenol blue (PubChem: 8272)
Sucrose (PubChem: 5988)

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ABSTRACT

The aim of this study was to assess the effects of fipronil insecticide on the Caspian kutum fish at different levels of biological organizations and to find possible relationship between these biomarkers. Different doses of fipronil (65, 130 and 200 mg/kg) were intraperitoneally administered to the fish for 2 weeks. After 7 and 14 days of exposure, alterations in organ-somatic index, tissue and DNA structure, oxidative stress and CYP1A gene expression in gill, liver, brain and kidney were studied. Determination of these parameters in the liver showed that the degree of tissue change (DTC), comet tail, superoxide dismutase (SOD) and relative CYP1A mRNA expression increased mostly in a time dependent manner whereas in the kidney increased mostly in a dose dependent manner. These parameters in the gill increased more in time and dose dependent manner. Apart from the changes in CYP1A expression and oxidative stress, no alterations was observed in the brain. Multiple regression analysis showed that the CYP1A had the most correlation with the organ-somatic index ($R^2 = 0.76$) and comet tail ($R^2 = 0.89$) in the liver, and with DTC ($R^2 = 0.93$) and oxidative stress ($R^2 = 0.87$) in the kidney. Generally, this study showed that CYP1A gene expression can be considered as one basic factor for fipronil toxicity in this fish. However, other possible factors also should be considered for future research.

1. Introduction

According to the global pesticide market, about 3 million tons of pesticides intentionally are released into the environment each year [1]. This wide use of pesticides can cause harmful effects on non-target organisms, especially marine organisms. Fipronil, which was produced by a French company in 1987, has raised concerns for its dangerous effects on human health and the environment [2], resulting from the increasing use in agricultural and residential zones.

Fish as the earliest class of vertebrates are extensively studied in aquatic toxicology. Moreover, scientific recommendations imply that the use of fish model in toxicology, both in ecological and biomedical studies, is a good option [3,4]. In the area south of the Caspian Sea, fipronil (under the trade name Regent®) is widely used in rice farms to kill pest insects and can reach the Caspian Sea and might threat aquatic life. Caspian white fish (*Rutilus frisii kutum*), classified in the cyprinidae

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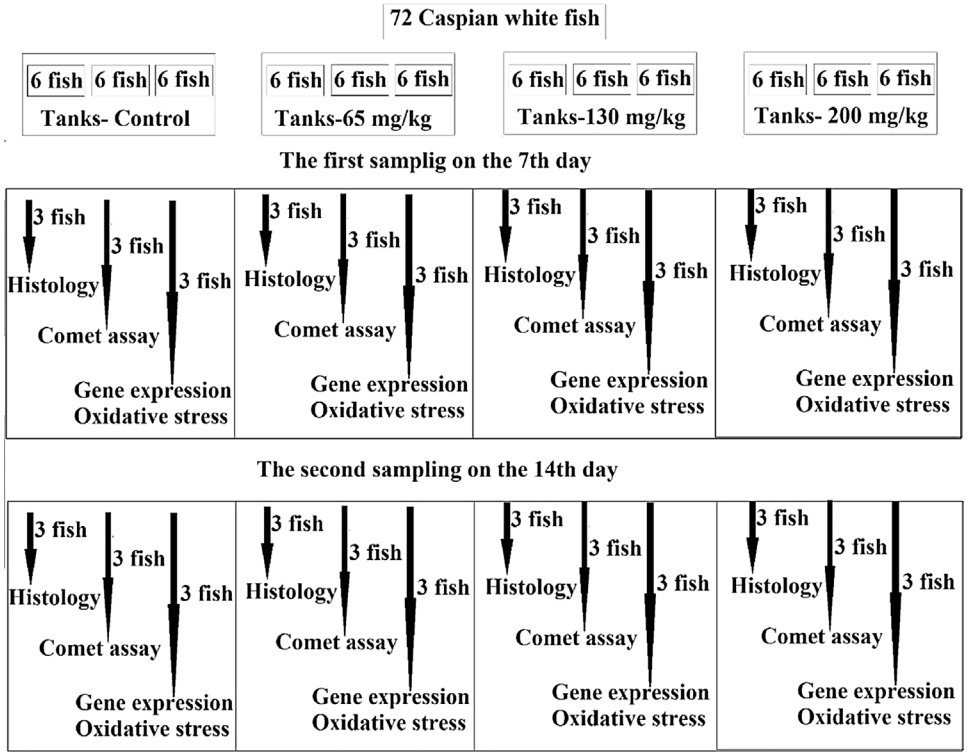

Fig. 1. Schematic picture of experimental design.

family, is the most popularly consumed fish in this region and cultured extensively. The life span of this species is about 9–10 years in southern part of Caspian Sea [5]. Thus, this fish was used as a biological model for studying toxicological effects of fipronil on aquatic life in this sea.

Fipronil can be exposed to fish through different routes and the most relevant route depends on the aim of the study and the physicochemical characteristics of the fipronil. Ardeshir et al. [6] compared the effects of waterborne and intraperitoneal (i.p.) routes of exposure to fipronil in the Caspian kutum and cited the advantages of i.p. route over waterborne exposure in fish. It seems that i.p. administration is the best route to study fipronil toxic effects on fish in a time/dose dependent manner.

Although the mechanism of fipronil toxicity in insects has been fully determined and is related to blocking gamma-aminobutyric acid-gated chloride channels of neurons in the central nervous system [7], there is not enough information about its mechanism in vertebrates due to the existing complex interactions. However, generally, mechanism of toxicity of persistent organochlorinated pesticides might be explained through some biological reactions including binding to some receptors such as aryl hydrocarbon receptor (AhR) and induction of biotransformation enzymes such as CYP1A, oxidative stress and triggering pathological-related condition and DNA damage [8]. Thus, exposure to fipronil in fish might exert changes in biological responses at different levels of biological organization including morphological and biochemical parameters, histopathology, genotoxicity and alteration in gene expression of biotransformation enzymes. The present study was carried out to investigate these parameters in gill, liver, brain and kidney of the Caspian kutum exposed to fipronil intraperitoneally and to show correlation between biomarkers at different levels of biological organization. To assess genotoxic effect of fipronil and measure DNA strand breaks, the comet assay as a rapid and reliable technique [9,10] was used and comets were analyzed by Cellprofiler software. This software is able to analysis and identify thousands of biological images by advanced algorithms, and causes to save time and eliminate objective errors [11,12]. At gene level, the expression of CYP1A gene, as a member of CYP gene superfamily, was assessed. In addition to the detoxification, previous studies also demonstrated the potential function of CYP1A as a tumor suppressor [13–15]. There is a little information about effect of fipronil on fish CYP gene expression and activity. Tang et al. [16] suggested that CYP3A4 is the major isoform responsible for fipronil oxidation in human. Afterwards, Das et al. [17] reported that fipronil can induce CYP3A4 and CYP1A1 by enhancing mRNA as well as protein expression in human hepatocytes. However, Caballero et al. [18] reported that fipronil has major effects on CYP1A activity in rat liver. In fish, CYP1A subfamily has important roles in the metabolism of exogenous chemicals, especially pesticides, and is extensively used as a biomarker to assess contamination of the aquatic environment [19–22]. Thus, in this study, gene expression of this enzyme was used as biomarker of fipronil toxicity as well.

2. Materials and methods

2.1. Fish

One hundred Caspian white fish fingerlings (16 ± 3 g and 11 ± 2 cm) were obtained from the Shahid Rajai Fish Proliferation and Culture Center (Sari, Mazandaran Province, Iran) and exposed to fipronil in this center. Prior to the test, fish were acclimated to the treatment condition for one week, and fed powdered fishmeal until the day before fipronil exposure. Non-chlorinated well water with water-shower aeration and a semi-static system in plastic tank (200 L) was used, along with 13 h light and 11 h dark as the photoperiod.

2.2. Experimental design

Fipronil (98% purity, 50:50 racemic mixture) was bought from Moshkfam Fars Chemical Company (Shiraz, Iran). Stock solutions of fipronil were made by dissolving 50, 100, 150 mg of this compound in 10 mL sunflower oil. Before the injection, the fish were anesthetized using 2-phenoxyethanol (0.2%), and their length and weight were measured. For each dose, 0.24 ± 0.04 mL of the stock solution was intraperitoneally injected into the fish using an insulin syringe based on the weight of each fish. Sub-lethal test doses of 10, 20, and 30% of LD_{50}–96 h (65, 130, 200 mg/kg) were used according to the i.p. LD_{50} of fipronil in the Caspian kutum (632 mg/kg) determined in the previous