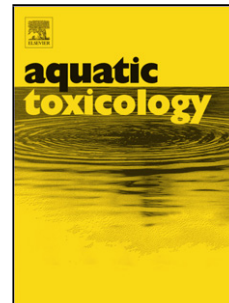


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Effects of diclofenac on the expression of Nrf2 and its downstream target genes in mosquito fish (*Gambusia affinis*)

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Highlights:

1. *Nrf2* and *NQO1*, *GCLC*, *GST*, *UGT* and *SOD2* genes in mosquito fish were first cloned;
2. Diclofenac could affect the expression of *Nrf2* and its mediating target genes;
3. Responses of *Nrf2* and its downstream genes are sensitive to diclofenac than their enzyme activity.

ABSTRACT: Diclofenac (DCF) is one of widely used non-steroidal anti-inflammatory drugs. Recently, this drug has been universally detected in aquatic environment. However, its potential adverse effects and oxidative stress toxic mechanisms on fish remain unclear. In the present study, we first cloned the crucial partial sequences of some key oxidative stress related genes, which include NF-E2-related factor 2 (*Nrf2*), NAD(P)H: quinoneoxidoreductase (*NQO1*), glutamate-cysteine ligase catalytic subunit (*GCLC*), Cu-Zn superoxide dismutase (*SOD2*), catalase (*CAT*), alpha-glutathione S-transferase (*GSTA*), and UDP-glucuronosyltransferases (*UGT*) in mosquito fish (*Gambusia affinis*). We also deduced amino acids of *Nrf2* and then constructed the phylogenetic trees of *Nrf2*, *NQO1* and *GCLC*, respectively. Results showed that a high identity percentage was founded between *G. affinis* and other bony fish species, such as *Xiphophorus maculatus* and *Poecilia reticulata*. The transcriptional expression of these genes and partly related enzymes activities were then investigated under the included environmental relevant concentration DCF exposure (0 μmolL^{-1} , $1.572 \times 10^{-3} \mu\text{molL}^{-1}$, $1.572 \times 10^{-2} \mu\text{molL}^{-1}$, $0.1572 \mu\text{molL}^{-1}$ and $1.572 \mu\text{molL}^{-1}$) for 24 h and 168 h. The expression of *Nrf2* was inhibited at 24 h but induced at 168 h, exhibiting a significant time and/or dose-effect relationship under DCF exposure. Similar observation was found in its downstream target genes. However, *Nrf2*-mediated antioxidant enzymes activities displayed differently under the same concentration of DCF exposure for the same time. Under DCF exposure for 168 h, the genes exhibited dramatic induction trend, but there were no significant changes in enzyme activities and MDA content. Overall, mRNA responses were more sensitive than enzyme changes in mosquito fish under DCF exposure.

Keywords: Diclofenac; Mosquito fish; *Nrf2*; Oxidative stress

Highlights:

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