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Short Communication

EVALUATING THE GENE EXPRESSION LINKED TO OXIDATIVE STRESS IN THE EYES OF ADULT MALE ZEBRAFISH (*DANIO RERIO*) AFTER TREATMENT WITH 4 - NONYLPHENOL

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ABSTRACT: The phenolic endocrine disruptor molecule 4-nonylphenol (4-NP) is prevalent in several industrial goods and the environment, adversely affecting the health of people, animals, and aquatic organisms. It is necessary to assess the harmful impact of this contaminant on the ocular systems of aquatic animals. The investigation was done on adult male zebrafish exposed to 4-nonylphenol for 21 days. At the conclusion of the research, the expression of oxidative tress-related genes (sod, cat, nrf2) was assessed in the zebrafish eye. One hundred twenty mature zebrafish were categorized into four categories. The zebrafish in the first group were maintained in standard reverse osmosis (RO) water, whilst the second group was subjected to ethanol at a concentration of 10 µl/L of water and designated as the vehicle group. Fish from two toxicity groups were subjected to 4-nonylphenol at concentrations of 100 and 200 µg per liter of water. The mRNA expression levels of superoxide dismutase (SOD), catalase (CAT), and nuclear factor erythroid 2 related factor 2 (Nrf2) in the eyes of zebrafish exposed to 200 µg of 4-nonylphenol per liter of water exhibited considerable downregulation.

Keywords: 4-nonylphenol, Eye, mRNA expression, Zebrafish.

Endocrine-disrupting compounds (EDCs) are molecules that can be made by humans or by nature. They can imitate, block, or intervene body's natural hormones, which are part of the endocrine system. A lot of health problems have been linked to these chemicals [16]. Although they may potentially have an impact on hormone synthesis and metabolism, the majority of EDCs directly mimic or inhibit hormone receptor action. Endocrine disruptors are found in many everyday products, including certain cosmetics, food and drink packaging, toys, carpets, and pesticides. Some chemicals that have flame-retardant qualities also are endocrine disruptors. These substances may enter the body via the air, food, skin, and water. Although it is impossible to totally prevent or eliminate EDCs, you may lower your exposure and the chance of any negative health consequences by making wisely or insightful decisions [11, 13]. EDCs are mostly used as raw materials in plastic packaging, cosmetics, detergents, paints, and lubricants. Examples of these include atrazine, polychlorinated biphenyls (PCBs), butyl benzyl phthalate, perchlorate, triclosan, phytoestrogens, phthalates, per- and poly-fluoroalkyl substances (PFAS), dioxins, phenanthrene, 4-tert-pentylphenol, 4-NP, octylphenol, bisphenol A, butyl phenol, and polybrominated diphenyl ethers (PBDE). Some are also undesirable byproducts, such as industrial intermediates, plasticizers, flame retardants, and results of burning fossil fuels [20, 21]. Intentionally and accidentally many rivers and inshore marine waters get

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large amounts of household and industrial effluents and chemicals from the surrounding land [30]. Chronic exposure to low EDC combinations is more likely to cause human diseases [16]. EDCs may influence numerous organ systems, including CNS pathophysiology and neurological diseases. EDCs are called neuro-disrupting agents because they impair neural transmission and network development [15]. In aquatic creatures, EDCs may cause neurological, reproductive, developmental as well as changes in the endocrine hormones [10]. 4-Nonylphenol (4NP) is a breakdown product of nonylphenol ethoxylates [11]. Due to its structural similarities to endogenous estrogens, particularly 17-oestradiol, and its ability to mimic or counteract hormonal reactions, it is an EDC [13]. 4-Nonylphenol is an estrogen-like antiandrogenic that affects animal reproduction. The lipophilic nature of 4-NP leads to bioaccumulates in cells and tissues [27]. The paper industries, textile industries, cleaning products, and plastics products use nonylphenol ethoxylates to make non-ionic surfactants [21]. The production of home cleaners, agricultural pesticides, papers, paints, cosmetics, plastics, wastewater treatment plant effluents, and sewage sludge have all released nonylphenol and compounds that are related to it into ecosystems. [20]. Marine plastic litter may potentially pollute aquatic environments [1]. Nonylphenol exposure alters fish's normal physiology, histological and behavioral traits according to several studies [2, 3, 5]. Nonylphenol alters animal and human neurodevelopment [23]. Inflammation and neurotoxicity result from oxidative stresses activation of the mitochondrial apoptosis and inflammatory signaling pathways, which change the expression of genes linked to apoptosis as well as inflammatory mediators including cyclooxygenase-2 and nitric oxide synthase [14]. Neurodegenerative diseases like Parkinson's and Alzheimer's may result from uncontrolled inflammation of the central nervous system. However, the causes of NP neurotoxicity and cognitive impairment require more study. A comprehensive behavioral evaluation may uncover brain function issues. Gonadal hormones regulate CNS development; hence, NP exposure may alter cognitive function and behavior in fish, reptiles, and birds [13]. Since it may cross the blood-brain barrier, NP can mimic estrogen and block its effects. Zebrafish are useful as toxicological model animals and in other fields [28, 31]. Because they are small, easy to manage and develop quickly, zebrafish are better toxicological models than other animals. There are various firms that specialize in zebrafish aquariums that can accommodate thousands of fish because of the low housing space and care requirements. Zebrafish have been inutile in labs for a long time therefore their breeding and maintenance conditions are well understood [9]. Small larval and adult zebrafish require less lab equipment and chemicals for assays (minuscule amounts of reagents) and histological evaluations (diminutive amounts of embedding materials) than large species [8].

The study

Each group's zebrafish were housed in a facility with regulated water temperature, pH, electrical conductivity and hardness. The experimental protocol has been authorized by the Institutional Animal Ethics Committee (IAEC) of the CVSAH, JAU, Junagadh, Gujarat, India (IAEC approval no: JAU/JVC/IAEC/SA/71/2020). There were 120 mature male zebrafish, all over three months old and that were randomly divided into 4 groups with each group including 30 fish and different doses of toxicant which is mentioned in Table 1. Ice cold method is used for graciously sacrificing the zebrafish after completion of exposure to 4 nonylphenol [33]. The collection of eyes from fish is performed with the help of a stereo-zoom microscope. The isolation of RNA and synthesis of cDNA as per standard protocol [25]. The amplification primers of the target genes sequence of β-actin (Housekeeping gene) are forward-CGA GCT GTC TTC CCA TCC A and reverse-TCA CCA ACG TAG CTG TCT TTC TG [4]; CAT forward-AGT TCC CTC TGA TTC CTG TG and reverse-ATG GCG ATG TGT GTC TGG [12]; SOD forward-CAA CAC AAA CGG CTG CAT CA and reverse-TTT GCA ACA CCA CTG GCA TC [26]; NRF2 forward-TGT TGG TTC GGA GGC TCT TAA and reverse-AGG CCA TGT CCA CAC GTA CA [32]. The housekeeping gene as well as target genes were amplified using the real-time polymerized chain reaction. The amplification size of β-actin, CAT, SOD, and NRF2 genes are 60 bp, 173 bp, 132 bp, and 62 bp respectively. The $2\text{-}\Delta\Delta C_t$ technique was used to compute the relative fold expression [22]. All data were statistically analyzed using an appropriate method with the help of Graph Pad Prism 9.0.

Results and discussion

Fig. 1 shows fold variations in CAT, SOD, and Nrf2 mRNA expression in zebrafish eyes. T1 zebrafish had no substantial downregulation of SOD, CAT and Nrf2 mRNA compared to C1. T2 zebrafish had considerably lower ocular SOD, CAT, and Nrf2 mRNA

Table 1. Different groups, treatments and numbers of zebrafish used.

	Name of group	Concentration	No. of fish
I	Control (C1)	RO water	30
II	Vehicle (C2)	Concentrate Ethanol @ 10 μl/L	30
III	Treatment One (T1)	Concentrate Ethanol @ 10 μl/L + 4-NP @ 100 μg/L	30
IV	Treatment Two (T2)	Concentrate Ethanol @ 10 μl/L + 4-NP @ 200 μg/L	30
		Total	120

* Total experimental period was 21 days.

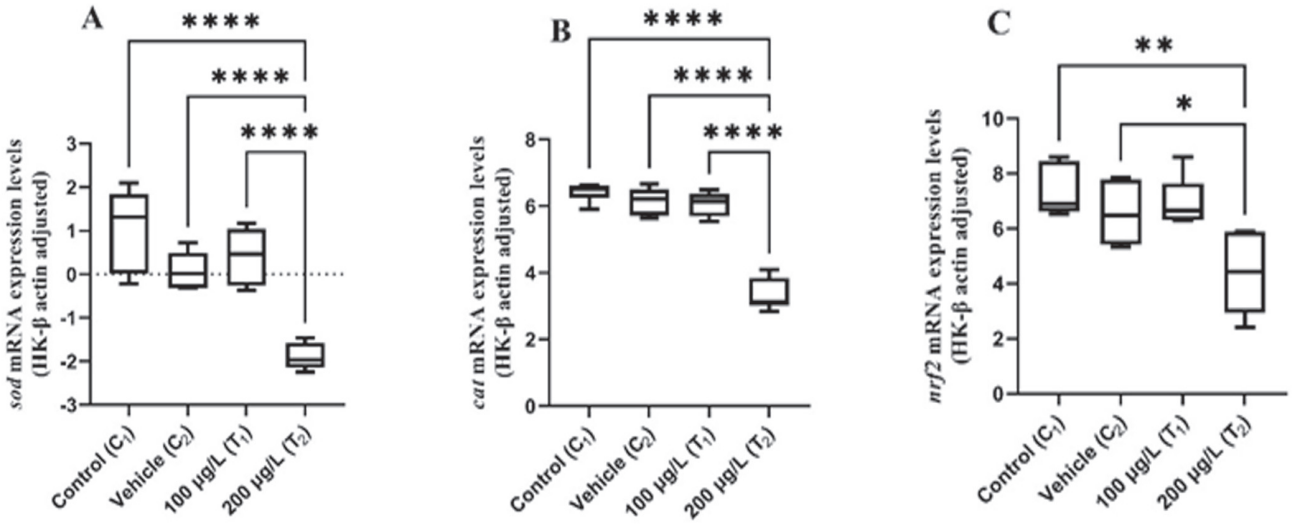

Fig. 1. mRNA expression level of enzyme in treated zebrafish eye tissue as compared to control group. [*indicates p<0.05, **indicates p< 0.01, ****indicates p< 0.001].

levels than the C1 group. We examined the link between oxidative stress-mediated modification processes in the CAT, SOD, and Nrf2 genes in zebrafish ocular tissue following extended 4-NP exposure. Higher 4-NP levels downregulated the Nrf2, SOD and CAT genes, suggesting oxidative stress in the zebrafish eye. This is confirmed by earlier studies that revealed lower SOD1 and CAT mRNA expression in male rat gonads after 20 days of 4-NP therapy [7] and in rat liver [19]. Nrf2 is essential for modulating antioxidant enzyme gene expression. The cytoplasmic negative regulator Keap1 normally suppresses Nrf2. All vertebrates including zebrafish have the Nrf2-Keap1 system which protects cells from oxidative stress [18, 17]. Reactive species from 4-nonylphenol influence signal transduction, gene expression, and DNA damage [7]. In the promoter regions of genes, Nrf2 interacts with the Antioxidant Response Element (ARE). This binding activates the transcription of genes responsible for producing detoxifying and antioxidant enzymes. Nrf2 help as a shield of cells from oxidative damage brought by free radicals and other reactive oxygen species (ROS) by promoting the expression of these genes [24]. Kelch-like-ECH-associated protein 1 (Keap1), a cytoskeletal protein, anchors and suppresses Nrf2 transcription [29]. Nrf2 is released from Keap1 and enters the nucleus to regulate antioxidant gene transcription after being triggered by oxidative stress [6]. According to our knowledge, this is the first research to examine Nrf2 in zebrafish eyes after long-term 4-NP exposure. The Nrf2 pathway plays a crucial role in countering this oxidative stress by activating antioxidant enzymes like SOD1/2, CAT, glutathione (GSH), glutathione peroxidase (GPX) and Heme oxygenase 1 (HO-1). However, overactive Nrf2 can also lead to tumorigenesis [34, 35].

Conclusion

Exposure of zebrafish to 4-nonylphenol at 200 µg/L of water for twenty one days downregulates the

expression of SOD, CAT and nuclear factor erythroid 2-related factor 2 (Nrf2) genes, which indicates oxidative stress mediated alterations in the eye.

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