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Oxidative damage in gills and liver in Nile tilapia (*Oreochromis niloticus*) exposed to diazinon☆

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ABSTRACT

Agricultural activity demands the use of pesticides for plague control and extermination. In that matter, diazinon is one of the most widely used organophosphorus pesticides (OPs). Despite its benefits, the use of OPs in agricultural activities can also have negative effects since the excessive use of these substances can represent a major contamination problem for water bodies and organisms that inhabit them. The aim of this paper was to evaluate oxidative damage in lipids and proteins of Nile tilapia (*Oreochromis niloticus*) exposed acutely to diazinon (0.97, 1.95 and 3.95 ppm) for 12 or 24 h. The evaluation of oxidative damage was determined by quantifying lipid hydroperoxides (Fox method) and oxidized proteins (DNPH method). The data from this study suggest that diazinon induces a concentration-dependent oxidative damage in proteins, but not lipids, of the liver and gills of Nile tilapia. Furthermore, the treatment leads to a decrease in the concentration of total proteins, which can have serious consequences in cell physiology and fish development.

1. Introduction

Pesticides are toxic substances released into the environment in large amounts with the potential to cause adverse effects on human and wildlife populations (Galloway and Handy, 2003). Organophosphorus pesticides (OPs) are insecticides used worldwide, designed as irreversible acetylcholinesterase (AChE) inhibitors. They were introduced as an alternative to the persistent and more bioaccumulative organochlorine pesticides. Diazinon (0,0-diethyl 0-(6-methyl-2-{1-methylethyl}-4-pyrimidinyl)phosphorothioate) is a broad spectrum OP insecticide effective against many pests from fruits, vegetables, tobacco, forage, field crops, pasture, grasslands and ornamental plants. Once diazinon is in the water it remains stable for up to six months and can accumulate in non-target tissues, causing undesirable biochemical changes in mammals (Dikshith et al., 1975; Tomokuni et al., 1985, García et al., 1995) and aquatic organisms like fishes (Al-Ghanim, 2014, Sharbidre et al., 2011, Isik and Celik, 2008).

Aquatic environments are potentially vulnerable to pollution since almost all kinds of chemicals used in anthropogenic activities, directly or indirectly, reach water bodies (Islam and Tanaka, 2004). Pollutants can induce an imbalance in the pro-oxidant and antioxidant mechanisms of living beings through the release of reactive oxygen species (ROS), such as H_2O_2, $O_2^{\bullet}$, OH^-, and $^{1/2}O_2$. These molecules have unpaired electrons in their bonding orbitals and are able to extract electrons from biomolecules, causing adverse effects like lipid peroxidation; proteins and DNA oxidation; altering cell physiology and inducing cell death (Masroor et al., 2000; Galloway and Depledge, 2001; Hermes-Lima, 2004; Basova et al., 2012).

Lipid peroxidation is a chain reaction in which ROS react with membrane phospholipids inducing the formation of conjugated dienes and lipid hydroperoxides that result in chemical changes in polyunsaturated fatty acids (PUFAs). This may cause a reduction in the fluidity of the membrane as well as cell membrane destruction. In addition, lipoperoxidation can affect internal membrane systems, such as endoplasmic reticulum and mitochondria (Hermes-Lima, 2004). Although proteins can be altered by ROS, there are few cellular mechanisms involved in protection against protein oxidation, such as the ubiquitin–proteasome pathway, a mechanism that reduces oxidative damage by removing the accumulation of damaged proteins (Goldberg, 2003) (Fig. 1).

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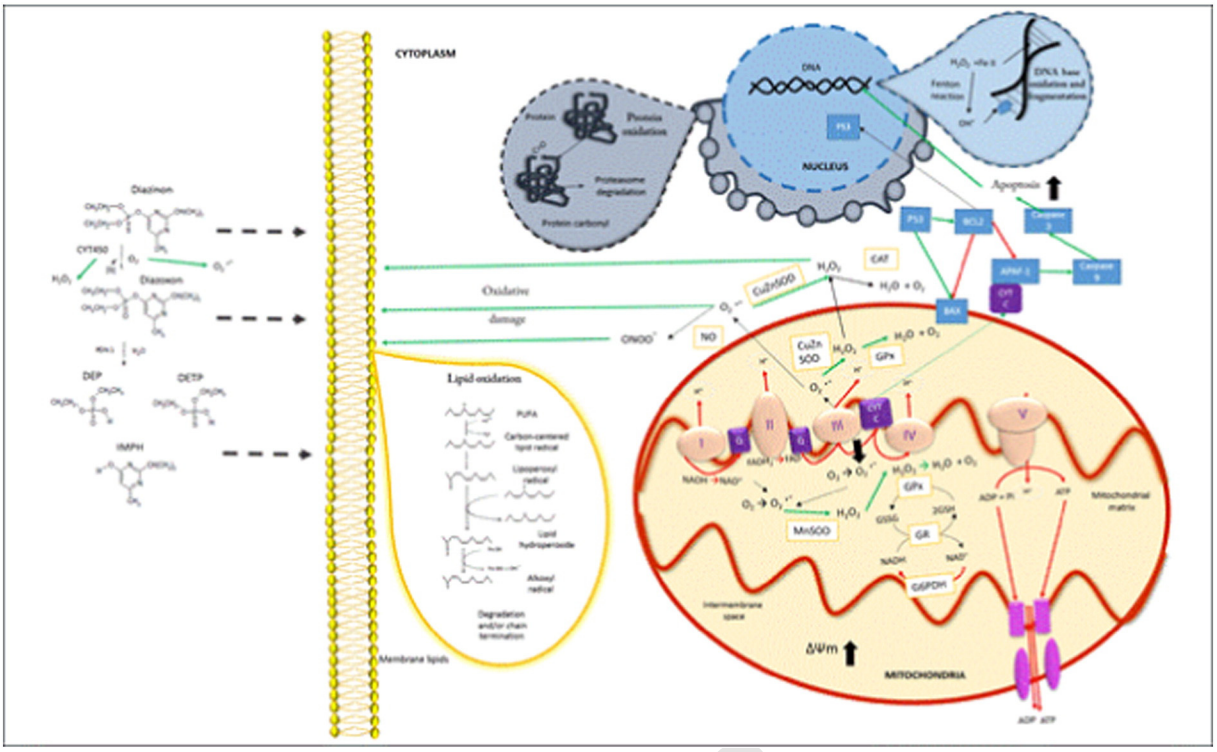

Fig. 1. Diagrammatic representation of effect of the organophosphorous (OPs) compounds on oxidative stress pathways over the cell and its different compartments as membrane lipids (yellow), nuclear proteins and DNA (blue), mitochondrial membrane proteins (red). The inhibited and induced pathways are shown by red and green arrows, respectively. Abbreviation: ADP: Adenosine diphosphate, APAF-1: Apoptotic protease activating factor 1, ATP: Adenosine triphosphate, BCL2: B-cell lymphoma 2, CYT C: Cytochrome C, CYT450: cytochrome 450 superfamily, DEP: diethyl phosphate, DETP: diethyl thiophosphate, FADH2: flavin adenine dinucleotide, GPx: Glutathione peroxidase, GR: Glutathione reductase, GSH: glutathione reduced, GSSG: glutathione oxidized, IMPH: 2-isopropyl-6-methyl-4-pyrimidinol, SOD: Superoxide dismutase, NADH: Nicotinamide adenine dinucleotide, P53: Tumor protein, PUFA: Polyunsaturated fatty acid, Q: Coenzyme Q, ΔΨm: Mitochondrial transmembrane potential.

Tilapia (*Oreochromis* spp.) is a teleost fish with a worldwide distribution. This species is cultured under extensive and intensive methods. Hence, this organism has shown great adaptability to virtually all environmental conditions present in tropical and subtropical countries and has become the main fish species to be exploited commercially within continental waters (Fitzsimmons, 2000). Therefore, tilapia fish is a good model for assessing aquatic ecosystems and for toxicological studies. Previous studies performed by our research group have revealed that the CL_{50} of diazinon for Nile tilapia is 7.83 ppm. In addition, it has been proven that sublethal concentrations of diazinon cause immunological alterations in Nile tilapia, but did not affect hepatic enzyme activities in this fish (Girón-Pérez et al., 2007, 2008, 2009). Thus, the aim of this study was to evaluate oxidative damage in lipids and proteins of Nile tilapia (*O. niloticus*) acutely exposed to diazinon.

2. Materials and methods

Nile tilapia fish (*O. niloticus*) were obtained from a local aquaculture farm, in Nayarit, Mexico. Selected male fish (3 months old, 273 ± 43 g and 20 ± 3 cm in length) were acclimated in 400 L tanks at constant temperature (26 ± 2 °C) and aeration for 4 weeks. The average values for water quality were: pH 8.0 ± 0.1, salinity 0.5 psu, dissolved oxygen 7.0 ± 0.2 mg/L, and oxygen saturation 85.4 ± 2.4%. Fish were fed two times/day with commercial feed Winfish®, corresponding 3% of their corporal weight.

To evaluate the effect of diazinon, commercial formulation Diazinon 25-E (Agrodelta®) was used. The organisms ($n = 10$/group) were exposed to sub-lethal concentrations of 0.97, 1.95 and 3.95 ppm of diazinon for 12 or 24 h. These concentrations were obtained from the CL_{50} of 7.83 ppm (Girón-Pérez et al., 2007). Before the exposure, organisms were individually acclimated for 24 h in 30 L fish tanks with constant temperature and aeration. Control fish ($n = 10$) were kept under the same conditions but without diazinon. After the exposure period, organisms were sacrificed by thermal shock in an ice bed. Liver and gills of each animal were dissected and stored in 40 mM butylated-hydroxytoluene (BHT) at a 1:10 w/v ratio for further analyses. For each parameter evaluated, all determinations were performed in triplicate.

2.1. Hydroperoxides

To determine the concentration of hydroperoxides (LHP) in the liver, tissue was placed in concentrated cold methanol at a 1:9 w/v ratio (one part of tissue: 9 parts methanol); to determine LHP concentration in gills, these were placed in cold methanol at a 1:5 w/v. Samples were then centrifuged at 1000 *g*/10 min at 4 °C, and the supernatant was separated for spectrophotometric analyses. The reaction mix was prepared in 96-well plates as follows:

90 μL of 1 mM $FeSO_4$ (prepared prior to use), 35 μL of 0.25 M H_2SO_4, 35 μL of 1 mM xilenol orange (dissolved in cold water).

The reaction mix was incubated for 30 min at room temperature (23 ± 2 °C); then, 20 μL of each sample was added to the wells and incubated for 1 h at room temperature in the dark. Absorbance was first measured at 545 nm; then, 5 μL of 1 mM cumene hydroperoxyde solution was added to each well, incubated for 1 h at room temperature, and absorbance was measured again at the same wavelength. The LPH were calculated using the following formula:

$$\text{CHPE/g tissue} = (\text{A545 nm sample/A545 nm 5 nmol CHP}) \times 5 \text{ nmol CHP} \times 300/\text{V1} \times 6.$$

Where CHPE = Cumene hydroperoxyde equivalents, CPH = Cumene hydroperoxyde, 300 = total volume (μL); V1 = sample volume

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