Science of the Total Environment 605–606 (2017) 811–823

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Sublethal effects induced by captopril on *Cyprinus carpio* as determined by oxidative stress biomarkers

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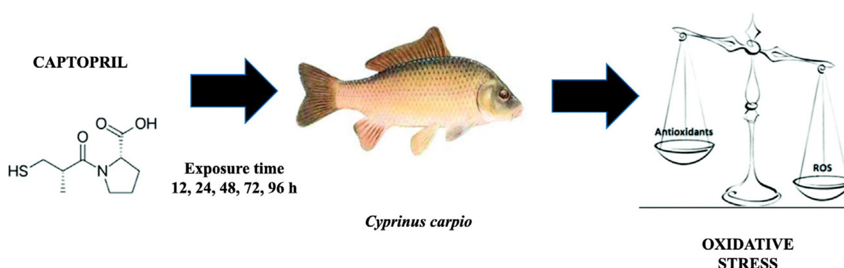
HIGHLIGHTS

- This study aimed to evaluate captopril-induced sublethal effects in *Cyprinus carpio*.
- Modifications were found in the activity of SOD and CAT.
- Significant increases in HPC, LPX and PCC occurred mainly in kidney, gill and brain.
- Captopril induces oxidative stress on *C. carpio*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 April 2017
Received in revised form 21 June 2017
Accepted 24 June 2017
Available online xxxx

Editor: D. Barcelo

Keywords:
Oxidative stress
Beta-blockers
Common carp
Emerging contaminants

ABSTRACT

To our knowledge, this is the first study to evaluate captopril-induced oxidative stress in fish, and specifically in the common carp *Cyprinus carpio*. At present, very few studies in the international literature evaluate the sublethal effects of captopril on aquatic organisms ~~such as fish~~, and available ones focus on determination of median lethal concentration in crustaceans and algae. Also, studies evaluating these effects do not make reference to the mechanism of action of this pharmaceutical or its toxicokinetics. This limits our knowledge of the characterization of the sublethal effects of this medication and of its potential ecological impact. The present study aimed to evaluate the sublethal effects induced by three different concentrations of captopril, on *C. carpio*), by determination of activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), as well as indicators of cellular oxidation: hydroperoxide content (HPC), lipid peroxidation (LPX) and protein carbonyl content (PCC). Specimens were exposed for 12, 24, 48, 72 and 96 h to three different captopril concentrations: 1 μg L^{-1}, 1 mg L^{-1} and 100 mg L^{-1} (the first one has been detected environmentally, the other two have been associated with diverse toxic effects in aquatic species), and brain, gill, liver, kidney and blood samples were evaluated. Significant increases in HPC and LPX were observed mainly in kidney and gill, while PCC also increased in brain. Modifications were found in the activity of SOD (mostly in kidney, brain and blood), CAT (all organs) and GPx (kidney and gill). In conclusion, captopril induces oxidative stress in *C. carpio*.

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http://dx.doi.org/10.1016/j.scitotenv.2017.06.208
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1. Introduction

The use of pharmaceuticals and personal care products (PPCPs) has increased to an extremely high degree. These products are eliminated from the body and enter sewage systems, eventually ending up in wastewater treatment plant effluent as well as the aquatic environment. Since substances with pharmacological action have been designed to carry out a biological function, depending on their physicochemical properties (*e.g.* high liposolubility) they have a tendency to bioaccumulate and can affect aquatic and terrestrial ecosystems (Halling-Sørensen et al., 1998; Brandão et al., 2014). Data on the ecotoxicity of pharmaceuticals and the effects induced by them on diverse aquatic species is scarce, and not all studies focus on the most commonly prescribed and used types of medications.

Wastewater treatment plants and municipal, hospital and pharmaceutical industry discharges are the main sources of environmental contamination by pharmaceuticals (Emmanuel et al., 2004; Joss et al., 2005; Verlicchi et al., 2010). The wastewater treatment systems currently used to reduce or eliminate the concentrations of these compounds are not sufficiently effective (Kümmerer, 2001), so that these products are now of environmental concern at world level and have been designated "emerging" contaminants (Fent et al., 2006; Richardson, 2009).

Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-l-proline, is one of these emerging contaminants, an angiotensin converting enzyme (ACE) inhibitor that acts by blocking the protein peptidase from the active center of the enzyme (Jackson, 2001). Captopril is used to treat arterial hypertension, congestive heart failure and myocardial infarction, and to preserve renal function in diabetic nephropathy (Armijo et al., 2010; Tzanavaras, 2010).

According to the World Health Organization, cardiovascular diseases (CVDs) are the main cause of death at world level (WHO, 2016). These disorders accounted for 31% of all deaths worldwide in 2012 (17.5 million people). Low- and middle-resource and income countries are the ones most affected by CVDs. Of the 16 million annual deaths in individuals under age 70, 82% occur in ~~low- and middle-income~~ these countries and 37% are caused by CVDs (WHO, 2016). In Mexico, diabetes, CVDs and heart disease remain the top causes of death, and together add up to > 50% of all deaths in the country. Information released by the National Council for the Prevention of Accidents (Consejo Nacional de Prevención contra Accidentes, CONAPRA), which includes figures up to 2011, reveals that the top causes of death in the country include diabetes (24.70%), heart disease (21.73%), CVDs (9.55%) and cirrhosis and other liver diseases (8.68%).

Captopril has been detected in aquatic ecosystems at concentrations of ng to μg L^{-1} (Chen et al., 2014). It has been shown to induce toxicity in organisms such as daphnids, with a 48-h LC_{50} (median lethal concentration) > 100 mg L^{-1}; *Desmodesmus subspicatus* (24-h LC_{50} = 168 mg L^{-1}); and *Lemna minor* (3 to 7-day LC_{50} = 25 mg L^{-1}) (Cleuvers, 2003, 2005; Nalecz-Jawecki and Sawicki, 2003; Nalecz-Jawecki and Persoone, 2006; Park, 2005; Cunningham et al., 2006; Kaza et al., 2007; NOAA, 2016).

Furthermore, captopril has been postulated as a free-radical scavenging agent due to the terminal sulfhydryl group in its molecular structure (Bagchi et al., 1989; Andreoli, 1993). *In vitro* studies indicate that captopril acts as an antioxidant due to these two properties: being a scavenger of reactive oxygen species (ROS) and capable of increasing the levels of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Andreoli, 1993; De Cavanagh et al., 1995). This medication has been shown to improve total antioxidant capacity in patients with hypertension (Schneider et al., 1990). A study by Chopra et al. (1992) indicates that the –SH group present in captopril structure is crucial in order for this pharmaceutical to be able to scavenge free radicals but not the proline portion.

In addition to its capacity to potentiate antioxidant activity in mammals, captopril can undergo phase I biotransformation reactions, producing ROS such as the superoxide anion (O2 • -) and hydrogen peroxide (H_2O_2). These reactive species induce oxidative stress, which is considered one of the main mechanisms of action of toxic substances. Oxidative damage induced by ROS on lipids, proteins and DNA, and the adverse effects of ROS on enzymatic antioxidant mechanisms in aerobic organisms have been used in recent years as biomarkers for monitoring of environmental pollution (Valavanidis et al., 2006). The major oxidative stress biomarkers used in toxicological studies of aquatic systems are lipid peroxidation (LPX), hydroperoxide content (HPC), protein oxidation, and enzymatic antioxidant activity (Droge, 2003).

Since no data are available regarding the concentrations of captopril required to induce toxicity on fish species, methodologies need to be established to determine the effects of this compound on species of ecological and economic importance such as the common carp *Cyprinus carpio.*

Bioindicators are used to evaluate the toxic impact of contaminants in water bodies. Toxicity assays on fish are one of the most effective methods for understanding the deleterious effects of environmental contaminants in aquatic systems. Fish play a major role in aquatic food webs, where they generally occupy an intermediate or higher position: they are not only fed upon by a variety of aquatic predators, but are also a major source of food for humans at world level. The species *C. carpio* is frequently used as a bioindicator, since cyprinids are quantitatively the most important group of teleost fishes cultured throughout the world for commercial purposes and are also very sensitive and easy to maintain organisms.

Biomarkers are measurable internal indicators of changes in organisms occurring at the molecular or cellular level, offering the possibility of understanding environmentally mediated disease (Valavanidis and Vlachogianni, 2010). Oxidative stress, one of the major mechanisms of action of toxicants, is among the most frequently used biomarkers since it is able to evaluate general damage induced on biomolecules such as lipids, proteins and DNA (Barata et al., 2005). Oxidative damage has been used in recent years as a biomarker for monitoring of environmental pollution (Valavanidis et al., 2006).

The present study aimed to evaluate the sublethal effects induced by captopril on brain, liver, kidney, gill and blood of *C. carpio* at three concentrations (1 μg L^{-1}, 1 mg L^{-1} and 100 mg L^{-1}), using oxidative stress biomarkers.

2. Materials and methods

2.1. Test specimens

Cyprinus carpio specimens were obtained from the carp culture facility in Tiacaque (State of Mexico) and transported to the Environmental Toxicology Laboratory at the Faculty of Chemistry (UAEMex). The characteristics of the organisms were: 21.3 ± 0.57 cm long and weighing 63.81 ± 5.7 g. These fish were bred and cultured at the facility, ensuring their being free of contaminants. During aquaculture, fish were not exposed to any metals. Concentrations of As, Cd, Cu, Cr, Hg, Ni, Pb and Zn were determined by atomic absorption spectrophotometry in water from the aquaculture center. None of these metals were detected in the water samples. Specimens used in assays were maintained in the laboratory and placed in 160-L aquaria filled with dechlorinated water and the necessary salts for maintenance of *C. carpio* for a 45-day acclimation period, and were fed Pedregal Silver™ fish food. The physicochemical characteristics of tap water reconstituted with salts during acclimation were: temperature 21 ± 1 °C, oxygen concentration 75–85%, pH 7.5–8.0, total alkalinity 18.1 ± 5.8 mg L^{-1}, total hardness 18.7 ± 0.6 mg L^{-1}. A natural light/dark photoperiod was maintained.

2.2. Experimental design

Captopril, [*N*-[(*S*)-3-Mercapto-2-methylpropionyl]-L-proline, ≥98% purity, was purchased from Sigma-Aldrich (St. Louis, MO)] in powder form for use in the present study. A 100 mg L^{-1} solution of captopril

in water was prepared, from which dilutions were made to obtain the 1 μg L^{-1} and 1 mg L^{-1} concentrations used in the sublethal toxicity assay. Test systems (10-L fish tanks with five carp each) were set up for each concentration (1 μg L^{-1}, 1 mg L^{-1}, 100 mg L^{-1}) and exposure time (12, 24, 48, 72 and 96 h) as well as one captopril-free system for each exposure time (also with five carp) which was used as the control group. The assay was performed in triplicate using a total of 300 fish. Test systems were static without renewal, in accordance with OECD (1992) guideline 203. At the end of each exposure time, specimens were removed from the systems and placed in a fish tank containing 50 mg L^{-1} of clove oil (Yamanaka et al., 2011) to anesthetize them prior to obtaining blood samples.

Blood was collected from the caudal vessel using a heparinized 1-mL hypodermic syringe, and 200 μL phosphate buffered saline (PBS) pH 7.4 was added per each 50 μL blood. Fish were weighed and measured at the end of the exposure period and no significant differences were found with respect to measurements taken during the acclimation period (Section 2.1). Specimens were ~~sacrificed~~ euthanized and entire organs (gill, liver, kidney and brain) were removed by dissection in an ice bath (to obtain the gills a cut was made in the operculum, then the fish was cut laterally from pectoral fin to anal fin to extract the other organs), weighed and homogenized in 1 mL PBS pH 7.4, and centrifuged at 12,500 rpm and −4 °C for 15 min. The supernatant was used to determine oxidative stress biomarkers. Antioxidant activity was estimated by determination of the activity of the enzymes SOD, catalase (CAT) and GPx. The following indicators of cellular oxidation were determined: hydroperoxide content (HPC), lipid peroxidation (LPX) and protein carbonyl content (PCC).

2.3. Antioxidant activity evaluation

2.3.1. SOD activity evaluation (method of Misra and Fridovich, 1972)

To 80 μL of supernatant in a 1-cm cuvette were added 520 μL carbonate buffer solution [50 mM sodium carbonate (Sigma-Aldrich), 0.1 mM EDTA (Vetec)] pH 10.2 plus 200 μL adrenaline (30 mM, Bayer). Absorbance was read at 480 nm, at 30 s and 5 min. SOD activity was determined by interpolating the data on a type curve. Results were calculated as IU SOD mg protein^{-1}.

2.3.2. CAT activity evaluation (method of Radi et al., 1991)

Supernatant (40 μL) was placed in a quartz cuvette and was added 2 mL isolation buffer solution [0.3 M sucrose (Vetec), 1 mM EDTA (Vetec), 5 mM HEPES (Sigma-Aldrich) and 5 mM KH_2PO_4 (Vetec)] plus 0.4 mL of an H_2O_2 solution (20 mM, Vetec). Absorbance was read at 240 nm, at 0 and 60 s. The absorbance values obtained for these times were substituted in the formula: CAT concentration $= [(A_0 - A_{60}) / MEC]$, where the MEC of H_2O_2 is 0.043 mM cm^{-1}, and results were expressed as μM H_2O_2 mg^{-1} protein.

2.3.3. GPx activity evaluation (method of Gunzler and Flohe-Clairborne, 1985 as modified by Stephensen et al., 2000)

To supernatant (100 μL) in a quartz cuvette was added 10 μL glutathione reductase [2 U glutathione reductase (Sigma-Aldrich)] plus 290 μL reaction buffer [50 mM K_2HPO_4 (Vetec), 50 mM KH_2PO_4 (pH 7.0, Vetec), 3.5 mM reduced glutathione (Sigma-Aldrich), 1 mM sodium azide (Sigma-Aldrich), 0.12 mM NADPH (Sigma-Aldrich)] and 100 μL H_2O_2 (0.8 mM, Vetec). Absorbance was read at 340 nm, at 0 and 60 s.

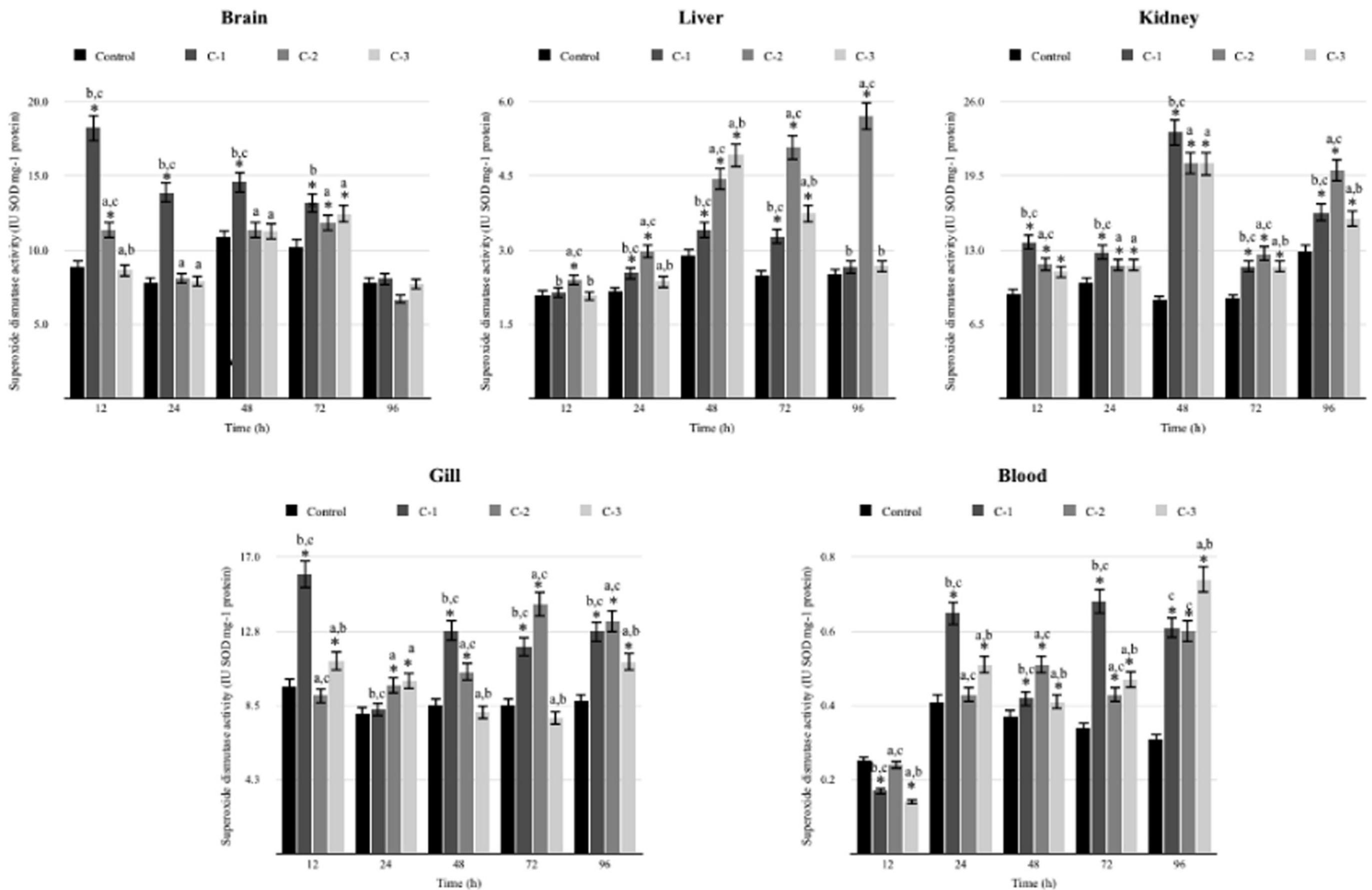

Fig. 1. Superoxide dismutase (SOD) activity in brain, liver, kidney, gill and blood of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to captopril concentrations of 1 μg L^{-1} (C-1), 1 mg L^{-1} (C-2) and 100 mg L^{-1} (C-3). Values are the mean of three replicates ± SEM for each concentration and exposure time. $N = 300$. * Significantly different ($p < 0.05$) from the control group. Lowercase letters indicate a significant difference relative to: [a]C-1, [b]C-2, [c]C-3, ANOVA and Tukey-Kramer.

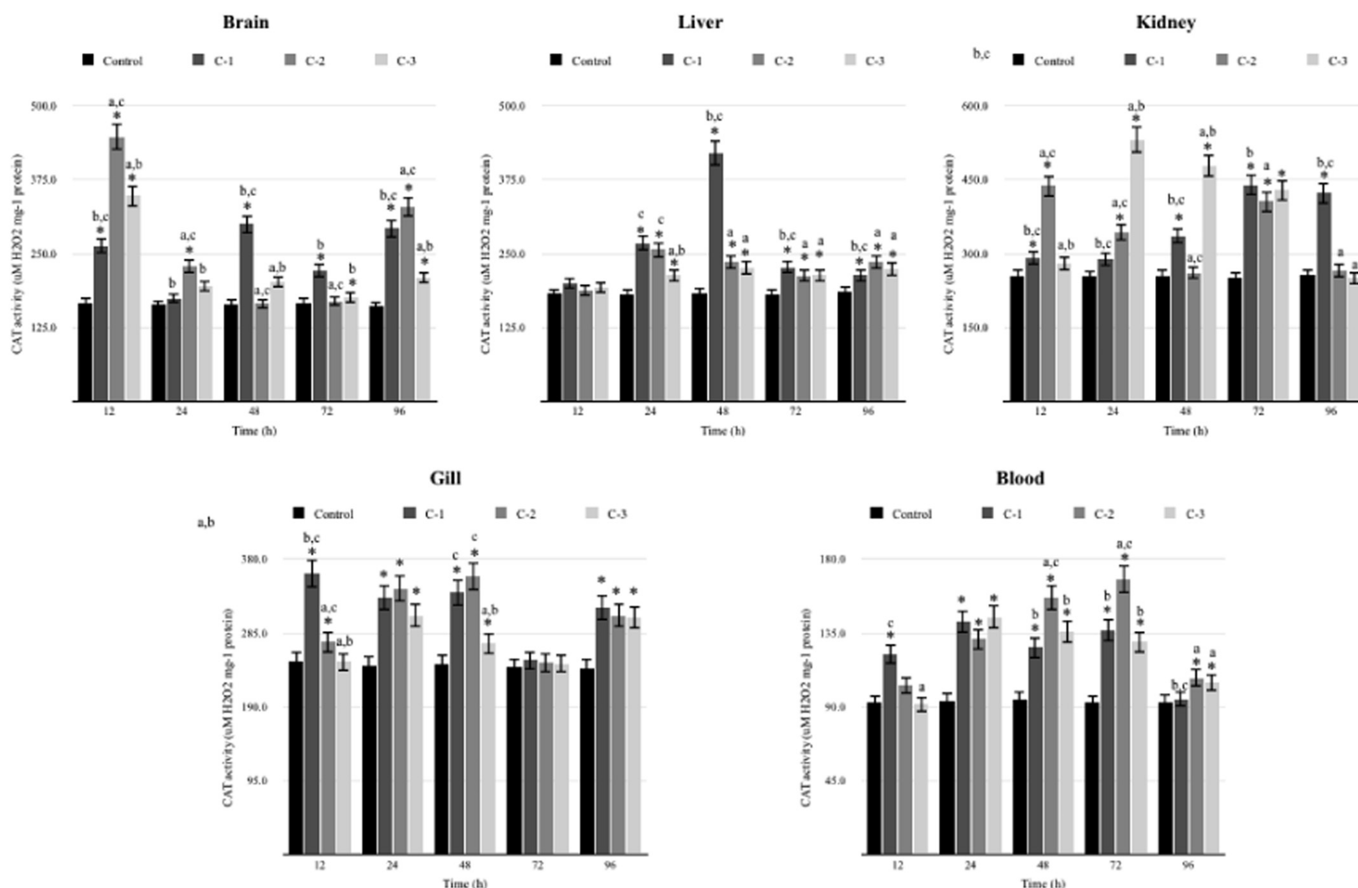

Fig. 2. Catalase (CAT) activity in brain, liver, kidney, gill and blood of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to captopril concentrations of 1 μg L^{-1} (C-1), 1 mg L^{-1} (C-2) and 100 mg L^{-1} (C-3). Values are the mean of three replicates ± SEM for each concentration and exposure time. $N = 300$. * Significantly different ($p < 0.05$) from the control group. Lowercase letters indicate a significant difference relative to: [a]C-1, [b]C-2, [c]C-3, ANOVA and Tukey-Kramer.

Results were obtained by using the equation: GPx concentration = $[(A_0 - A_{60}) / MEC]$, where the MEC of NADPH is 6.2 mM cm^{-1}, and were expressed as mM NADPH mg^{-1} protein.

2.3.4. Total protein content determination (method of Bradford, 1976)

To 50 μL of supernatant was added 150 μL distilled water and 2.5 mL Bradford's reagent. This solution was shaken in the vortex and allowed to rest for 5 min at ambient temperature while light protected. Absorbance was read at 595 nm. The results were interpolated on a bovine albumin curve. Total protein content was used to express the results of the biomarkers used.

2.4. Indicators of cellular oxidation

2.4.1. HPC evaluation (method of Jiang et al., 1992)

To 50 μL of sample [previously deproteinized with 10% trichloroacetic acid (TCA, Sigma-Aldrich)] was added 450 μL of reaction mixture [0.25 mM $FeSO_4$, 25 mM H_2SO_4, 0.1 mM xylenol orange and 4 mM butyl hydroxytoluene in 90% (*v*/v) methanol (all Sigma-Aldrich)]. The mixture was incubated for 60 min at ambient temperature, and absorbance was determined at 560 nm against a blank containing only reaction mixture. Results were interpolated on a type curve and expressed as nM cumene hydroperoxide (CHP) mg^{-1} protein.

2.4.2. Thiobarbituric acid reactive substances assay (TBARS, method of Büege and Aust, 1978)

To 100 μL of uncentrifuged sample was added Tris-HCl buffer solution (150 mM pH 7.4, Sigma-Aldrich) until a 1-mL volume was attained. This solution was incubated at 37 °C for 30 min, then supplemented with 2 mL TCA-TBA reagent [0.375% thiobarbituric acid in 15% TCA (both Sigma-Aldrich)]. The solution was heated for 15 min in a boiling water bath, following which the sample was centrifuged at 3000 rpm for 10 min, and absorbance was read at 535. Results were expressed as mM malondialdehyde (MDA) mg^{-1} protein, using the MEC of 1.56 $\times 10^5 M^{-1} cm^{-1}$.

2.4.3. PCC evaluation (method of Levine et al., 1994 as modified by Parvez and Raisuddin, 2005 and Burcham, 2007)

To supernatant (50 μL) was added 75 μL DNPH (10 mM, Sigma-Aldrich) in HCl (2 M, Sigma-Aldrich). This was incubated at ambient temperature for 1 h in the dark. Next, 250 μL of 20% TCA was added, and the sample was allowed to rest for 15 min at 4 °C. The precipitate was centrifuged at 16,000 rpm for 5 min. The bud was washed several times with 1:1 ethanol/ethyl acetate (Sigma-Aldrich), then dissolved in 1 mL guanidine solution (6 M, Sigma-Aldrich) pH 2.3, and incubated at 37 °C for 30 min. Absorbance was read at 366 nm. Results were expressed as μM reactive carbonyls (C = O) mg^{-1} protein, using the MEC of 21,000 M cm^{-1}.

2.5. Determination of captopril in water

2.5.1. Standards

The standard solution was a mixture of 550 mL methanol and 450 mL water which was added 0.05% of 85% phosphoric acid at pH 2.85 ± 0.05 (37:63 *v*/v), and was then filtered and degassed. A captopril standard solution (100 ppm) was prepared and stored at 4 °C. Solutions of 0.001, 0.1, 0.5, 1, 5, 10, 50, 75 and 100 ppm captopril were prepared to calibrate the equipment.

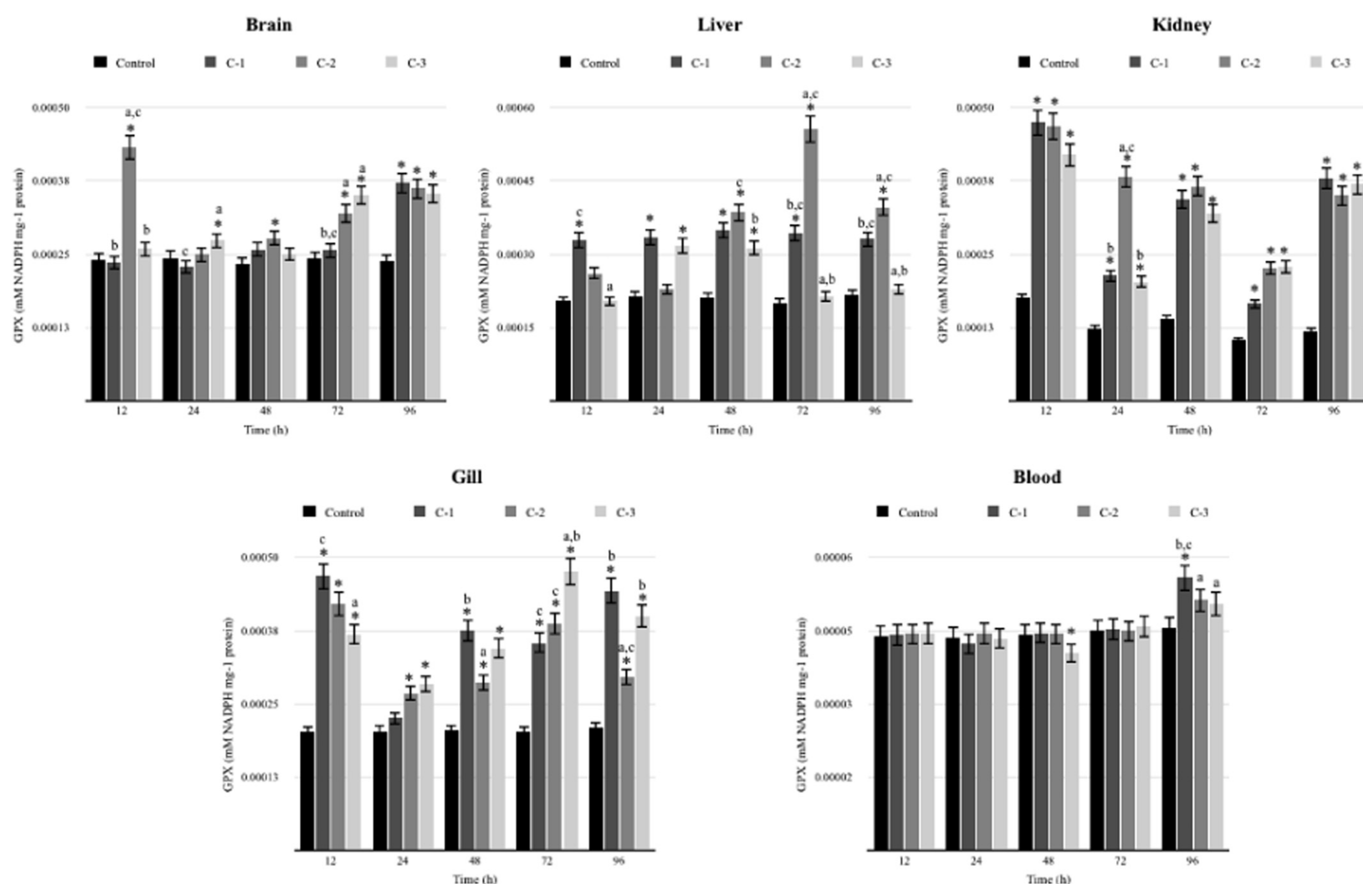

Fig. 3. Glutathione peroxidase (GPx) activity in brain, liver, kidney, gill and blood of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to captopril concentrations of 1 μg L^{-1} (C-1), 1 mg L^{-1} (C-2) and 100 mg L^{-1} (C-3). Values are the mean of three replicates ± SEM for each concentration and exposure time. $N = 300$. * Significantly different ($p < 0.05$) from the control group. Lowercase letters indicate a significant difference relative to: [a]C-1, [b]C-2, [c]C-3, ANOVA and Tukey-Kramer.

2.5.2. Equipment

Captopril was analyzed in a high-performance liquid chromatography (HPLC) system (Thermo Finnigan Surveyor LC Pump) with automated injector, UV–Vis PDA detector and surveyor autosampler. The stationary phase was analyzed in a Kromasil 5 μm C18 column (150 × 4.6 mm) at ambient temperature. The mobile phase was: 550 mL methanol plus 450 mL water which was added 0.05% of 85% phosphoric acid, pH adjusted to 2.85 ± 0.05 (37:63 *v*/v), and was then filtered and degassed. Operating conditions were: flow rate 1 mL min^{-1}, injection volume 100 μL, wavelength 220 nm. Chromatography was performed at ambient temperature. The detection limit was 0.01 μg mL^{-1} and the quantification limit was 0.076 μg mL^{-1}.

2.5.3. Calibration curves

The calibration curve for captopril was determined using standard solutions (0.001, 0.1, 0.5, 1, 5, 10, 50, 75 and 100 ppm), with a 55:45 mixture of methanol/water and phosphoric acid at pH 2.85 ± 0.05 ($R^2 = 0.9982$). Analyzed samples were prepared by dilution of the stock solution on the day of analysis. The mobile phase was passed at regular intervals to clean the detector.

2.5.4. Water samples from test systems

Water samples (2 mL) were collected in glass vials directly from the test systems at 12, 24, 48, 72 and 96 h, and refrigerated at 4 °C for subsequent determination of captopril concentrations. Volumes of 100 μL were injected in the HPLC system, which was operated as described above. The procedure was performed in triplicate. When analyzed samples fell outside the range of the type curve, dilutions were performed. Results were multiplied by the dilution factor and expressed as time-weighted mean concentrations of captopril.

2.6. Animal welfare

This protocol was reviewed and approved by the Bioethics Committee of the Universidad Autónoma del Estado de México (UAEM) to ensure that it was carried out in accordance with institutional standards for the care of animal subjects. Provisions in the official Mexican norm on breeding, care and use of laboratory animals (NOM-062-ZOO-1999) were also taken into account.

2.7. Statistical analysis

To evaluate sublethal toxicity assay results (oxidative stress biomarkers), after replication of data normality and homogeneity of variance (verified by Shapiro-Wilk and Levene's tests), results were examined by one-way analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparisons test with a 95% confidence limit to determine differences between means. Pearson's correlation analysis was performed to determine potential correlations between captopril concentrations at the different exposure times and oxidative stress biomarkers. Sigmastat v2.03 was used for all statistical calculations.

3. Results

3.1. Antioxidant activity

3.1.1. SOD activity

SOD activity results are shown in Fig. 1. A significant increase compared to the control group was observed with C-1 (1 μg L^{-1}) at 12, 24 and 48 h in brain; at 72 and 96 h in liver; at all exposure times in kidney; at 12, 48, 72 and 96 h in gill; and from 24 h up to 96 h in blood. With C-2

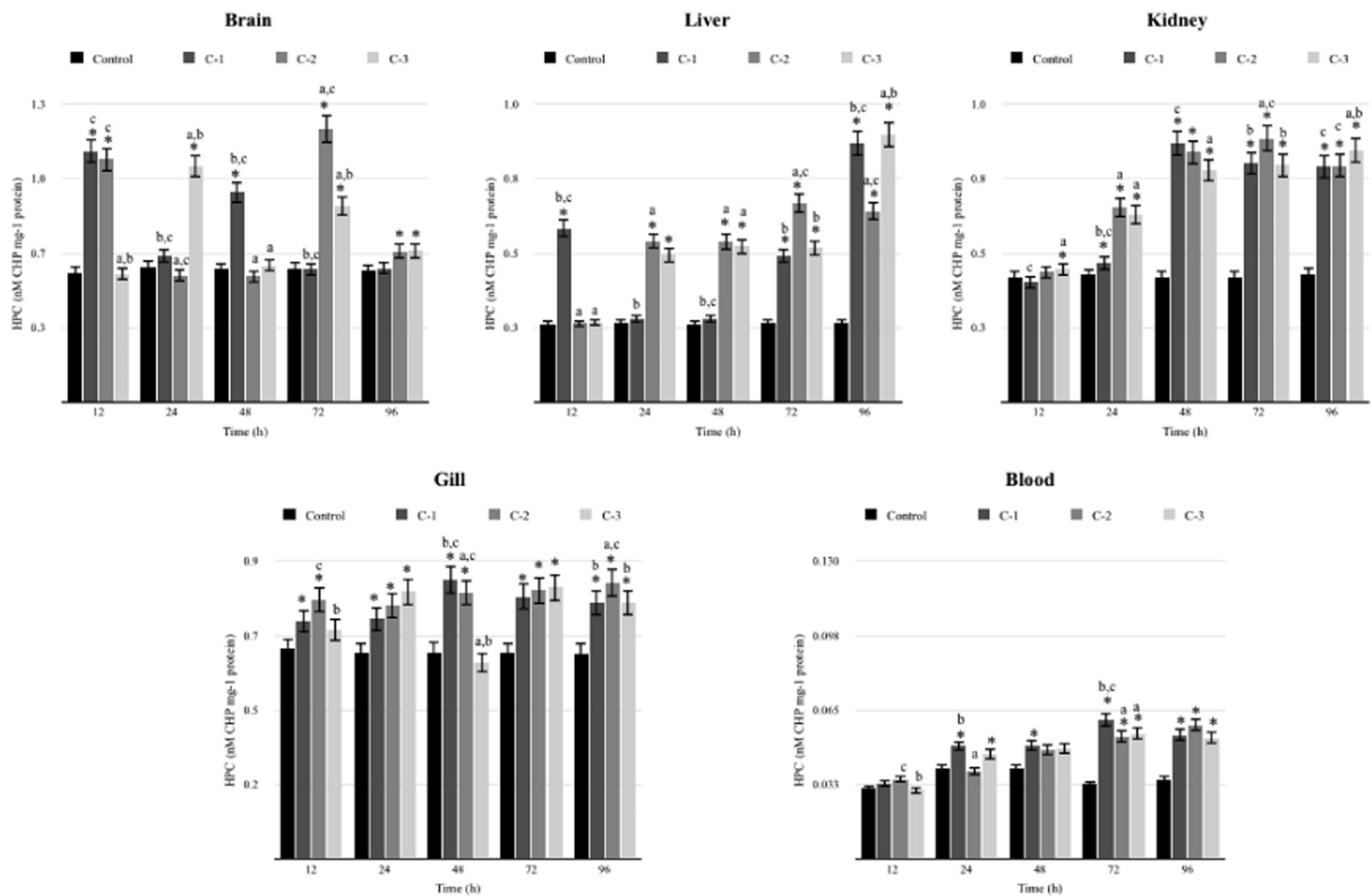

Fig. 4. Hydroperoxide content (HPC) in brain, liver, kidney, gill and blood of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to captopril concentrations of 1 μg L^{-1} (C-1), 1 mg L^{-1} (C-2) and 100 mg L^{-1} (C-3). Values are the mean of three replicates ± SEM for each concentration and exposure time. $N = 300$. CHP = cumene hydroperoxide. * Significantly different ($p < 0.05$) from the control group. Lowercase letters indicate a significant difference relative to: [a]C-1, [b]C-2, [c]C-3, ANOVA and Tukey-Kramer.

(1 mg L^{-1}), significant increases were found from 12 h to 72 h in brain; at 12, 48, 72 and 96 h in liver; from 24 h to 96 h in kidney; at 72 and 96 h in gill, and at 48 h in blood. With C-3 (100 mg L^{-1}), increases with respect to the control group occurred at 12, 24 and 72 h in brain; at 48 and 96 h in liver; from 48 h to 96 h in kidney; at 96 h in gill; and at 24, 72 and 96 h in blood.

3.1.2. CAT activity

Fig. 2 shows CAT activity results. Significant increases compared to the control group were observed with C-1 (1 μg L^{-1}) at 12, 48, 72 and 96 h in brain and kidney; from 24 h to 96 h in liver; at 12, 24, 48 and 96 h in gill; and from 12 h to 72 h in blood. With C-2 (1 mg L^{-1}), significant increases were found at 12, 24 and 96 h in brain; from 24 h to 96 h in liver and blood; at 12, 24 and 72 h in kidney; and at 12, 24, 48 and 96 h in gill. With C-3 (100 mg L^{-1}), significant increases occurred at 12, 72 and 96 h in brain; from 24 h to 96 h in liver and blood; from 24 h to 72 h in kidney; and at 24, 48 and 96 h in gill.

3.1.3. GPx activity

Results of GPx activity are shown in Fig. 3. Significant increases with respect to the control group were seen with C-1 (1 μg L^{-1}) at 96 h in brain and blood; at all exposure times in liver and kidney; and at 12, 48, 72 and 96 h in gill. With C-2 (1 mg L^{-1}), increases relative to the control group were observed at 12, 72 and 96 h in brain; at 48, 72 and 96 h in liver; and at all exposure times in kidney and gill, while in blood no modifications were found with respect to the control group. With C-3 (100 mg L^{-1}), increases were also seen at 24, 72 and 96 h in brain; at 24 and 48 h in liver; and at all exposure times in kidney and gill, while in blood a decrease was observed at 48 h.

3.2. Indicators of cellular oxidation

3.2.1. HPC

HPC results are shown in Fig. 4. Significant increases were seen in comparison to the control group with C-1 (1 μg L^{-1}) at 12 and 48 h in brain; at 12, 72 and 96 h in liver; at 24, 48, 72 and 96 h in kidney and blood; and at all exposure times in gill. With C-2 (1 mg L^{-1}), significant increases were found at 12 and 72 h in brain; at 24, 48, 72 and 96 h in liver and kidney; at all exposure times in gill; and at 72 and 96 h in blood. With C-3 (100 mg L^{-1}), significant increases occurred at 24, 72 and 96 h in brain and gill; from 24 h to 96 h in blood; and at all exposure times in liver and kidney.

3.2.2. LPX

LPX values are shown in Fig. 5. Significant increases compared to the control group were observed with C-1 (1 μg L^{-1}) at 12, 24 and 48 h in brain; at 48, 72 and 96 h in liver; at 12, 48, 72 and 96 h in kidney; and at all exposure times in gill, while no significant differences ($p > 0.05$) were found in blood. With C-2 (1 mg L^{-1}), significant increases were also observed at 12, 48, 72 and 96 h in brain; from 48 h to 96 h in liver; at all exposure times in kidney; and from 12 h to 72 h in gill, while no significant increases were found in blood. With C-3 (100 mg L^{-1}), significant increases occurred at 24, 72 and 96 h in

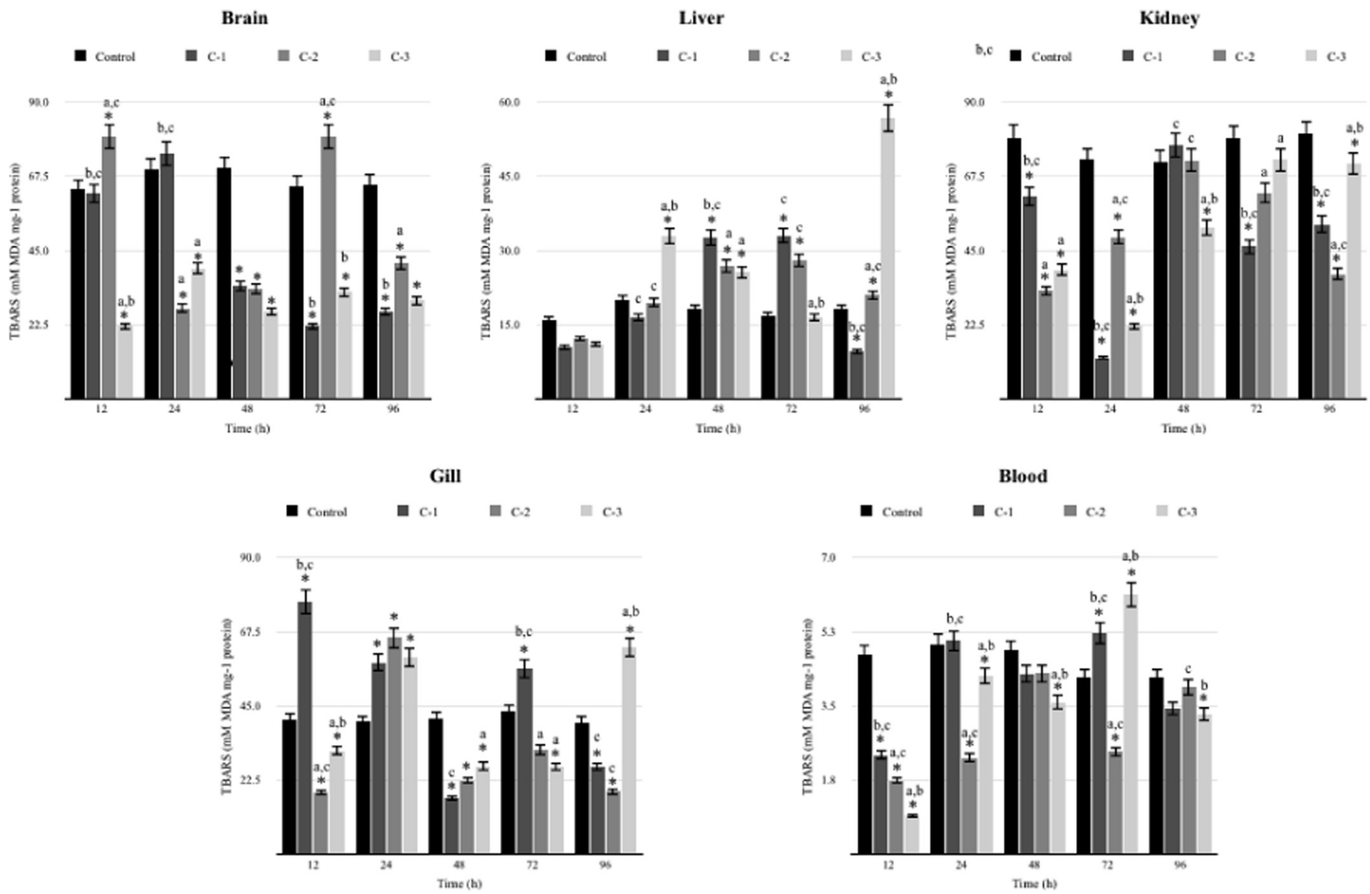

Fig. 5. Lipid peroxidation (LPX) in brain, liver, kidney, gill and blood of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to captopril concentrations of 1 μg L^{-1} (C-1), 1 mg L^{-1} (C-2) and 100 mg L^{-1} (C-3). Values are the mean of three replicates ± SEM for each concentration and exposure time. $N = 300$. MDA = malondialdehyde. * Significantly different ($p < 0.05$) from the control group. Lowercase letters indicate a significant difference relative to: [a]C-1, [b]C-2, [c]C-3, ANOVA and Tukey-Kramer.

brain and gill; at 24, 48 and 96 h in liver; at 12, 48, 72 and 96 h in kidney; and at 96 h in blood.

3.2.3. PCC

PCC values are shown in Fig. 6. Significant increases with respect to the control group were observed with C-1 (1 μg L^{-1}) at 24, 48 and 96 h in brain; at 48, 72 and 96 h in liver and blood; and at all exposure times in kidney and gill. With C-2 (1 mg L^{-1}), significant increases were observed at 12, 24, 72 and 96 h in brain; at 24, 48 and 96 h in liver; at all exposure times in kidney; from 12 h to 72 h in gill; and at 72 and 96 h in blood. With C-3 (100 mg L^{-1}), significant increases occurred at 12, 72 and 96 h in brain; at 24 and 96 h in liver; at all exposure times in kidney and gill; and at 72 and 96 h in blood.

3.2.4. Changes in oxidative stress biomarkers

Table 1 shows the percentage change in each oxidative stress biomarker in each organ at the different exposure times. Positive values indicate increases in the specific biomarker and negative values decreases with respect to the corresponding control group.

3.3. Captopril concentrations in test systems

Table 2 shows the captopril concentrations determined in water from test systems at the different exposure times. Fully 56–62% of the initial captopril concentration placed in the test systems had been lost by 12 h of exposure and this loss increased gradually thereafter reaching 86–89% by the end of the assay at 96 h.

3.4. Pearson's correlation analysis

Table 3 shows the correlations found between oxidative stress biomarkers assayed in the different organs, and exposure times and captopril concentration in water. Values in bold denote a greater degree of correlation between the variables analyzed. Enzymatic activity shows a greater degree of correlation with C-1 and C-3, suggesting the absence of concentration-dependent effects. Similarly, in indicators of cellular oxidation, there is also a greater degree of correlation with C-1 and C-3. As regards exposure times, enzymatic activity was more closely correlated with 12 and 96 h, while indicators of cellular oxidation correlated most with 12 h.

4. Discussion

Captopril, ~~is~~ an antihypertensive of high consumption worldwide, can enter aquatic systems through hospital, industrial and municipal wastewater discharge, eliciting sublethal effects on hydrobionts and having an ecological impact. To our knowledge, the present study is the first to evaluate captopril-induced oxidative stress in fish, and specifically in *C. carpio*. Currently, there are few studies in the international literature on the occurrence and sublethal effects of this medication, and existing ones focus on determination of the LC_{50} in crustaceans and algae. There are no studies on its mechanism of action or toxicokinetics. This limits our knowledge of the characterization of the sublethal effects of this pharmaceutical and its potential impact as an environmental pollutant. Therefore, it is of fundamental importance to conduct studies evaluating the effects of captopril on higher aquatic species such as fishes of commercial interest like *C. carpio*. The results of our study show

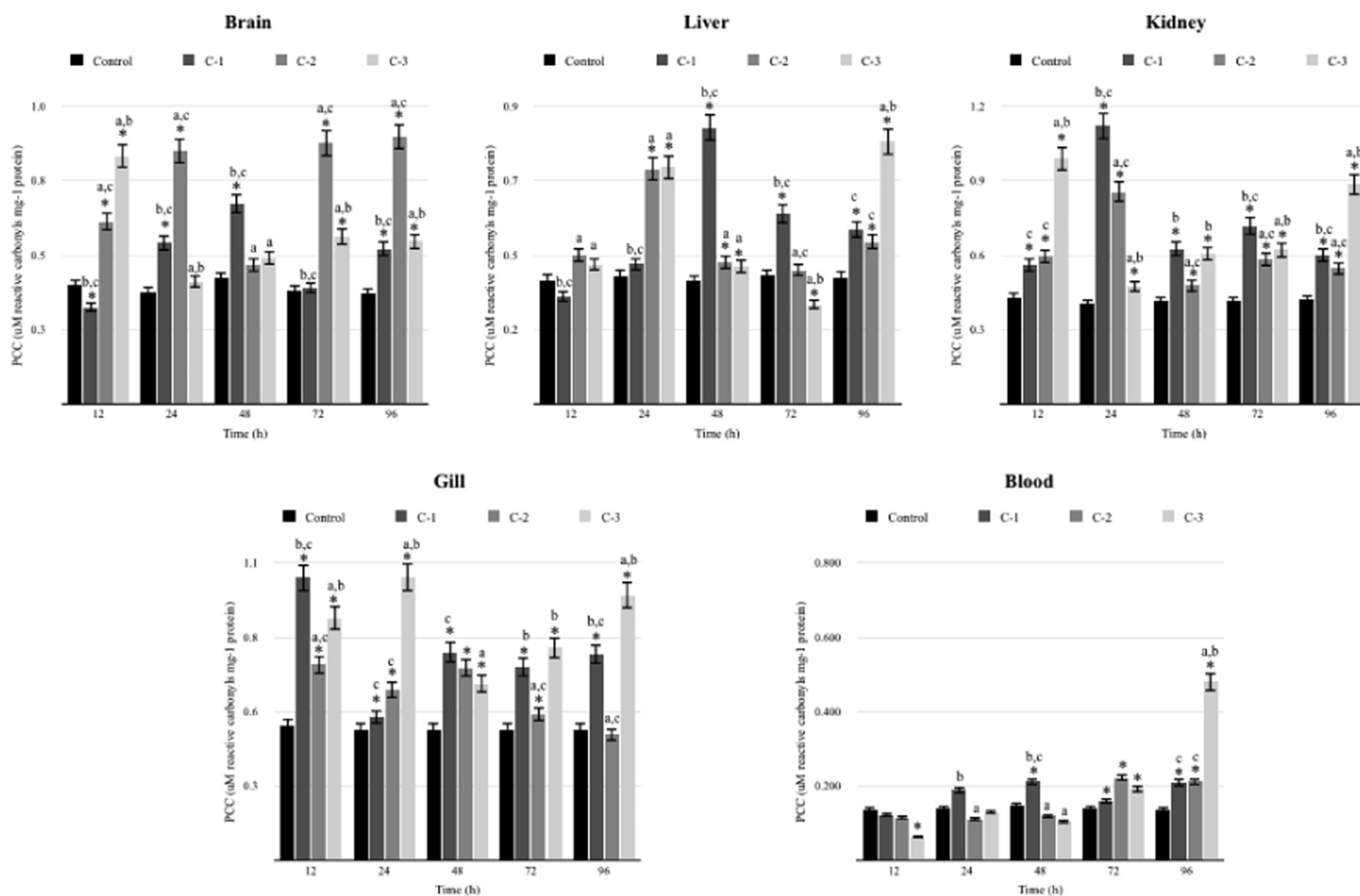

Fig. 6. Protein carbonyl content (PCC) in brain, liver, kidney, gill and blood of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to captopril concentrations of 1 μg L^{-1} (C-1), 1 mg L^{-1} (C-2) and 100 mg L^{-1} (C-3). Values are the mean of three replicates ± SEM for each concentration and exposure time. $N = 300$. * Significantly different ($p < 0.05$) from the control group. Lowercase letters indicate a significant difference relative to: [a]C-1, [b]C-2, [c]C-3, ANOVA and Tukey-Kramer.

that captopril induces oxidative stress on the common carp, affecting biomolecules such as lipids and proteins. This model may be useful in other fish species and may help determine the ecological impact of this compound in aquatic systems.

A large variety of pharmaceutical substances have been detected in diverse environmental compartments, particularly water bodies (Halling-Sørensen et al., 1998; Heberer, 2002; Heberer et al., 2002; Nikolaou et al., 2007; Jiang et al., 2011). Within this group of substances are some pharmaceutical products with cardiovascular action, and prominent among the latter are beta-blockers, captopril is one of them (Gros et al., 2007; Valcárcel et al., 2011). These products induce toxicity on zooplankton (in crustaceans such as *Daphnia magna*) and benthic organisms (Fent et al., 2006). As has been stated, there are few studies regarding the sublethal effects of captopril and its environmental occurrence, despite the fact that this pharmaceutical is extensively used globally due to its great efficacy as well as the elevated incidence of CVD.

Results of the present study are consistent with previous findings in mammals (De Cavanagh et al., 1995) since captopril induced significant increases in SOD, CAT and GPx activity. SOD activity increased in almost all organs evaluated at all exposure times and concentrations; kidney, liver and gill being the organs in which this effect was most evident. A similar behavior was seen with CAT and GPx activity, with important increases in the activity of both enzymes occurring also in brain. Increased SOD, CAT and GPx activity has also been observed in the presence of emerging contaminants (Islas-Flores et al., 2013, 2014; SanJuan-Reyes et al., 2013, 2015; Elizalde-Velázquez et al., 2016). Although the studies cited used medications from other pharmaceutical classes, they all have in common that most of the pharmaceuticals undergo cytochrome P450 (CYP)-mediated phase I biotransformation, in the process of which ROS are produced that are responsible for induction of antioxidant enzymes and increases in indicators of cellular oxidation. It should be noted that the genome of *C. carpio* has been sequenced and CYP is known to be present in the fish (Stegeman and Livingstone, 1998).

The enzymes evaluated in our study constitute the principal scavenging mechanisms of the main species of ROS produced by cellular activity and are therefore the first line of defense against oxidative stress. The enzyme SOD catalyzes the dismutation of superoxide anion (O_2^*) to hydrogen peroxide (H_2O_2), which is converted to O_2 and water by CAT and GPx (Van der Oost et al., 2003). Although several *in vitro* studies refer to captopril as a ROS scavenger (Egan et al., 1988), not all studies support this idea (Kukreja et al., 1990).

Increased production of ROS implies not only an increase in the levels of antioxidant enzymes, it can also induce cellular damage through oxidation of important biomolecules such as lipids and proteins, resulting in higher levels of LPX and oxidized proteins (Shacter, 2000; Gómez-Oliván et al., 2014).

The LPX process involves a series of chain reactions in which prooxidant agents, such as free radicals or non-radical species with a high oxidant capacity, attack lipids that have carbon-carbon double bonds, in particular polyunsaturated fatty acids. This implies the removal of one hydrogen atom from a CH_3 group, with oxygen interstation, resulting in peroxyl and hydroperoxide radicals. In turn, hydroperoxides readily break down to diverse chemical species such as lipid alkoxy radicals, aldehydes (MDA), alkenes (4-hydroxynonenal), lipid epoxides and alcohols, most of which are toxic (Porter et al., 1995; Yin et al., 2011; Ayala et al., 2014). Among the substrates of these products are DNA and proteins that are especially sensitive to oxidation by these compounds.

Table 1
Percentage change in oxidative stress biomarkers, induced by exposure to captopril.

ORGAN	ASSAY	SOD			CAT			GPX			HPX			LPX			PCC		
	Time (h)	C-1 (%)	C-2 (%)	C-3 (%)	C-1 (%)	C-2 (%)	C-3 (%)	C-1 (%)	C-2 (%)	C-3 (%)	C-1 (%)	C-2 (%)	C-3 (%)	C-1 (%)	C-2 (%)	C-3 (%)	C-1 (%)	C-2 (%)	C-3 (%)
Brain	12	105.3	27.8	−2.7	−109.7	−204.2	−1801.7	782.3	−813	29,981.9	−3787.7	−584.2	−67.6	−88.4	−97.7	−100.3	2.7	−103.1	52.5
	24	77.4	3.2	1.1	−64	−182.7	−861.2	371.4	−680.3	−59,287.6	8614.7	2219.5	−357.7	−116.1	−101.3	−99.8	−1.5	−98.7	−72.4
	48	34.3	4.9	4	−18.9	−155	−423	172.8	−1016	−25,737.1	2433.1	1307.7	−409.2	−131.3	−105.4	−99.6	−5.5	−95.8	−76.6
	72	29	15.7	21.6	37.3	28.4	−60.6	−313.7	−941.7	−4459	373.5	−219.1	261.5	−219.4	−158.7	−96.4	−39.2	−82.1	−131.4
	96	3.3	−14.1	−0.6	−95.5	−2963.6	−3187.1	7.5	−107.9	16,732.4	−15,607.3	−207,069	6397.1	−103.1	−99.3	−100.6	1.3	−101.2	−101.6
Liver	12	2.4	14.8	−0.5	−103.2	−4414.8	−36,890.3	735.6	−812.6	169,735.7	−20,987.7	−2953.1	−92	−96.9	−99.5	−100.1	0.5	−100.5	9.3
	24	17.6	37.5	9.3	−75.3	−528.1	−2300.3	335.6	−545.6	−5992.9	998.3	197.5	−108.6	−155	−115.5	−98.1	−15.1	−90.3	−16.9
	48	18	53.6	70.2	31	72.1	50.2	−30.3	−198	−381.9	92.9	−406.1	−908.4	123.7	33.2	−108.7	−427.2	−445.3	−51
	72	31.3	103.6	50.6	−51.2	−263.3	−465.7	76.9	−250.2	−594.5	137.6	79	−117	−248.1	−280.3	−52.9	−81.1	−67.3	−42.5
	96	6	127.5	6.8	−94.7	−1684.4	−1854.6	10.1	−110.7	−1734	1466.8	14,418.2	−877.4	−106.1	−107.2	−93.8	−12.5	−88.2	−89.9
Kidney	12	49.2	28.4	21.5	−24.2	−149.3	−1343.9	800.3	−3402.9	−15,905.5	367.4	−54.1	−96	77.4	−78.9	−99.5	26.1	−66.3	−30.9
	24	26.8	15.4	15.7	1.9	−92.8	−486.7	424.4	21,969	139,727.8	536	26.3	−105.4	−500.8	−193.4	−100.1	−48.2	−90.4	−14.3
	48	169.4	138.3	137.8	−0.3	−100.2	−308.7	208.1	−62,332.7	−45,333.1	−27.3	−113.1	−63.4	−44	61.2	−100.1	−263.5	499.2	−887.9
	72	32.3	45.3	32.3	−28.8	−189.2	−362.8	91.7	−418.7	−1397.6	233.8	154.9	−142.7	−192.1	−182.2	−87	−52.3	−72.8	−49
	96	26.9	55.2	22.6	−59	−319	−432.2	35.5	−160.2	−807.6	404	1037.4	−340	−132.8	−132.9	−83.5	−37.1	−72	−78.8
Gill	12	67.3	−5.3	15.6	−392.2	−682.8	−5789.6	748	−290.7	−1967.3	576.7	−22.9	−99.6	334.9	−41.9	−97.9	133.4	−60.2	−39.6
	24	2.7	19.9	23.2	16.9	518.2	2059.3	297.4	1662.2	7064.3	325	9.3	−99.5	−1171.4	−460.4	−106.5	−76.9	−93.4	−6.1
	48	50.4	22.7	−4.8	−121.2	−340.5	−809.4	137.7	−213.6	4333	−2128.8	−1645.9	103.4	−106.3	−95	−102.2	7.6	−107.1	−203.6
	72	39.7	68.7	−8.4	−112.2	−382.6	−631.4	65	−158	1788.9	−1232.5	−1995.4	216	−110.8	−91	−105.1	15.5	−114	−152.8
	96	45.3	52.2	25.6	−51	−212.5	−321.4	51.2	−200.5	−884	341	565.6	−276	−148.8	−143.6	−83.8	−41.7	−72	−73.9
Blood	12	−32	−4	−44	1000	−3225	−26,975	736.4	−26.4	−40.1	52.1	−92.9	−99.7	7.2	−86.1	114.7	−233.2	−3317.7	3229.1
	24	58.5	4.9	24.4	400	583.3	2330.6	299.5	−25.1	−203	708.1	136.4	−94.1	−169	−123.9	−39	−68.5	−59.5	−36.9
	48	13.5	37.8	10.8	−71.4	−628.6	−1409.5	124.2	−273.9	−2633.9	861.5	593.4	−142.1	−123.9	−114.4	−95.7	−16.4	−86.8	−38.9
	72	100	26.5	38.2	44.4	−55.6	−177.2	218.9	392.5	926.5	136.1	−37.8	−78.6	107.8	−20.8	−102.2	392.6	264.1	−435.9
	96	96.8	93.5	138.7	48.3	−50.1	−152.2	203.7	322	132.1	−59	−128.9	−15.3	−88.2	49.5	−62.5	−226.4	156.8	−1126.1

(−) Decrease of the specific biomarker. SOD = superoxide dismutase activity, CAT = catalase activity, GPx = glutathione peroxidase activity, HPX = hydroperoxide content, LPX = lipid peroxidation, PCC = protein carbonyl content.

Table 2
Captopril concentrations in water from the test systems at the different exposure times.

	Initial concentration	Time	Final concentration (mg L^{-1})	% Captopril loss
Captopril	1 μg L^{-1}	12	0.3849 ± 0.0002 μg L^{-1}	61.51
		24	0.3512 ± 0.0001 μg L^{-1}	64.88
		48	0.3162 ± 0.0001 μg L^{-1}	68.38
		72	0.2236 ± 0.0001 μg L^{-1}	77.64
		96	0.1127 ± 0.0001 μg L^{-1}	88.73
	1 mg L^{-1}	12	0.4351 ± 0.0001 mg L^{-1}	56.49
		24	0.3602 ± 0.0001 mg L^{-1}	63.98
		72	0.2186 ± 0.0001 mg L^{-1}	78.14
		96	0.1368 ± 0.0001 mg L^{-1}	86.32
	100 mg L^{-1}	12	41.534 ± 0.01 mg L^{-1}	58.47
		24	34.046 ± 0.01 mg L^{-1}	65.96
		48	33.742 ± 0.01 mg L^{-1}	66.26
		72	20.844 ± 0.01 mg L^{-1}	73.16
		96	11.656 ± 0.01 mg L^{-1}	88.35

In particular, MDA and 4-hydroxynonenal can form adducts that play a major role in many cell processes and may be involved in secondary deleterious reactions by promoting intra- or intermolecular protein-DNA crosslinks that can induce profound alterations in the biochemical properties of biomolecules, facilitating the development of diverse pathological conditions (Ayala et al., 2014).

Our results show that captopril exposure induced overall increases in LPX and HPC at all three concentrations in all organs evaluated. This may be due to an increment in the release of free radicals formed during captopril biotransformation in the fish by CYP activity.

In general, the biotransformation reactions of pharmaceuticals such as captopril, in which isoenzymes such as CYP participate, produce more polar and hydrosoluble inactive metabolites that are readily eliminated. However, in some cases, the metabolites produced have higher levels of biological activity and consequently toxic properties which can induce oxidative stress.

Table 3
Pearson's correlation analysis between oxidative stress biomarkers, and captopril concentrations and exposure times.

Antioxidant activity					
Biomarkers	Organs	Time (h)	Concentration of captopril in water		
			C-1	C-2	C-3
SOD	BRAIN	12	−0.413	**0.988**	**0.968**
		24	**0.799**	**0.970**	**0.996**
		48	**0.876**	**0.932**	**0.900**
		72	**0.965**	**0.981**	**0.975**
		96	**0.734**	**0.853**	**0.996**
	LIVER	12	**0.987**	**0.851**	**0.919**
		24	**0.970**	**0.847**	**0.944**
		48	**0.836**	**0.789**	**0.900**
		72	**0.709**	**0.760**	**0.945**
		96	**0.922**	**0.873**	**0.901**
	KIDNEY	12	**0.757**	**0.993**	**0.950**
		24	**0.908**	**0.988**	**0.973**
		48	**0.888**	**0.949**	**0.899**
		72	**0.871**	**0.964**	**0.983**
		96	**0.964**	**0.798**	**0.812**
	GILL	12	**0.676**	**0.910**	**0.901**
		24	**0.783**	**0.501**	**0.844**
		48	**0.998**	**0.840**	**0.971**
		72	**0.868**	**0.922**	**0.843**
		96	**0.946**	**0.872**	**0.880**
	BLOOD	12	**0.969**	**0.993**	**0.996**
		24	**0.933**	**0.930**	**0.964**
		48	**0.831**	**0.600**	**0.742**
		72	**0.812**	**0.726**	**0.871**
		96	**0.791**	**0.894**	−0.066
CAT	BRAIN	12	−0.431	−0.401	0.375
		24	0.108	0.388	−0.210
		48	0.285	−0.477	0.296
		72	−0.397	−0.361	−0.450
		96	−0.382	0.397	0.040
	LIVER	12	−0.480	0.270	0.142
		24	0.146	0.389	0.409
		48	0.300	−0.114	0.239
		72	0.374	−0.347	0.412
		96	−0.282	0.242	−0.346
	KIDNEY	12	0.395	−0.327	−0.360
		24	0.406	−0.411	0.276
		48	−0.440	−0.442	0.186
		72	−0.372	0.348	−0.195
		96	−0.435	−0.429	−0.123
	GILL	12	−0.147	−0.465	−0.087
		24	0.303	−0.428	0.413
		48	0.010	−0.346	0.162
		72	0.368	0.405	0.414
		96	−0.343	−0.355	0.278
	BLOOD	12	−0.350	−0.172	0.297
		24	−0.164	−0.325	−0.035
		48	−0.407	−0.425	−0.360
		72	0.395	−0.336	0.266
		96	0.390	−0.454	−0.359

Oxidative damage					
Biomarkers	Organs	Time (h)	Concentration of captopril in water		
			C-1	C-2	C-3
HPX	BRAIN	12	**0.865**	**0.959**	−0.361
		24	0.497	0.374	**−0.803**
		48	**−0.621**	−0.367	**−0.784**
		72	0.312	**0.956**	0.498
		96	**−0.681**	**−0.561**	0.276
	LIVER	12	**−1.000**	**−0.586**	**0.575**
		24	**0.931**	**0.636**	−0.407
		48	**−0.978**	**0.559**	−0.009
		72	0.140	**0.751**	**−0.817**
		96	−0.133	0.218	0.213
	KIDNEY	12	**0.897**	0.218	**0.618**
		24	**0.870**	**−0.813**	−0.487
		48	**0.987**	−0.134	0.496
		72	0.199	−0.146	0.484
		96	**0.911**	**0.873**	−0.056
	GILL	12	**−0.765**	**−0.736**	**−0.921**
		24	−0.097	**0.714**	**0.833**
		48	−0.545	−0.152	**0.869**
		72	0.431	−0.006	**−0.986**
		96	**−0.926**	−0.029	**0.855**
	BLOOD	12	0.352	−0.182	**0.770**
		24	**−0.960**	0.127	**−0.904**
		48	−0.281	−0.400	**0.652**
		72	**0.931**	**0.822**	**0.877**
		96	**0.869**	−0.433	−0.031
TBARS	BRAIN	12	**−0.576**	−0.033	0.320
		24	**0.552**	**0.523**	−0.394
		48	**0.500**	**0.552**	−0.337
		72	0.010	−0.520	−0.465
		96	**0.506**	−0.462	−0.349
	LIVER	12	**0.562**	**0.556**	−0.003
		24	−0.012	0.138	−0.447
		48	0.199	−0.012	**0.575**
		72	**0.576**	−0.339	0.159
		96	−0.332	**0.567**	**−0.530**
	KIDNEY	12	**0.577**	**0.574**	−0.447
		24	−0.351	**−0.504**	0.520
		48	**−0.558**	0.408	0.461
		72	**0.568**	**0.562**	**−0.550**
		96	**0.570**	−0.190	−0.047
	GILL	12	**0.566**	−0.360	0.553
		24	0.311	**0.545**	**0.570**
		48	−0.315	0.444	0.377
		72	**−0.508**	−0.272	**0.574**
		96	**−0.549**	−0.174	0.408
	BLOOD	12	**0.520**	−0.461	−0.139
		24	−0.398	0.436	−0.410
		48	−0.061	−0.439	**−0.588**
		72	**0.572**	0.036	0.172
		96	−0.419	**0.940**	−0.276

Table 3 (*continued*)

Antioxidant activity						Oxidative damage					
Biomarkers	Organs	Time (h)	Concentration of captopril in water			Biomarkers	Organs	Time (h)	Concentration of captopril in water		
			C-1	C-2	C-3				C-1	C-2	C-3
GPX	BRAIN	12	0.211	0.211	0.427	**PCC**	BRAIN	12	**0.828**	**0.935**	**0.690**
		24	0.211	0.211	0.427			24	**0.876**	**0.870**	0.106
		48	0.211	0.211	0.427			48	**0.728**	**0.897**	**0.961**
		72	0.211	0.211	0.427			72	**0.984**	0.120	**0.714**
		96	0.211	0.211	0.427			96	**0.827**	**0.713**	**0.991**
	LIVER	12	0.211	0.211	0.427		LIVER	12	**0.958**	**0.777**	**0.673**
		24	0.211	0.211	0.427			24	**0.881**	**0.545**	**0.772**
		48	0.211	0.211	0.427			48	**0.904**	0.327	**0.944**
		72	0.211	0.211	0.427			72	**0.898**	**0.560**	0.467
		96	0.211	0.211	0.427			96	**0.929**	**0.841**	**0.826**
	KIDNEY	12	0.211	0.211	0.427		KIDNEY	12	**0.784**	**0.536**	**0.961**
		24	0.211	0.211	0.427			24	**0.798**	**0.528**	**0.734**
		48	0.211	0.211	0.427			48	**0.845**	**0.997**	**0.912**
		72	0.211	0.211	0.427			72	**0.943**	**0.842**	**0.914**
		96	0.211	0.211	0.427			96	**0.962**	**0.769**	**0.972**
	GILL	12	0.211	0.211	0.427		GILL	12	**0.867**	**0.783**	**0.709**
		24	0.211	0.211	0.427			24	**0.921**	**0.929**	0.371
		48	0.211	0.211	0.427			48	**0.832**	**0.767**	0.419
		72	0.211	0.211	0.427			72	**0.698**	**0.991**	**0.841**
		96	0.211	0.211	0.427			96	**0.790**	**0.827**	**0.877**
	BLOOD	12	**0.781**	**0.781**	**0.570**		BLOOD	12	**0.983**	**0.999**	**0.997**
		24	**0.781**	**0.781**	**0.570**			24	**0.959**	**0.999**	**1.000**
		48	**0.781**	**0.781**	**0.570**			48	**0.959**	**0.973**	**0.974**
		72	**0.781**	**0.781**	**0.570**			72	**0.896**	**0.552**	**0.873**
		96	**0.781**	**0.781**	**0.570**			96	**0.818**	**0.933**	**−0.673**

Correlation coefficients >0.5 are significant (shown in bold).

Antihypertensive medications are metabolized mainly by enzymes of the P450 family that are present in different body tissues such as the kidneys, lungs, skin, gut, adrenal cortex, testicles, placenta and others, but are particularly active in the liver. Different P450 families have been characterized in fishes such as *C. carpio* including CYP1, CYP2, CYP3, CYP4, CYP11, CYP17 and CYP19 (Stegeman and Livingstone, 1998). Captopril is metabolized by the enzyme CYP2D6.

In the biotransformation of captopril by the CYP2D6 family, ROS are formed. These ROS can be *OH or oxygenated intermediates, such as the oxy-cytochrome P450 complex [P450 (Fe^{3+}) O^2] resulting from release of the superoxide anion by reaction decoupling. In both cases, ROS production is increased, which may explain the LPX and HPC values obtained in the present study.

Protein damage is induced through oxidation of the side chains of amino acids; many ROS species can oxidize protein sulfhydryl groups. In the present study, PCC increases were observed in both liver and gill at all exposure times, as well as damage induced by all concentrations at 96 h in brain and blood. Blood transports proteins and other molecules to tissues, while the brain contains a large number of proteins essential for brain activity and is sensitive to oxidative damage.

These results may be explained by the presence of ROS and reactive nitrogen species (RNS) — *OH and $ONOO^-$ — formed in organs such as the liver, kidneys and gills during captopril biotransformation and in arginine metabolism, respectively. In particular, $ONOO^-$ is highly reactive and has a high affinity for binding to protein sulfhydryl groups, oxidizing the protein.

ROS and RNS can remove protons from methylene groups in amino acids, leading to formation of carbonyls which have a tendency to ligate protein amines, also damaging nucleophilic centers and inducing sulfhydryl group oxidation, disulfide reduction, peptide fragmentation, modification of prosthetic groups and protein nitration. These modifications lead to loss of protein function (Cabiscol et al., 2010; Van der Oost et al., 2003; Glusczak et al., 2007) and therefore also of body integrity (Parvez and Raisuddin, 2005).

Our results indicate that in most of the organs evaluated and at the different exposure times, captopril showed no concentration-dependent effects, since in most cases the lowest concentration induced higher increases in antioxidant enzymes. In the case of indicators of cellular oxidation, the two lowest captopril concentrations assayed induced greater change. Although diverse studies (*i.e.* Cleuvers, 2003, 2005; Nalecz-Jawecki and Sawicki, 2003; Nalecz-Jawecki and Persoone, 2006; Park, 2005; Cunningham et al., 2006; Kaza et al., 2007; NOAA, 2016) state that acute toxicity measured by LC_{50} in mostly crustaceans and algae is >100 mg L^{-1}, our study shows that fish species such as *C. carpio* are more sensitive to low concentrations of this contaminant. This makes *C. carpio* a good candidate for use in evaluating other biomarkers of exposure to captopril as well as the ecological impact of this pharmaceutical in aquatic systems.

In our study, captopril concentrations in water from the test systems decreased from 12 h on (Table 1) at the two higher concentrations. These reductions accounted for >70% of the initial concentrations after 96 h. It is worth noting that the HPLC detection limit did not permit determination in test systems where the lowest concentration (1 μg L^{-1}) was used. Captopril loss in water from the test systems leads us to assume that there was biotransformation of the compound in fish or else that it underwent photodegradation in the system with possible presence of metabolites such as captopril disulfide, captopril sulfonic acid, *S*-methyl captopril or captopril disulfide S-dioxide (Mahmoud and Kümmerer, 2012) that were not detected in our study. Further studies are needed to confirm the formation of captopril metabolites and degradation products in test systems using *C. carpio*.

The present study shows that common carp and oxidative stress are respectively an excellent bioindicator and biomarker for assessment of the risk associated with captopril presence in aquatic systems even at very low concentrations.

5. Conclusion

Captopril induced oxidative stress on *C. carpio*, inducing damage on lipids mainly in kidney and gill, while protein oxidation occurred in all organs evaluated except kidney. Antioxidant enzyme activity increased in all organs, particularly liver, kidney and gill. The biomarkers used clearly evidence the risk posed by this emerging contaminant to teleost fishes such as *C. carpio*.

Conflict of interest

The authors declare they have no actual or potential competing financial interests.

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