Ecotoxicity and genotoxicity assessment of losartan after UV/H$_2$O$_2$ and UVC/photolysis treatments

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Abstract

Losartan potassium (LOS) is one of the most antihypertensives used in the world, and its presence in environmental matrices can cause impacts to biota. In this study, the ecotoxicity and genotoxicity of LOS was assessed before and after treatment by UVC/ photolysis and UV/H$_2$O$_2$. The photodegradations were carried out at LOS solutions (2.5 mg L^{-1}; 4.6 µM) for 30, 60, 90, 120, 240, and 480 min of treatment. For chromatographic analysis, the samples were submitted to solid-phase extraction (SPE) and analyzed by HPLC-DAD. Ecotoxicity bioassays were conducted using *Daphnia magna* (acute) and *Desmodesmus subspicatus* (chronic) for all the degradation times. To evaluate the genotoxicity, the comet assay was performed with a *D. magna* whole organism cell suspension applying the alkaline gel electrophoresis technique. For both process, the degradation rate was over 99% at 30 min, which reduced the acute toxicity of LOS to *D. magna*. In addition, only the sample treated at 240 min by UV/ H$_2$O$_2$ showed significant chronic and acute toxicity. However, the genotoxicity effect was observed for samples treated LOS before treatment and at 480 min by UV/H$_2$O$_2$. Therefore, even reaching high LOS degradation rates, for both processes, the bioassays demonstrated the importance of ecotoxicological analyses by AOPs treatment.

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Introduction

According to the World Health Organization (WHO), it is estimated that in 2020 about 1.5 billion people will suffer from hypertension in the world (Kaur and Dulova 2020). Among the most consumed antihypertensive drugs, losartan potassium (LOS) is one of the pharmaceutics listed as essential (WHO 2019) classified as vasodilator and angiotensin II receptor blockers (ARBs). Its consumption has doubled in the USA in recent years and is the main hypertensive drug used in Brazil in the public health system (de Andrade et al. 2020). In addition, as an antihypertensive, LOS has been studied as a possible treatment for COVID-19, which could lead to an even greater consumption of the drug around the world (Altay et al. 2020; Khan et al. 2020). Once consumed, LOS is only partially metabolized, and about 4% of the drug is excreted as the parent compound (Al-Majed et al. 2015; Wielens Becker et al. 2020). This means a considerable

supply of LOS and its metabolites in effluents and consequently in the environment.

Several sources of pharmaceutics in the environment have been reported, including wastewater treatment plants (WWTPs), landfills, hospitals effluents, and pharmaceutical manufacturing facilities (PMFs) (Scott et al. 2018). LOS has been detected in several environmental matrices, usually in the range of ng L^{-1} to ug L^{-1} (Kot-Wasik et al. 2016; Botero-Coy et al. 2018; Cortez et al. 2018; Wang et al. 2018; Castiglioni et al. 2020; Golovko et al. 2020). However, Larsson et al. (2007) detected LOS in extremely higher concentrations (2.4-2.5 mg L^{-1}) in an effluent from a pharmaceutical manufacturing facility. As consequence, it is classified as hazardous to the aquatic environment. In addition, due its high stability to hydrolysis band biodegradation, LOS has been considered for monitoring micropollutants by environmental agencies in several EU countries (de Andrade et al. 2020; Kosek et al. 2020).

Since LOS is present in the environment, it is crucial to investigate its toxic effects on non-target organisms, especially chronic ones. Previous studies have reported toxic effects of LOS to different organisms, such as microcrustaceans, fish and microalgae (FDA 2002), macrophytes (Godoy et al. 2015), and marine invertebrates (Yamamoto et al. 2014; Cortez et al. 2018). However, studies that evaluate sublethal effects of LOS are still scarce.

Since conventional processes were not designed to total removal of micropollutants, such as pharmaceuticals (Kosek et al. 2020), several technologies have been used for degradation and/or removal LOS in waters and effluents. In this context, advanced oxidation processes (AOPs) such as UVC/ photolysis and UV/H$_2$O$_2$ (Starling et al. 2019; Kaur and Dulova 2020), UV/Fe^{2+}- activated persulfate (Kaur and Dulova 2020), heterogeneous catalysis with peroxymonosulfate (PMS) (de Andrade et al. 2020), UVC/S$_2$O$_8$$^{-2}$ (Starling et al. 2019), photo-electro-Fenton (Martínez-Pachón et al. 2019), and electrochemical oxidation (Salazar et al. 2016) have recently been studied as an alternative for removing LOS. However, partial oxidation can generate more toxic by-products than the parent compound (Rizzo 2011; Utzig et al. 2019). For this reason, the efficiency of the process must be monitored not only by analytical parameters but also through toxicity bioassays (Rizzo et al. 2019).

Although AOPs have shown great potential for degradation of LOS, few studies have evaluated their application in environmentally relevant concentrations. In addition, the evolution of toxicity during and after AOPs, when monitored, generally uses a single acute bioassay. In this sense, the present study aimed to evaluate the acute and chronic ecotoxicity besides the genotoxicity of LOS before and after treatment by UVC/ photolysis and UV/H$_2$O$_2$ processes. For this purpose, organisms of different trophic levels were used for the first time in the literature: the microalgae *Desmodesmus subspicatus* (chronic ecotoxicity) and the microcrustacean *Daphnia magna* (acute ecotoxicity and genotoxicity).

Materials and methods

Chemicals reagents and solutions

The losartan potassium (LOS, ≥ 99% purity) was supplied by Delaware Ltda. (Brazil). LOS solutions (2.5 mg L^{-1}/4.6 μM) were prepared in water purified by reverse osmosis (ROW). Due to the high solubility of LOS in water (> 500 mg L^{-1}), no solvent was added (de Andrade et al. 2020). Until use, the solutions were kept in amber flasks, at 2–8 °C for a maximum of 48 h. Hydrogen peroxide (35% w/v) and Allper® reagent were obtained from Peróxidos do Brasil Ltda. (Brazil). Bovine liver catalase (4% w/v) was supplied by Sigma-Aldrich.

Degradation experiments

LOS solutions used in photodegradation experiments were prepared in purified water (ROW) (2.5 mg L^{-1}; 4.6 μM). The solutions were used at natural pH (6.3 ± 0.2). UVC/ photolysis and UV/H$_2$O$_2$ experiments were conducted out in a bench borosilicate reactor with 600 mL equipped with water recirculation and magnetically stirred. The radiation was provided by a high-pressure mercury vapor lamp (125 W) without its original glass bulb and covered with a quartz bulb, which was immersed into the solution. The UV/H$_2$O$_2$ process was performed with 50 mg L^{-1} (1.5 mM) of the H$_2$O$_2$, and after every 30 min of treatment, the H$_2$O$_2$ amount was re-added to the initial concentration. The residual H$_2$O$_2$ was monitored by the spectrophotometric methodology using Allper® reagent (Brandhuber and Korshin 2009) with LOD 0.5 mg L^{-1} at λ = 395 nm. In both treatments, samples were collected in fixed time interval (0, 30, 60, 90, 120, 240, and 480 min). For each treatment time, aliquots of 3 mL were collected, and the residual H$_2$O$_2$ was removed by adding 0.01 mol L^{-1} of bovine liver catalase and stored at − 20 °C in an amber flask. All the experiments were in triplicate.

Chromatographic conditions

All chemicals used were of analytical-reagent grade (J.T.Baker), and all solutions were prepared with ultrapure water (MegaPurity). The detection and quantification of LOS were performed by a high-performance liquid chromatography (Prominence, Shimadzu) coupled to a diode array detector (DAD) set at 254 nm. Chromatographic separation took place in an ODS analytical column (Hypersyl, 150 × 46 mm d.i, particle size diameter 5 μm). An isocratic method was performed by mobile phase 63:37 (v/v) of methanol and orthophosphoric acid (0.1 %), injection volume of 50 μL, and

0.5 mL min^{-1} of flow rate. The column temperature was kept at 30 °C. In these chromatographic conditions, the LOS was eluted at the retention time (R_T) of 10 min.

The linearity of a method can be observed by the equation y = 106,181.75x − 3055.092 (r^2 = 0.9992). The calibration curve was obtained in linear range (between 0.075 and 9.0 μM). For samples below the LOQ, a pre-concentrated factor (PCF) of 40 was performed by solid-phase extraction (SPE) using ODS cartridges (500 mg and 6 mL, Chromabond). The SPE column was conditioned by 10 mL of methanol and ultrapure water, 20 mL of the sample, then dried for 2 min under vacuum, and eluted with 10 mL of methanol. The eluates were dried and then dissolved in 0.5 mL of methanol and ultrapure water (1:1 v/v).

Bioassays

For ecotoxicity and genotoxicity assays, all samples were collected in clean glass flasks and analyzed immediately, or, when it was not possible, they were kept frozen at − 20 °C for a maximum of 20 days until the tests were performed (ABNT 2015). Bovine liver catalase (1% w/v) was added for residual H_2O_2 removal. Two organisms of different trophic levels were used: *Desmodesmus subspicatus* microalgae and *Daphnia magna* microcrustacean.

Acute ecotoxicity with *Daphnia magna*

The tests with *Daphnia magna* were carried out following the NBR 12713 (ABNT 2016). Previously to the ecotoxicity tests, the pH of the samples was measure and, when necessary, adjusted to 7.0 using NaOH (0.01 mol L^{-1}). Samples for each treatment time were diluted in culture medium at 100 (sample without dilution), 50, 25, 12.5, 6.25, and 3.125% (v/v). The results were expressed in terms of the toxicity factor (TF), which corresponds to the highest sample solution which no toxic effect is observed, represented as 1 (sample without dilution), 2, 4, 8, 16, and 32 (highest dilution) (ABNT 2016). Ten neonates were exposed in 20 mL of the test solutions and maintained for 48 h at 20 °C on the dark, in three replicates. The negative control was the culture medium, and the positive control was potassium chloride. After 48 h of exposure, the number of immobile organisms was recorded to calculate the percentage of immobility and toxicity factor (TF).

Chronic ecotoxicity with *Desmodesmus subspicatus*

The algae growth inhibition test was conducted according to NBR 12648 (ABNT 2018). *D. subspicatus* cultures were kept in a climate chamber at 23 ± 1 °C and 24-h photoperiod with 6000–6500 lux. The algae were cultivated in narrow glass columns, filled with 500 mL algae medium (AM) and aerated from the bottom. For the tests, Erlenmeyer flasks (100 mL) were filled with 50 mL algal medium (5×10^4 cells mL^{-1}); three replicates for each group (control and treatment) were performed. The flasks were agitated on a horizontal shaker at 145 g min^{-1} (Tecnal, TE—1400). Cell densities were determined after 72 h by spectrophotometric indirect method. The absorbance (750 nm) and the cell density (cells mL^{-1}) were established according to analytical curve (r^2 = 0.9993). Spectrophotometric analyzes were performed in a microplate reader (BMG Labtech, FluoStar Omega) using 96-well plates, with 4 replicates for each dilution (50 μL). By calculating the percentage of cell density between test and control solution, percentage of inhibition of algae growth was obtained. The results were expressed in FT, which were attributed to the highest concentration in which there was no significant percentage of algal growth inhibition (ICA > 20%).

Comet assay on *Daphnia magna*

Comet assay was performed as described by Knapik and Ramsdorf (2020). Ten neonates (≤ 24 h) were exposed to sublethal LOS (2.5 mg L^{-1}) concentrations before (0 min) and at the end (480 min) of UVC/photolysis and UV/H_2O_2 processes. The culture medium (ABNT 2016) was used as negative control. After 48 h of exposure (20 ± 0,5 °C), a pool of 30 neonates were homogenized (whole organisms) in a rotary tissue homogenizer (8000 rpm, for 5 s, in 30 μL PBS buffer pH 7.4), generating a cell suspension. Homogenization occurred directly in 2 mL microtubes, to avoid loss of cellular and genetic material. Then, 80 μL of 0.05% liquefied low melting point (LMP) agarose was and added into the microtubes. The cell suspension was transferred to slides previously covered with agarose (1.5%) and refrigerated at 4 °C for 20 min. For each concentration tested, 24 slides were obtained for analysis, which were kept at least 24 h in a lysis solution (2.5 M NaCl, 100 mM EDTA, and 10 mM Tris) and next, placed in an electrophoresis vat and immersed in an electrophoresis buffer solution (300 mM NaOH and 200 mM EDTA) at a 25 V and 300 mA. After, the slides were neutralized (3 × 5 min) with TRIS (0.4 mol L^{-1}) solution, dried, and fixed with ethanol. Then, 25 μL of ethidium bromide dye (20 μl mL^{-1}) was added over the slides, and the colorizing was analyzed. The scores attribution for the nucleoids was performed using epifluorescence microscope (Leica DMLS, × 400 magnification). DNA damage quantification was performed visually by analyzing 100 nucleoids per slide (9 slides per sample/control), with classes ranging from 0 (no apparent damage) to 3 (damaged DNA), adapted from Parrella et al. (2015). To determine the damage, the intensity of tail fragmentation was considered. Then, a score representing the damage for each slide was calculated (Collins et al. 1997) according to Eq. (1):

$$Score = (n\ class\ 0 \times 0) + (n\ class\ 1 \times 1)$$
$$+ (n\ class\ 2 \times 2) + (n\ class\ 3 \times 3) \qquad (1)$$

where n class = total damage rated as 0, 1, 2, or 3.

Statistics data were analyzed by GraphPad Prism 5® software. The Shapiro-Wilk normality test was applied, and then the Kruskal-Wallis nonparametric test was performed followed by Dunn's multiple comparison ($p < 0.05$).

Results and Discussion

The initial concentration of LOS (2.5 mg L^{-1}) was used in this work as an extreme environmental situation, where it was detected in an effluent from a pharmaceutical manufacturing facility (Larsson et al. 2007). To the best of our knowledge, this is the first study to assess the degradation of LOS by AOPs in an environmentally relevant concentration.

Figure 1 shows the LOS spectra profile during the UVC/photolysis treatment. At 0 min, the retention time at 10 min corresponds to LOS initial concentration of the 2.5 mg L^{-1} (4.6 μM) and also observed smaller peaks at R_T of 11.3 e 12 min that could be attributed to impurities or the instability of the LOS solution during the manipulation (Rao et al. 2012). The authors investigated the stability of antihypertensive drugs and degradation by-products from solutions prepared under laboratory conditions, similarly to the present study. They verified unknown chemical structure at m/z 827 ratio after 24 and 48 h of LOS monitoring. However, LOS is considered a molecule stable to hydrolysis and biodegradation (de Andrade et al. 2020).

According to Fig. 1a, the LOS was completely degraded in the first 30 min of treatment by UVC/photolysis. This result is in agreement with previous studies that show the LOS

instability under 30 min of photoirradiation (Ali et al. 2017; Starling et al. 2019). The UV-Vis absorption spectra for LOS shows higher intensity at 265 nm indicating the electron delocalization of the imidazole and tetrazole rings on its structure. The electronic transitions under UV-C radiation can destabilize the LOS structure and make it susceptible to degradation (Ali et al. 2017; Starling et al. 2019).

In order to verify the by-products formed throughout the LOS degradation process, aliquots were collected at 60 and 480 min of treatment and pre-concentrated (× 40) by SPE. As observed in Fig. 1b, only at 480 min a peak appeared at R_T of 11.9 that suggest the presence of by-product.

In relation to the UV/H$_2$O$_2$ process (Fig. 2a), the LOS degradation achieved removal > 80% at 30 min of reaction. The LOS and de H$_2$O$_2$ ratio 20:1 (H$_2$O$_2$: LOS) used in this work were according to Borba et al. (2018). However, the difference of LOS degradation obtained in this work could be assigned to power of the radiation source (mercury vapor lamp) and its position related to reactor as reported by Yuan et al. (2009, 2011), Starling et al. (2019), and Borba et al. (2018).

After 30 min, the LOS was completely removed, and a by-product peak was identified in the same retention time (12 min) and chromatographic conditions to UVC/photolysis process. An increase on the intensity of this chromatographic peak was observed until 90 min, which decrease the intensity at 120 min and was no longer detected at 480 min (Fig. 2b). Also, other unknown by-products were formed throughout the UV/H$_2$O$_2$ process at retention times from 4.5 to 6.5 min (Fig. 2a). Due to polarity differences and hydrophilic of the by-products, they have been eluted at shorter retention times. These intermediate compounds can present oxygenated functional groups in its structure as identified by GC-MS (Borba et al. 2018). For the samples at 240 and 480 min, the SPE method was performed, and no significant difference in the chromatographic profile was observed when compared to Fig. 2a.
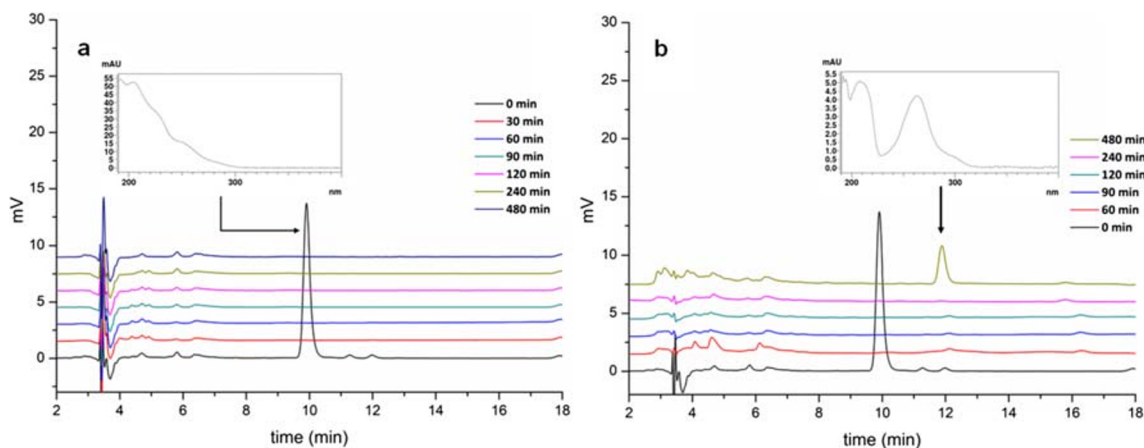

Fig. 1 Chromatographic analysis ($\lambda = 254$ nm) of potassic losartan before (2.5 mg L^{-1}) and during UVC/photolysis process: samples analyzed without SPE (**a**) and after SPE with a concentration factor of 40 times for aliquots obtained during the treatment (**b**). Inside of figures: UV-Vis spectra in the potassic losartan retention time (**a**) and in the retention time of a possible by-product. Analytical method, mobile phase MeOH:H$_3$PO$_4$ 0.1% (63:37 v/v); flux rate, 0.5 mL min^{-1}; injection volume, 50 μL
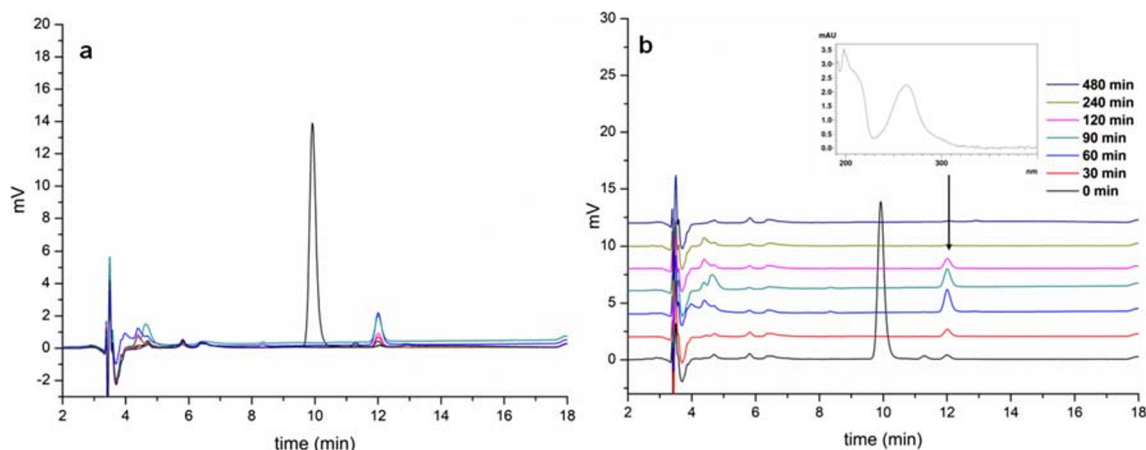
Fig. 2 Chromatographic analysis ($\lambda = 254$ nm) of potassic losartan before (2.5 mg L^{-1}) and during the UV/H$_2$O$_2$ process: overlapping of the chromatograms (**a**) and with waterfall projection on the y-axis (**b**).

Inside of "b" is the UV-Vis spectra in the retention time of a possible by-product. Analytical method, mobile phase MeOH:H$_3$PO$_4$ 0.1% (63:37 v/v); flux rate, 0.5 mL min^{-1}; injection volume, 50 μL

Yuan et al. (2009) identified intermediate products formed by some pharmaceuticals during UV/H$_2$O$_2$ process such as benzoic acid and its derivatives and 1.4-benzenedicarboxylic acid. In fact, it was observed that at the end of the UV/H$_2$O$_2$ process, the pH dropped from 6.5 to close to 4.0. This is probably due to the acids by-products and also to the acid character of the oxidant (Kaur and Dulova 2020). As describe by Starling et al. (2019), the presence of chlorine and nitrogen on LOS structure can form organochlorine compounds and cyanide ions during the treatment. Kaur and Dulova (2020) identified eight LOS transformation products after the UV/H$_2$O$_2$ process. It is important to note that all the identified TPs had already been reported in the literature. The authors suggest two possible pathways for the LOS degradation: the reaction between hydroxyl radical and the alcohol moiety of LOS or the addition of HO$^\bullet$ to the carbon double bond, forming several isomers of biphenyl hydroxylation. Then, chlorine is removed and followed by hydroxylation of carbon at 2 and 5 position, and consequently the opening of the imidazole ring (Carpinteiro et al. 2019). Considering that LOS contain chlorine atoms in its structure, the Cl radicals may be generated, which would contribute to the degradation of the molecule under UVC-irradiation (Starling et al. 2019). These chemicals can cause acute, chronic, and/or genotoxic effects, even at low concentrations.

Acute toxicity with *Daphnia magna*

For UVC/photolysis process, the untreated LOS solution (0 minute) resulted on average of immobility by acute tests of the 76.67 for the pure solution (100%) and 13.33 % at 50% of dilution (Table 1). After the treatments, the diluted samples did not show acute effect. In this sense, for undiluted samples (100%), a lowest average of immobility (20%) at 240 min was observed and then increases up to 93% at 480 min. The

highest average of immobility was detected at 90, 120, and 480 min of treatment. These results indicate the hormesis effect, a nonlinear relationship between the exposure dose and the test organism responses, which presence of by-products at very low concentrations (not detectable chromatographically), may generate an acute effect for *D. magna*. Studies report that some pharmaceuticals can exhibit this behavior, since they cause cellular stress, generated by adaptation responses (Calabrese 2008; Nielsen and Roslev 2018). The hormesis effect is in agreement with Fig. 1 that the presence of by-products at $R_T = 12$ min at 480 min of treatment was not detectable at chromatographic conditions.

For the UV/H$_2$O$_2$ process, after 30 min of treatment was observed an acute effect below 10% of immobility for 50% of dilution, which indicate a reduction of toxicity compared to LOS chemical (Table 2). Despite the rate immobility reduced for undiluted samples (100%) at 60 and 90 min of treatment, an increase from 120 min was notice. Fig. 2 shows peaks in by-products of LOS degradation that may have been

Table 1 Average of immobility (%) and toxicity factor (TF) of losartan potassium before and after treatment by UVC/photolysis to *Daphnia magna*

		Average of immobility (%)				
Time (min)	NC	100%	50%	25%	CV (%)	FT
0	0	76.67 ± 2.83	13.33	0	1.89	4
30	0	66.67 ± 2.58	0	0	2.32	2
60	0	63.33 ± 2.41	0	0	2.29	2
90	0	100 ± 3.73	0	0	2.24	2
120	0	80 ± 2.98	0	0	2.24	2
240	0	20 ± 1.00	0	0	3.00	2
480	0	93.33 ± 3.48	0	0	2.24	2

NC negative control, *CV* coefficient of variation, *TF* toxicity factor

Table 2 Average of immobility (%) and toxicity factor (TF) of losartan potassium before and after treatment by UV/H$_2$O$_2$ to *Daphnia magna*

| Time (min) | Average of immobility (%) | | | | | | | | |
	NC	100%	50%	25%	12.5%	6.25%	3.125%	CV (%)	FT
0	0	76.67 ± 2.83	13.33	0	0	0	0	1.89	4
30	0	100 ± 3.69	6.67	0	0	0	0	2.08	2
60	0	3.33 ± 0.23	0	0	0	0	0	4.12	1
90	0	0	0	0	0	0	0	0	1
120	0	100 ± 3.73	0	0	0	0	0	2.24	2
240	0	100 ± 0.31	100	100	100	100	93.33	0.03	> 32
480	0	56.67 ± 2.15	0	0	0	0	0	2.27	2

NC negative control, *CV* coefficient of variation, *TF* toxicity factor

responsible for potentiating this acute effect. At 240-min treatment, the toxicity was above of 90% for all dilution's samples. Thus, although the analysis chromatographic limitations on detectability of compounds at low concentrations, the toxicity observed may be due to the presence of by-products that were not chemically characterized by this technique.

The $^{\bullet}$OH radicals from the UV/H$_2$O$_2$ treatment can react with organic compounds containing π-bonds, as unsaturated hydrocarbons and aromatic rings, by hydroxylation reaction (Melo et al. 2009) and generate toxic by-products to *D. magna*. Carpinteiro et al. (2019) verified that the LOS degradation by free chlorine treatment can also form toxic by-products to *D. magna* via electrophilic halogenation and hydroxylation reactions. According to the authors, the CE$_{50/48h}$ in silico of by-products, using the ECOSAR *software*, was at ranged from 8 to 0.008 mg L^{-1}. This can explain the high in vivo toxicity at 240 min for the microcrustacean in this work for UV/H$_2$O$_2$ process.

The calculate CE$_{50/48h}$ value of the standard LOS *D. magna* resulted in 331 mg L^{-1} (FDA 2002; Cortez et al. 2018). Also, it is noticed that among the sartan drugs, the CE$_{50/48h}$ for olmesartan and valsartan was greater than 120 mg L^{-1} and 580 mg L^{-1}, respectively, showing high acute toxicity for these class of compounds (Bayer et al. 2014). However, the results obtained in this study demonstrate that the CE$_{50/48h}$ value are over 100 mg L^{-1} and may be underestimated once 76.67% of the exposed organisms was immobilized at 2.5 mg L^{-1} of LOS (initial concentration).

Chronic ecotoxicity with *Desmodesmus subspicatus*

First of all, the inhibition of algae growth (IAG) was calculated from dilution factor (DF) for both processes at all treatment times. In this sense, before UV/H$_2$O$_2$ and UVC/photolysis treatments (0 min), the IAG was 16% at highest sample concentration (DF = 80%), which was considering no significant (ANBT 2018) (Fig. 3, Table S1). After 30, 90, and 120 min of

treatment times for UVC/photolysis process, the IAG values decrease to 10, 7, and 2%, respectively. However, at 60, 240, and 480 min, a stimulus in algal growth was observed in the order of 5, 3, and 3%, respectively. Therefore, the UVC/photolysis not showed a significative chronic effect to *D. subspicatus*.

The microalgae growth stimulus was observed at 60, 240, and 480 min. This may have occurred due to the potassium, nitrogen, and carbon by-products formed during the UVC/photolysis process which would be assimilable by the microalgae. Thus, the presence of microalgae on the environmental is beneficial owing to its metabolism that converts CO$_2$ into O$_2$, which became the aquatic environment aerobic, ensuring its ecosystem dynamics. Nevertheless, an excessive proliferation can cause eutrophication that prevents the light penetration and, as a consequence, a drop on dissolved oxygen levels and death of fixed and free submerged macrophytes, among other aquatic plants. Also, an increase on the heterotrophic aerobes activity can consumes the O$_2$ present in the aquatic environment and becomes anaerobic system and inappropriate for aerobic organisms such as fishes (WHO 1997).
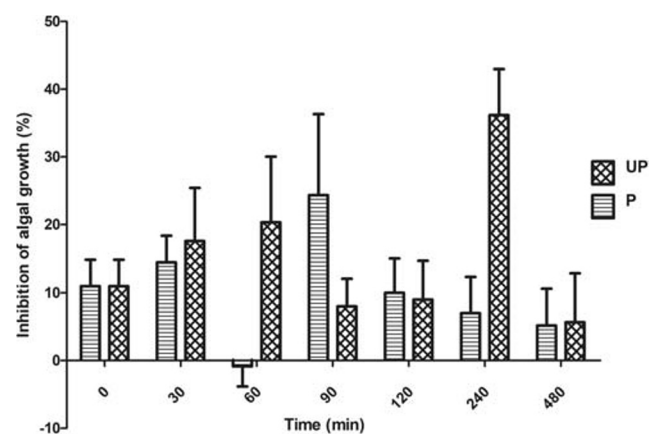

Fig. 3 Inhibition of *Desmodesmus subspicatus* growth of potassic losartan before (2.5 mg L^{-1}) and during the treatment by UVC/photolysis (P) and UV/H$_2$O$_2$ (UP) processes

Table 3 summarized the toxicity factor after and before LOS by UVC/photolysis and UV/H_2O_2 process, which the IGA values was calculated from TF data. For the UVC/photolysis treatment, the IGA values for all samples were not greater than 20% at the highest concentration analyzed (DF = 80%); therefore, the TF values were equal to 1 for this process.

Regarding to UV/H_2O_2, a significant chronic effect was observed for samples after the photodegradation treatment and IGA value up to 41% at 240 min (see Table S2). At 30 and 60 min of treatment was also verified an adverse effect on microalgae related to an increase of the IGA by the increase of the sample concentration. The reduction of this chronic effect was achieved at DF of 80%. A growth stimulus of algal biomass at DF of 3.125 and 6.25% at 480 min was observed, which the toxicity is increasing as the increase of the DF (Fig. 3; Table S2). Thus, the TF before and after LOS degradation by UV/H_2O_2 (Table 3) indicates that, except for the 240 min of treatment, the IGA values were not greater than 20% at the highest concentration analyzed (DF = 80%), indicating non-chronic toxicity for these samples.

It is interesting to note that for both organisms (D. magna and D. subspicatus), an increase in toxicity after 240 min in the UV/H_2O_2 process was observed, even the LOS was no longer detected, which could correspond to the by-products. This result shows the importance of using different organisms to assess the ecotoxicity as well as the monitoring AOPs not only by analytical parameters but also by toxicity bioassays.

Bouissou-Schurtz et al. (2014) verified that the LOS did not exhibit ecological risk for the Selenastrum capricornutum microalgae (known as Raphidocelis subcapitata). The authors consider the ratio between the environmental concentration (0.011 μg L^{-1}) and the estimated concentration that has no effect on the organism (14.3 mg L^{-1}), which was 7.6×10^{-7}. Larsson et al. (2007) quantified the LOS concentration in the pharmaceutical industries effluents and verified that the risk quotient (QR) was 0.17, which there are no potential ecological risk. Likewise, Godoy et al. (2015) detected a QR in range from 6×10^{-3} to 9×10^{-3} to the Lemna minor macrophyte for LOS in surface waters and effluents with QR = 39.2 as described by Larsson et al. (2007). According the QR, the estimated environmental concentration (EEC) is compared to an effect level, and the QR value must be higher than 1 to infer that the chemical is presenting a risk to the ecosystem. Therefore, despite the low ecological risk, environmental damage can be caused by the presence of LOS in aquatic organisms, such as macrophytes results showed. Hence, this parameter explains the low ecotoxicity (TF = 1) of the LOS for D. subspicatus obtained in this work for both UV/H_2O_2 and UVC/photolysis processes, except for UV/H_2O_2 at 240 min.

The no observed effect concentration (NOEC) for green microalgae Selenastrum capricornutum and LOS was 143 mg L^{-1} according to FDA (2002). Bayer et al. (2014) evaluated the chronic effect for D. subspicatus and among the sartan drugs, which show that $EC_{50/72h} > 120$ mg L^{-1} to olmesartan and > 115 mg L^{-1} to valsartan. Therefore, the LOS concentration of 2.5 mg L^{-1} used in this work is lower than the concentration that causes 50% inhibition of algae biomass growth, which justifies the no chronic effect by TF values for D. subspicatus, before and after degradation experiments.

Genotoxicity with Daphnia magna

The genotoxicity of LOS before and after treatment was evaluated by the comet assay with D. magna. The number of damaged cells for all groups, for the 3600 cells analyzed, are presented in the Table S3. Damage to control groups was presumed to cultivar conditions and intrinsic conditions from the organisms. Figure 4 shows the scores of DNA damage in D. magna cells after exposure to LOS and after 480 min of

Table 3 Toxicity factor (TF) values of *Desmodesmus subspicatus* after and before losartan potassium treatment by UVC/photolysis and UV/H_2O_2 process

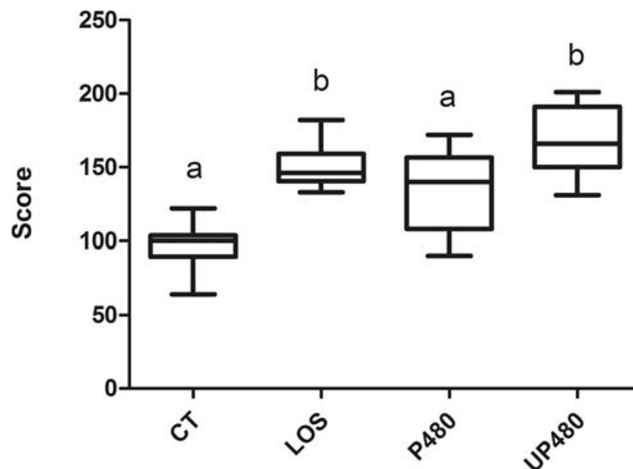| Treatment time (min)	TF	
	UVC photolysis	UV/H_2O_2
0	1	1
30	1	1
60	1	1
90	1	1
120	1	1
240	1	> 32
480	1	1

TF toxicity factor

Fig. 4 Medians obtained from damage scores after exposure of *D. magna* to LOS before and after 480 min by UVC/photolysis and UV/H_2O_2. Note: CT, control; LOS, losartan (2.5 mg L^{-1}); P480, LOS after UVC/Photolysis (480 min); UP480, LOS after UV/H_2O_2 (480 min). Equal letters indicate no statistically significant difference related compared to the control after Dunn's test ($p < 0.05$)

both treatments, UVC/photolysis and UV/H$_2$O$_2$. The medians obtained were 100, 146, 140, and 166 for the control, LOS, UVC/photolysis (480 min), and UV/H$_2$O$_2$ (480 min), respectively. The genotoxic effect was observed for LOS before the treatment and for UV/H$_2$O$_2$ (480 min).

According to Fig. 4, the by-products formed after 480 min of treatment by UV/H$_2$O$_2$ process showed the genotoxic effect for *D. magna*, although acute and chronic effects was not observed at this treatment time. This result demonstrates the importance of assessing the sublethal toxic effects of a compound using biomarkers of greater sensitivity. Recent studies have shown a genotoxic effect to several pharmaceuticals found in the environment (Borba et al. 2018; López-Pacheco et al. 2019; Ao et al. 2019). However, there are very few reports on the genotoxic effect of LOS at environmentally relevant concentrations. Cortez et al. (2018) observed DNA damage in mussel *Perna perna* gills cells after exposure to LOS at 3000 ng L^{-1}. Still, the evaluation of the genotoxicity of environmental contaminants remains little explored in the risk assessment. This is an extremely relevant parameter, because in addition to being associated with low concentrations (sublethal), it can be related to other effects on biomarkers, such as reproductive, endocrine, biochemical, and morphological (Pellegri et al. 2020). The use of the comet test with *D. magna* in the investigation of contaminants genotoxicity, whether in environmental monitoring or associated with treatment processes such as AOPs, has several advantages. It is a fast, sensitive, and easy procedure to be applied in the routine, especially in laboratories that already use *D. magna* in bioassays (Knapik and Ramsdorf 2020; Pellegri et al. 2020). *D. magna* is one of the most representative organisms in terms of aquatic ecotoxicity and also one of the most used in bioassays in the world. Finally, the efficiency of AOPs in the treatment of contaminants and environmental samples must be monitored not only by analytical parameters, but especially by bioanalytical tools. Advanced partial oxidation can generate several by-products that are combined in a complex mixture, which can cause toxic effects at different ecotoxicological levels. Hence the importance of associating analytical control with acute and chronic toxicity bioassays in addition to investigating the effects on biomarkers (Neale et al. 2017; Ghosh et al. 2017; Utzig et al. 2019; Xu et al. 2020).

Conclusion

In this study, the acute and chronic ecotoxicity as well as the genotoxicity of losartan potassium was evaluated before and after UVC/photolysis and UV/H$_2$O$_2$ processes. The efficient degradation for LOS achieved rates higher than 99.99% after 30 min for both processes. Also, throughout the treatments, unknown by-products formed during the degradation were observed. Both processes reduced acute and chronic toxicity

after 480 min. However, *D. magna* and *D. subspicatus* were sensitive to by-products formed after 240 min in the UV/H$_2$O$_2$ process. LOS, before and after 480 min UV/H$_2$O$_2$, caused genotoxicity to *D. magna* cells. This demonstrates the importance of (i) the bioanalytical monitoring of AOPs; (ii) the use of organisms of different trophic levels; and (iii) investigating sublethal toxic effects through biomarkers such as genotoxicity. Considering the results of acute and chronic ecotoxicity besides the genotoxicity, the 480 min of treatment by UVC/photolysis is the most suitable for the removal of LOS under the conditions used in this work.

Authors' contributions Eliane Adams was responsible to perform degradation experiments and ecotoxicological bioassays. Bruno B. Neves and Lucia R. Rocha Martins were responsible for the chromatographic analyses and validation methods. Liziê D. T. Prola was responsible for analyzing the results and editing and revising the manuscript. Wanessa A. Ramsdorf and Marcus V. de Liz were responsible for supervising the genotoxicity and degradation experiments, respectively. Adriane M. de Freitas was responsible for supervising and funding the project and editing and revising the manuscript. All authors contributed to the analysis and writing of the manuscript.

Data availability Not applicable.

Compliance with ethical standards

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