Original Article

Assessment of carbamazepine acute toxicity in the cockle *Cerastoderma edule* through chemical, physiological and biochemical tools

Avaliação da toxicidade aguda da carbamazepina no berbigão *Cerastoderma edule* por meio de ferramentas químicas, fisiológicas e bioquímicas

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Abstract

The cockle *Cerastoderma edule* was exposed to four concentrations (5, 10, 20 and 70 µg L^{-1}) of carbamazepine (CBZ). This anticonvulsant was found to alter the mussel behavior of by reducing its clearance rate (CR). Analysis of CBZ accumulation in tissues of *C. edule* was carried out using HPLC-UV after 48 or 96 hours of exposure. In addition, an overproduction of H_2O_2 by the bivalves was detected following exposure to CBZ but nitrite levels remained unchanged. Moreover, superoxide dismutase and catalase activities showed a significant increase in relation to their contact with CBZ. The activity of the biotransformation enzyme gluthatione-S-transferase did not change during exposure. Malondialdehyde (MDA) levels indicating cellular damage, increased when bivalves were exposed to 20 and 70 µg l^{-1} of carbamazepine for 96 h CBZ. The results also indicate that acetylcholinesterase activity (AChE) was inhibited in all CBZ concentrations during the 48 h exposure period. However, during the 96 h exposure period, AChE was only inhibited at the highest concentration. Further studies are needed now for more exploration of the toxicity of CBZ since it could be bioaccumulable throughout the food web and may affect non-target organisms.

Keywords: Carbamazepine, *Cerastoderma edule*, acute toxicity, Clearance rate, biomarkers.

Resumo

O berbigão *Cerastoderma edule* foi exposto a quatro concentrações (5, 10, 20 e 70 µg L-1) de carbamazepina (CBZ). Este anticonvulsivante alterou o comportamento do mexilhão, reduzindo sua taxa de depuração (CR). A análise do acúmulo de CBZ nos tecidos de *C. edule* foi realizada por HPLC-UV após 48 ou 96 horas de exposição. Além disso, uma superprodução de H_2O_2 pelos bivalves foi detectada após a exposição à CBZ, mas os níveis de nitrito permaneceram inalterados. Além disso, as atividades de superóxido dismutase e catalase apresentaram aumento significativo em relação ao contato com CBZ. A atividade da enzima de biotransformação glutationa-S-transferase não se alterou durante a exposição. Os níveis de malondialdeído (MDA), indicando dano celular, aumentaram quando os bivalves foram expostos a 20 e 70 µg l-1 de carbamazepina por 96 h CBZ. Os resultados também indicam que a atividade da acetilcolinesterase (AChE) foi inibida em todas as concentrações de CBZ durante o período de exposição de 48 horas. No entanto, durante o período de exposição de 96 horas, a AChE foi inibida apenas na concentração mais alta. Mais estudos são necessários agora para uma maior exploração da toxicidade da CBZ, uma vez que pode ser bioacumulável em toda a cadeia alimentar e pode afetar organismos não alvo.

Palavras-chave: Carbamazepina, *Cerastoderma edule*, toxicidade aguda, taxa de liberação, biomarcadores.

1. Introduction

A large variety of contaminants (e.g., industrial additives, pharmaceuticals, personal care products, steroids) have been identified in wastewater and surface waters (Noguera-Oviedo and Aga, 2016). Pharmaceuticals and their metabolites can reach water bodies through effluents from wastewater treatment plants (WWTPs), sewage systems, industrial discharge, aquaculture, and agriculture (Sui et al., 2015). These drugs are not completely removed by WWTPs and, after the effluent is discharged into the wider environment, they may have direct effects

on numerous species and ecosystems (Sui et al., 2015). Drugs could also harmful to non-target biota, especially filter-feeding taxa, as they are continuously exposed to micropollutants into the ecosystem (Santos et al., 2010). Fortunately, recent progress in analytical techniques has allowed a more accurate identification and quantification of these molecules in water, soil, and biota.

Pharmaceuticals have been considered as contaminants of emerging concern during the last twenty or more years. Carbamazepine (CBZ) is primarily used in human medicine in the treatment of epilepsy with an annual consumption rate of about 2,235,000 pounds worldwide (Zhang et al. 2008). It acts physiologically to stabilize the inactivated state of sodium channels, thereby rendering brain cells less excitable (Contardo-Jara et al., 2011). For these reasons, CBZ is a drug commonly found in aquatic areas (Khazri et al., 2019a). Concentrations of 60.58, 93.19, and 132 µg l^{-1} were recorded in wastewater collected from northern Tunisia (Khazri et al., 2019a). In other countries (Germany, China, Spain, Belgium, The Netherlands, and France), concentrations ranged from 0.03 to 11.6 µg l^{-1} in wastewater effluents, surface and ground waters (Liu et al., 2015; Huerta-Fontela et al., 2011).

According to Martin-Diaz et al. (2009), CBZ was resistant to biodegradation even the measured concentrations were low. CBZ exhibits extremely slow photodegradation and its ubiquitous distribution in aquatic areas. Numerous studies have focused on its effects on non-target organisms such as soil microorganisms, insects, fish, birds, and various bivalve species (*Ruditapes decussatus* Linnaeus, 1758, *Mytilus galloprovincialis* Lamarck, 1819) (Clara et al., 2004). It has been found that exposure to CBZ induces oxidative stress, immunotoxicity, cytotoxicity, and alteration of metabolism and health status of organisms (Gagné et al., 2006; Contardo-Jara et al., 2011; Aguirre-Martínez et al., 2013; Tsiaka et al., 2013).

Filter feeders are also the most used marine organisms as indicators due to their tolerance and potential transport of pollutants through the food chain (Dellali et al., 2001). The cockle *Cerastoderma edule* Linnaeus, 1758 has been used as an indicator species in environmental health status assessments due to its large filtration capacity, sessile habit, wide geographical distribution, easy handling and collection, and its sensitivity to various pollutants (Paul-Pont et al., 2010). In addition, *C. edule* is a species of commercial and economic interests around the Mediterranean Sea, due to its abundance and the quality of the flesh (De Montaudouin et al., 2010). It is also relatively tolerant to different classes of pollutants and environmental stressors, making it suitable for marine biomonitoring and bioassays (Dellali et al., 2001).

The Ghar El Melh Lagoon is situated in northeastern Tunisia and is directly connected to the Mediterranean Sea. It is a multi-contaminated site because it is the watershed of several rivers, including the Oued Medjerda, and receives various types of household and industrial effluent (Mahmoudi et al., 2002). This has led to a progressive deterioration in biodiversity in this ecosystem (Guasmi et al., 2006). *Cerastoderma edule* is an abundant and typical bivalve species in this lagoon.

In this work, the choice of CBZ concentrations was based on previously reported CBZ levels in different aquatic ecosystems in Tunisia and across the globe (Khazri et al., 2019a; Oliveira et al., 2017). For example, Khazri et al. (2019a) founded a CBZ concentration of 60.58 µg l^{-1} in effluent samples from a WWTP in Bizerte (Tunisia). The aim of this study was to investigate the possible effects of the environmentally relevant concentrations of CBZ ranging from 5 µg l^{-1} to 70 µg l^{-1} on the cockle *C. edule*. First, we evaluated the ability of the cockle to accumulate CBZ. Consequently, this bivalve would provide the scientific community an effective and timely indicator of the probable risks deriving from the consumption of contaminated seafood from coastal countries where CBZ is recommended and used as treatment of epilepsy. Second, we assessed the possible toxicity of CBZ through different biochemical biomarkers of exposure and effect. In details, we investigated the effects of CBZ on bivalves (*C. edule*) in the laboratory by using a multi-biomarker approach (Khazri et al., 2018; Oliveira et al., 2017; Almeida et al., 2015). The intensity of oxidative stress was measured after evaluating levels of the reactive- (ROS) and nitrous- (NOS) oxygen species produced, and the activities of enzymes of defense and those related to lipid peroxidation. Neurotoxicity and respiratory capacity were also measured by quantifying acetylcholinesterase activity (AChE) and clearance rate (CR), respectively.

2. Material and Methods

2.1. Acclimation in the laboratory

Specimens of *C. edule* (shell length: 2.48 ± 0.28 cm, shell width: 2.5 ± 0.48 cm) were collected on February 4, 2017 from Ghar El Melh lagoon (37°8′36.73″N and 10°12′38.66″E), Tunisia. After transportation to the laboratory, the specimens were placed in tanks of 2L (five bivalves per tank) for a week under a photoperiod regime of 16 h light:8 h darkness. Each tank contained 2 L of natural filtered seawater from the Bay of Bizerte (Tunisia). The media was renewed every two days during the acclimatization with a continuous aeration. The exposure design was selected based on previous published studies (Khazri et al., 2016; Sellami et al., 2017). The laboratory temperature was maintained at 19 °C (field temperature) and the water temperature was maintained at 17 °C, and pH was 8.7 (Khazri et al., 2017). Measurements were conducted by means of a temperature/conductivity meter (WTW LF 196, Weilheim, Germany), and pH meter (WTW, model pH 330/SET-1).

The tank water was replaced on a daily basis to feed the specimens with the nutrients present in natural seawater from the Bay of Bizerte (Salhi et al., 2018) and, also, to avoid accumulation of organic matter and the possible appearance of nitrogen compounds such as ammonium, nitrite or nitrate in the tanks. An absence of mortality was observed throughout the experiment and all bivalves fed normally.

2.2. Bioassay

A CBZ concentration range was selected based on concentrations previously measured in WWTPs from

northern Tunisia (Khazri et al., 2019a). Following the acclimation period, five treatments: (1) control (natural seawater only), (2) CBZ: 5 µg l⁻¹, (3) CBZ: 10 µg l⁻¹, (4) CBZ: 20 µg l⁻¹, and (5) CBZ: 70 µg l⁻¹ were established in a triplicate design (three tanks per treatment, five individuals per tank) and maintained for two exposure periods, 48 and 96 h. In this study, only the acute toxicity of CBZ was targeted for evaluation which explain that the experiment was ended after these two time slots. Tank water was not replaced during both exposure periods. Water parameters were maintained as follows: temperature = 17 °C, pH = 8, and salinity = 32 PSU. No mortalities were observed in either exposure period.

2.3. Quantification of carbamazepine

Three replicates of water (200 ml) were sampled from the control and CBZ microcosms, and analytical samples were taken by SPE using C18 cartridges (OASIS HLB) purchased from Waters (Milford, MA). These steps were followed by an elution with of methanol ≥99.9% (CAS No. 67-56-1) (6 ml). At the end, an evaporation of these extracts was applied, followed by a reconstitution in methanol (1 ml) as described by Khazri et al. (2019b).

The CBZ concentrations in both extracts from water and tissues of *C. edule* were quantified at 285 nm by high performance liquid chromatography (Agilent 1100 series HPLC system) equipped with a reversed-phase C18 column (5 µm, 4.6 × 250 mm), a quaternary pump, an auto sampler, and a UV detector. A mixture of 70:30 (v:v) methanol and milli-Q water (flow rate = 1 ml min⁻¹) was used to make up the mobile phase. All samples and standards were analyzed in triplicate.

2.4. Clearance Rate (CR)

The CR was evaluated by measuring the neutral red dyed particles lost from the water column (Coughlan, 1969). At the end of all exposure phases, specimens were taken from each treatment applied (*n* = 4) and put in 200 ml of neutral red dye (1 mg l⁻¹) following the rule of one clam per beaker (CAS No. 553-24-2) purchased from SLIM LAB Tunisia. In this work, CR was only measured after exposure of *C. edule* to 0-20 µg CBZ l⁻¹. For 70 µg CBZ l⁻¹, this parameter was not considered because it is very high compared to environmentally concentrations.

2.5. Biomarker measurements

All biomarker quantification procedures were carried out at temperatures between 0 and 4 °C. First of all, the soft tissues were homogenized by using an Ultra Turrax disperser (model IKA T18 Basic) in K_3PO_4 buffer (pH 7.4, 0.1 mM). The result was then centrifuged (9000 rpm, 4 °C, 30 min) and the protein content in each supernatant was estimated using the Bradford method (Bradford, 1976).

As reported Ellman et al. (1961), the acetylcholinesterase activity (AChE) was determined in nMol min⁻¹ mg⁻¹ protein after measuring the absorbance (412 nm, 5 min) in presence of 1 mM acetylthiocholine. The evaluation of SOD activity (U mg⁻¹ protein) followed the indirect method (i.e. the epinephrine/adenochrome system) according to Misra and Fridovich (1972). According to Aebi (1974), CAT

activity can be determined by using the spectrophotometric method which measures absorbance at 240 nm during the degradation of hydrogen peroxide (H_2O_2). The CAT activity was given in nmol min⁻¹ mg⁻¹ protein. The measurement of this molecule was made using a spectrophotometer with an absorbance of 546 nm.

Hydrogen peroxide (H_2O_2) concentrations were measured according to methods described by Kakinuma et al. (1979) and Green et al. (1982) using an adapted kit (Biomagreb, Tunisia). Glutathione-S-Transferase (GST) activity was spectrophotometrically evaluated (at 340 nm, ε = 9.6 Mm cm⁻¹). Thus, the production of 1-glutathion-2,4-dinitrobenzene was followed as proposed Habig et al. (1974). This compound comes from the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with a glutathione reduced form (GSH).

Lipid peroxidation (LPO) was determined through quantifying malondialdehyde (MDA) which is a lipid peroxidation product (Draper and Hadley, 1990). The absorbance was measured at 535 nm (ε = 156 Mm cm⁻¹). At the end, the LPO levels were given as the MDA produced in nMol min⁻¹ mg⁻¹ protein.

2.6. Statistical processing

Data, given in mean ± standard deviation (SD), were first integrated into STATISTICA v8. Bartlett, Levene and Brown-Forsythe tests were used to check parametric test criteria, normality and variance homogeneity, respectively. Thereafter, two parametric tests, one-way analysis of variance (ANOVA) and Tukey HSD, were applied to detect significant differences in multiple comparisons between the controls and different treatments.

3. Results

3.1. Chemical analysis

CBZ was not detected in water samples originating from Ghar El Melh lagoon where the *C. edule* specimens were collected. There were no changes in CBZ concentrations in any tank water between the beginning and the end of all treatments (one control and four CBZ exposure levels, 48 and 96 h time periods) (Table 1). After 48 h, all the soft tissues of *C. edule* specimens exposed to 10 µg CBZ l⁻¹ contained CBZ (Table 1).
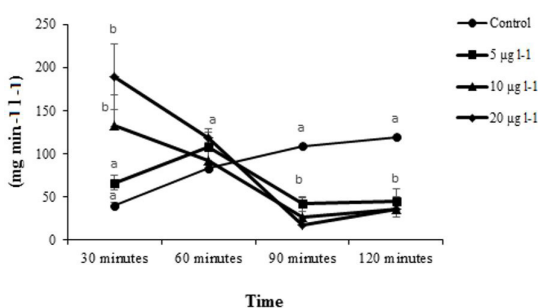
3.2. Clearance rate

The results obtained for the CR are shown in Figure 1. A high CR was observed during the first 30 min of the experiment for specimens exposed to 10 and 20 µg l⁻¹ of CBZ. It was clearly noted that 1.5 and 2 hours of exposure were followed by a discernible decline of the CR values for mussels collected from treatments 5, 10, and 20 µg CBZ l⁻¹.

3.3. Neurotoxicity

The AChE activity results for specimens exposed to CBZ for 48 or 96 h are presented in Figures 2A and 2B, respectively. Following 48 h of exposure, AChE activity significantly decreased in all CBZ exposure treatments

Table 1. Carbamazepine concentrations (5-70 µg l^{-1}) in the water phase where *Cerastoderma edule* were exposed, after 48 h and 96 h. Values are the mean of 2 samples per concentration × 3 replicates of the HPLC-UV quantification. Same letters indicate no significant differences between measured concentrations after 48 h and 96 h ($p < 0.05$).

Conditions	Nominal concentration	Measured concentration (Exposure medium)	Measured concentration (Exposure medium)
		48 hours	96 hours
Control	0	Not detected	Not detected
C1 (µg l^{-1})	5	4.54 ± 0.40[(a)]	4.28 ± 0.88[(a)]
C2 (µg l^{-1})	10	9.46 ± 0.14[(a)]	9.20 ± 0.59[(a)]
C3 (µg l^{-1})	20	19.4 ± 0.11[(a)]	16.6 ± 0.24[(a)]
C4 (µg l^{-1})	70	69.4 ± 0.35[(a)]	68.7 ± 0.67[(a)]

Figure 1. Clearance rate in *Cerastoderma edule* exposed to three concentrations of CBZ (5, 10, 20 µg.L^{-1}) during 2 h. Values are the mean of 3 replicates. Letters (a-b) indicate significant differences (p ≤0.05) between control and exposure conditions.

compared to the controls. At the end of 96 h of exposure, only the high concentration (70 µg l^{-1}) was associated with a reduced AChE activity.

3.4. Antioxidant enzymes

Results of antioxidant enzymes (SOD, CAT) levels measured in *C. edule* specimens following their laboratory exposure to CBZ for 48 and 96 h are shown in Figure 2. SOD activity (Figure 2C, D) was induced only specimens located in the 20 µg l^{-1} treatment after 48 h of exposure. Nevertheless, after 96 h, all treatments considered were followed by a significant enhance in terms of SOD activity. In relation to CAT activity, no significant difference was observed across all treatments, except in the highest exposure treatment (70 µg l^{-1}) after 96 h, where it increased significantly compared with the control (Figure 2E, F).

3.5. Biotransformation enzyme

No significant changes in GST were detected in any treatment following 48 and 96 h of exposure (Figures 2G, H).

3.6. Pro-oxidant

Following 48 h of exposure, the level of H_2O_2 (Figure 3A) increased significantly from an average of 0.247 (± 0.017 SD) nmol mg^{-1} protein in specimens from the lowest exposure treatment (5 µg l^{-1}) to an average of 0.312 (± 0.030 SD) nmol mg^{-1} protein in the highest exposure treatment (70 µg l^{-1}).

Compared with the controls, these increases correspond to increases of 89.29% and 136.56%, respectively. Following 96 h of exposure (Figure 3B), a significant increase was recorded in the level of H_2O_2 production in specimens from the highest exposure treatment (70 µg l^{-1}). Intracellular H_2O_2 (mean ± SD: 0.376 ± 0.068 nmol^{-1} mg^{-1} protein) was observed to be three-times higher compared with the control. Nitrate (NO) levels did not vary at any exposure treatment compared to controls whatever was the time slot considered (48 or 96 h) (Figures 3C, D).

3.7. Indicators of cellular damage

No significant difference in MDA levels were detected between treatments following 48 h of exposure. However, after 96 h, MDA levels significantly increased in the two highest exposure treatments (20 and 70 µg l^{-1}) compared with the controls (Figures 3E, F).

4. Discussion

4.1. Chemical analysis

WWTPs generally do not remove all CBZ from wastewater (Almeida et al., 2015). Consequently, CBZ can enter the aquatic environment *via* treated effluent. Almeida et al. (2015) observed that CBZ was not absorbable by bivalve shells which confirm the obtained results herein. The goal of our work was to evaluate the toxicity of CBZ on the cockle using various biomarkers involved in neurotoxicity, oxidative stress, and physiological responses following exposure to four CBZ concentrations over 48 and 96 h.

4.2. Clearance rate

The presence of CBZ significantly reduced the CR of *C. edule* specimens. This could be attributed to the fact that *C. edule* animals are protected from predators by a shell, which may offer additional protection from harmful pharmaceuticals such as CBZ (Gosling, 2003). Furthermore, in the presence of CBZ, siphon closure has been observed in the freshwater mussel *Corbicula fluminea* Muller, 1774 exposed to 5 and 50 µg l^{-1} for 30 days (Chen et al., 2014). Similar to previous findings for the bivalve *Sinanodonta*
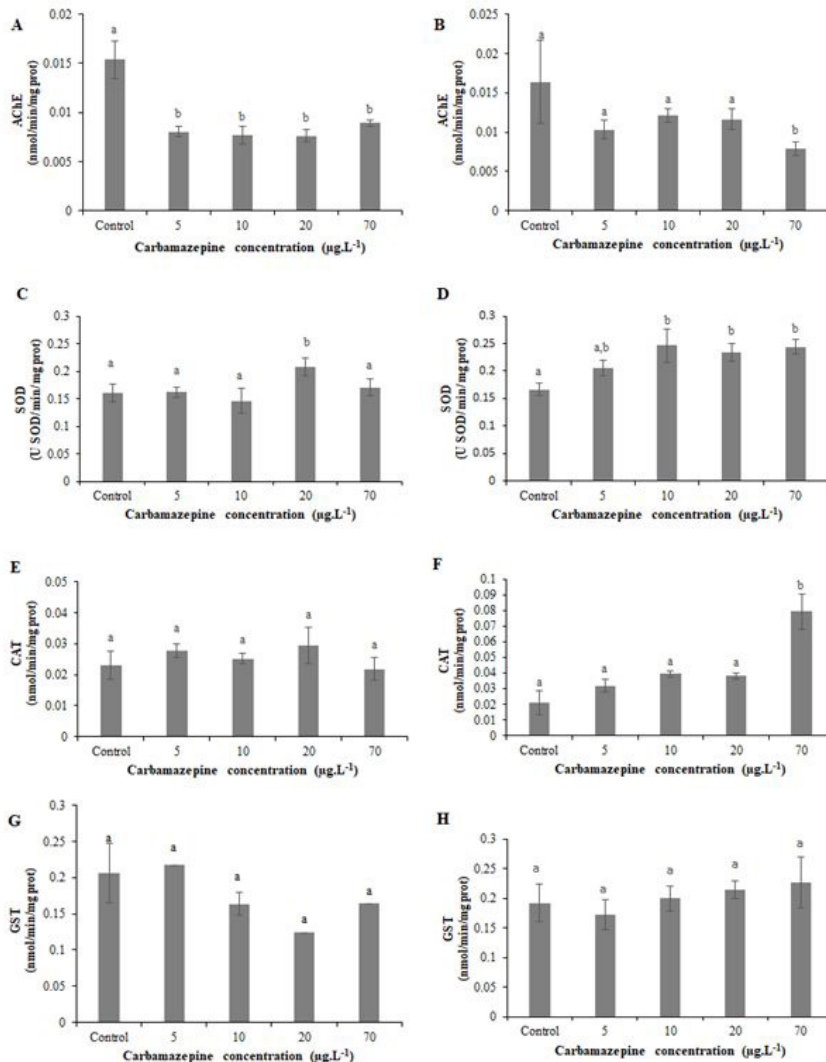
Figure 2. Effect of carbamazepine on enzymatic biomarkers, and AChE activity 48 h (A), 96 h (B), Antioxidant enzyme activities, SOD activity 48 h (C), 96h (D), CAT activity 48 h (E), 96 h (F) and GST activity 48 h (G), 96 h (H). Values are the mean (± SD) of 5 replicates. Significant differences (p≤0.05) among exposure concentrations, for each condition, are presented with different letters (a-b).

woodiana Lea, 1834 (Corsi et al., 2007), the significant decrease of AChE activity observed in the present study (from the lowest treatment concentration ($5\,\mu g\,l^{-1}$) onwards reinforces a theory that the opening and closing movements of valves may be mediated by cholinergic transmission in cockles. Consequently, following exposure to CBZ, cockle metabolism may slow down, which, in the long term, may be reflected in a reduction in their condition index and general health status (Farcy et al., 2011).

4.3. Oxidative stress

Several studies have shown that some contaminants (including pharmaceutical compounds) induce a production of ROS once they penetrate into the organism's tissues (Almeida et al., 2015; Oliveira et al., 2017; Khazri et al., 2018). For this reason, it is of particular interest for environmental biomonitoring programs to follow the response of this type of compound in order to predict contaminant exposure effects.

Living organisms protect their integrity by enzymatic and non-enzymatic antioxidant defense systems. These systems play a detoxifying role for ROS. The majority of pharmaceuticals are designed to be persistent and lipophilic so that they can retain their chemical structure in bodies of aquatic organisms like crustaceans and fish but also after their excretion in the environment for a prolonged period by bioaccumulation and biomagnification throughout the food chain (Martin-Diaz et al., 2009; Aguirre-Martínez et al., 2013; Tsiaka et al., 2013; Chen et al., 2014).

Following the exposure of *C. edule* specimens to CBZ, levels of H_2O_2 increased significantly in all exposure treatments after 48 h and in the highest exposure treatment ($70\,\mu g\,l^{-1}$) after 96 h. These high levels of H_2O_2 indicate oxidative stress and an overproduction of ROS. SOD activity
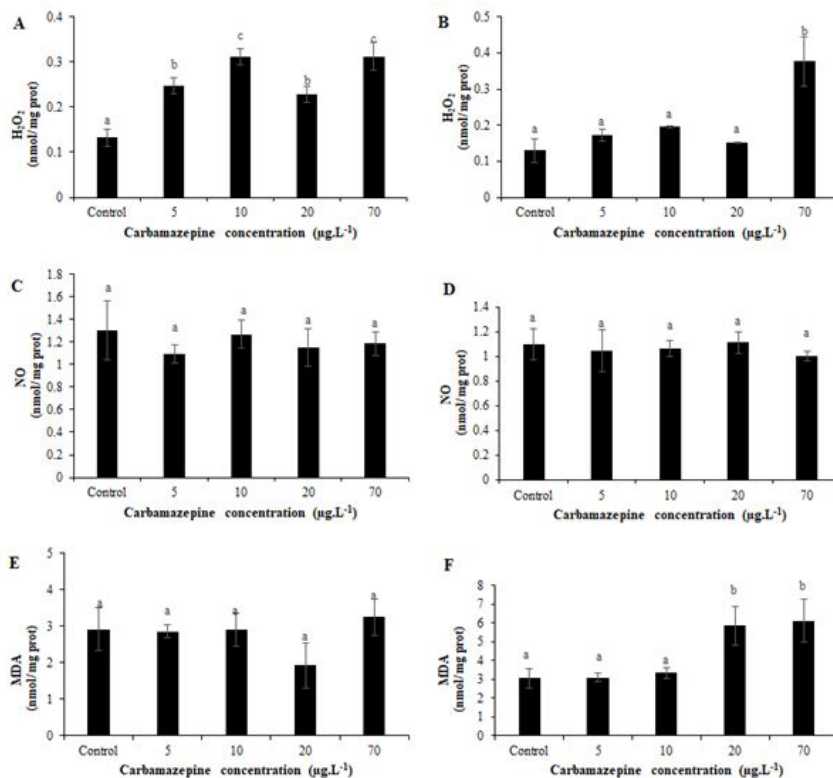
Figure 3. Effect of carbamazepine on proxydants and cellular damage, H_2O_2 level 48 h (A), 96 h (B), nitrite level NO 48 h (C), 96 h (D) and malondialdehyde level MDA 48 h (E), 96 h (F). Values are the mean (± SD) of 5 replicates. Significant differences (p ≤ 0.05) among exposure concentrations, for each condition, are presented with different letters (a-b).

was characterized by a concentration of time-dependent fluctuations following exposure to CBZ. The increase in SOD activity may be explained by an overproduction of the superoxide anion due to the alteration of the oxygen metabolism. The presence of the superoxide anion induces the production of SOD, which then converts it into H_2O_2. The observed increase in SOD activity indicates that this enzyme plays an important role in neutralizing the production of ROS. In this context, Almeida et al. (2014, 2015) and Khazri et al. (2018) previously observed an increase in SOD after clam species' (*R. philippinarum* Adams and Reeve, 1850 and *R. decussatus*) exposure to CBZ. However, Li et al. (2009) reported that SOD activity in rainbow trout, *Oncorhynchus mykiss,* tissues decreased with a CBZ concentration gradient of 0.1, 0.2, and 2 mg l^{-1} following 21 days of exposure. After prolonged exposure (42 days), a strong inhibition of SOD activity was observed and attributed to the overproduction of ROS and the relatively low activity of the antioxidant system. Similarly, Oliveira et al. (2017) and Freitas et al. (2016) reported decreases in SOD activities in relation to CBZ concentrations in a mussel *(M. galloprovincialis)* and clam (*Scrobicularia plana* da Costa, 1778*)* species following exposure.

CAT activity in organisms is known to be strongly influenced by pharmaceutical exposure (Freitas et al., 2015). Herein, an increase of H_2O_2 levels (which induce CAT activity) was induced in specimens from the same

exposure treatment (70 µg l^{-1}) following 96 h of exposure. Studies by Almeida et al. (2015) and Martin-Diaz et al. (2009) have shown that CAT activity was not significantly altered following exposure to CBZ in *R. philippinarum* and *M. galloprovincialis* mussel species. Almeida et al. (2014) found that the H_2O_2 produced by SOD activity was not transformed by CAT but possibly by a GPX enzyme which has similar functions to CAT. In contrast, Chen et al. (2014) noticed opposite results and points of view in the case for specimens belonging to *C. fluminea* after their exposure to 5 and 50 µg l^{-1} CBZ during one month. Moreover, Li et al. (2011) reported for *O. mykiss* that CBZ caused after 96 h an increase in CAT and SOD activities.

The Glutathione-S-Transferase (GST) is a biotransformation enzyme, widely studied in the animal kingdom (Livingstone 1991). Radwan et al. (1992) demonstrated GST activity was induced following exposure of a terrestrial gastropod, *Theba pisana* Muller, 1774, to carbamates. The authors associated this increase in GST activity with a strong detoxification activity in the body in response to carbamates.

We found no significant variation in GST activity after exposure of *C. edule* to carbamazepine. This suggests that GST activity may only be induced above a certain concentration of CBZ, depending on the species (Oliveira et al., 2017). The absorption of this enzyme is metabolized mainly by cytochrome P450 3A4 isoenzymes (Almeida et al.,

2015). These isoenzymes convert them first into inactive metabolites (10,11-epoxycarbamazepine) and then to excretable metabolites (10,11-dihydroxycarbamazepine). In the present study, there was no significant variation in GST in specimens following 48 and 96 h of exposure to CBZ, suggesting that CBZ was not transformed by GST into metabolites. It has also been found that GST isoenzymes oxidize GSH in order to neutralize lipoperoxidation products (Regoli and Giuliani, 2014).

At the highest CBZ treatment (70 µg l^{-1}), MDA levels increased significantly. These results suggest that the production of ROS exceeded their neutralization by antioxidants, thus exhausting the trapping mechanisms and leaving the cellular constituents (lipids and proteins) to be attacked by the overproduction of ROS (Marnett, 1999). Tsiaka et al. (2013) observed an increase in LPO in *M. galloprovincialis* specimens exposed to CBZ (concentrations: 0.01–0 g l^{-1}) for 1 h. In addition, Gagné et al. (2006) found a similar increase in LPO in the hepatocyte of *O. mykiss* following exposure to CBZ for 48 h. An increase of MDA activities was also recorded by Khazri et al. (2018) in the case of *R. decussatus* after its exposure to the CBZ concentrations of 30 and 50 µg l^{-1}.

Cholinesterases, in particular, AChE, have been found in tissues from our test species, *C. edule* (Guinot et al., 2012; Nilin et al., 2012). This enzyme is inhibited by several neurotoxicants. AChE activity is considered to be a specific indicator of exposure to environmental contaminants, such as pesticides and hydrocarbons (Day and Scott, 1990; Bocquené and Galgani, 1991; Guilhermino et al., 2000). In the present study, following exposure to CBZ, there was a significant decrease in AChE activity observed in specimens from the lowest concentration treatment (5 µg l^{-1}) which could be explained in three ways. First, H$_2$O$_2$, produced by organisms when their environment is contaminated with CBZ, can be fixed to active sites of AChE (Schallreuter et al., 2004). Second, the CBZ molecule may be attached to this site because its chemical configuration contains nitrile nitrogen. Alternatively, CBZ may decrease the excitability of neurons by stabilizing the inactivated state of voltage-gated sodium channels (Contardo-Jara et al., 2011).

5. Conclusions

This study elucidates the acute effects of CBZ, an omnipresent pharmaceutical drug in the aquatic environment, on the cockle *C. edule*. The quantification of CBZ by HPLC-UV proved to be an effective method and gave positive results, not only in the water samples, but also in complex matrices such as *C. edule* tissues. The BCF results show that CBZ is a bioaccumulative compound.

In relation to physiological parameters, our CR results for *C. edule* showed a significant decrease in filtration capacity following exposure to CBZ. Even at low concentrations, CBZ induced oxidative stress. Overall, our results highlight the need to improve knowledge on bioaccumulation and the metabolism of pharmaceutical compounds in non-target organisms in aquatic environments. Moreover, our results suggest a dose-dependent effect. SOD and CAT levels changed according to CBZ treatment concentration,

particularly after 98 h of exposure. Nevertheless, the antioxidant system defenses seemed to be appropriately activated in these conditions. Ultimately, when *C. edule* individuals are exposed to CBZ, they exhibit the signs of cellular damage and an inhibition of acetylcholinesterase activity.

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