International Scholars Journals

African Journal of Nursing and Midwifery ISSN 2198-4638 Vol. 7 (12), pp. 001-011, December, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Aquatic toxicity of various pharmaceuticals on some isolated plankton species

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Accepted 21 June, 2019

Four drugs belonging to different therapeutic classes were chosen to examine their toxicity on some plankton organisms of different trophic levels. They include algae (*Chlorella vulgaris* and *Ankestrodesmus falcatus*), protozoa (*Paramecium caudatum*), rotifers (*Brachionus calyciflorus*) and cladocerans (*Daphnia longispina*). The tested drugs are erythromycin (antibiotic), fluoxetine (antidepressant), naproxen (a nonsteroidal anti-inflammatory) and gemfibrozil (lipid regulator). Algae were found to be more sensitive than other tested species to all tested drugs. The antibiotic erythromycin seriously affected the tested organisms even at low concentrations causing remarkable drop in their growth rates compared to the Control of each species. The acute exposure to fluoxetine during the present experiment exerted dramatic effect on both tested algae, with average LC_{50} values of 36 and 40 µg $l^{-1}$for *A. falcatus* and *C. vulgaris*, respectively. On the contrary, fluoxetine enhanced the growth rates for both *B. calyciflorus* and *D. longispina* at low concentrations. *P. caudatum* was the most sensitive species to naproxen exposure, recording the lowest average LC_{50} value of 36 mg l^{-1} among the tested species after 24 h. Gemfibrozil had the least effect on the tested aquatic organisms with average LC_{50} values ranging between a minimum value of 56 mg l^{-1} recorded for *A. falcatus* and 78 mg l^{-1} for *B. calyciflorus.* All the tested organisms suffered from oxidative stress in different degrees as a result of drug exposure. Our results showed reduction in the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) activities and elevation in lipid peroxidation (TBARS) levels in all tested species after exposure when compared with their activities in the control group. The results presented in this study suggest that the drugs investigated have toxic effects on the aquatic organisms tested. We concluded that there is a potential risk for non-target organisms associated with low levels of pharmaceuticals in surface waters. Chronic responses of non-target biota are not fully expected. Therefore, safe ways of discarding drug residues should be developed in order to protect the aquatic fauna.

Key words: Catalase, lipid peroxidation, algae, zooplankton, pharmaceuticals.

INTRODUCTION

Drugs are not environmentally different from other chemicals. In fact, high quantities of pharmaceuticals are discharged into sewage treatment plants when used for human cure and directly into surface waters or on soil when used for veterinary purposes (Isidori et al., 2005). They are biologically active compounds that may interfere with specific biological systems (for example, enzymes) or generically act depending on their properties (Wiegel et al., 2004). Water-soluble compounds may contaminate ground waters because of their mobility, while lipophilic compounds may accumulate in sediments or soils

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(Fent et al., 2006). Because of their physicochemical and biological properties, there are concerns about the potential for their impacts to non-target species (Park, 2005).

Literature data rarely provide qualitative and quantitative information on the processes that determine fate and effects of these active substances (Halling-Sbrensen et al., 1998; Ternes, 1998) and their derivatives from biotic or abiotic transformations, that may make by-products more harmful than parent compounds (DellaGreca et al., 2003; Andreozzi et al., 2003). Some authors reported information on the environmental occurrence of drugs, pointing out those significant amounts of several pharmaceuticals discharged in the environment and not completely removed by waste water treatment plants (DellaGreca et al., 2007) have been detected in surface waters and occasionally in ground waters (Ternes, 1998; Heberer et al., 2002; Zuccato et al., 2006). Different studies suggest that therapeutic substances at concentrations found in the environment may have effects on aquatic organisms (Daughton and Ternes, 1999; Ferrari et al., 2003; Isidori et al., 2005). The disturbance they cause to the microbial life in surface waters was reported by Ku¨mmerer (2001b), while their effects at low concentrations on other organisms were examined by Baguer et al. (2000) and Halling-Sbrensen (2000). During the present study, 4 drugs belonging to different therapeutic classes were chosen to examine their toxicity on some plankton organisms of different trophic levels. The tested drugs are erythromycin (antibiotic), fluoxetine (antidepressant), naproxen (a nonsteroidal anti-inflammatory) and gemfibrozil (lipid regulator).

Recently, several studies have detected the presence of these pharmaceuticals in sewage, surface, ground and drinking waters. Erythromycin was present at all the sampling sites in the rivers Po and Lambro in Northern Italy at concentrations between 10 and 100 ng L^{-1} (Calamari et al., 2003; Castiglioni et al., 2004). The few literature data regarding erythromycin report that its toxicity increased with time and not with concentration (Migliore et al., 1997). A chronic experiment showed that algae, rotifers and crustaceans were particularly affected by erythromycin with EC_{50} in the range of 10–100 mg L^{-1}, while acute EC_{50} were in the range of 10–30 mg L^{-1} for all the organisms (Isidori et al., 2005a).

Fluoxetine is the active ingredient of Prozac, a commonly prescribed selective serotonin reuptake inhibitor (SSRI). SSRIs are primarily indicated for depression, but are also prescribed to treat compulsive behavior, and eating and personality disorders. Fluoxetine is detected in surface water in concentrations ranging from 12 ng L^{-1} in streams (Kolpin et al., 2002) to 46 ng L^{-1} near STP effluents (Metcalfe et al., 2003b). Brooks et al. (2003) indicated that an increase in the crustacean *Ceriodaphnia dubia* fecundity was also observed with 56 mg/l fluoxetine treatment. Flaherty et al. (2001) observed a comparable reproductive stimulation when *Daphnia magna* were exposed to 36 mg l^{-1} fluoxetine for 30 days. The SSRIs fluoxetine, fluvoxamine, and sertraline were toxic to algae in 96 h acute growth inhibition assays (Johnson et al., 2007). The lowest reported effect level for aquatic organisms is 13.6 g L^{-1}, at which growth of the alga *Pseudokirchneriella subcapitata* was significantly reduced after 5 days (Brooks et al., 2003; De Lange et al., 2006).

Naproxen is a non-steroidal anti-inflammatory drugs (NSAID), known for the anti-inflammatory, analgesic and antipyretic properties, rapidly phototransformed and biodegraded in the environment. Nevertheless, the naproxen daily load in municipal sewage treatment plants is in the order of grams (Ternes, 1998; Tixier et al., 2003). So as to be considered as a persistent compound due to its constant discharge into the aquatic environment with continuous exposure of non-target aquatic organisms (Isidori et al., 2005b).

Gemfibrozil is one of the fibric acid derivatives (fibrates), which is a class of medicines that lower blood triglyceride levels. Most fibrates administered are absorbed (90%) and excreted unmodified (Isidori et al., 2007). In Brazil, the highest concentrations of pharmaceuticals in sewage and surface waters were found for the fibrates. These lipid regulators were found at concentrations of up to 4.76 (gemfibrozil), 1.07 (bezafibrate), 0.16 µg l^{-1} (fenofibrate) in the effluents from WWTPs in Italy, France and Greece, respectively (Andreozzi et al., 2003). Their persistence against biodegradation could be one of the major reasons for their occurrence (Stumpf et al., 1999).

Aquatic drug toxicity affects many aspects of cellular metabolism. In recent years, growing attention has been devoted to the damage induced by oxygen radicals to cells under stress conditions. Superoxide radicals, O^{2-}, are formed as by-products of cell metabolism. These oxidant radicals can oxidize membrane lipids and cause denaturing of nucleic acids and proteins (Perelman et al., 2006). To minimize oxidative damage to cellular components, organisms have developed antioxidant defenses. Important antioxidant enzymes are the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Halliwell and Gutteridge, 1999; Barata et al., 2005). Organisms can adapt to increasing reactive oxygen species (ROS) production by up-regulating antioxidant defenses, such as the activities of antioxidant enzymes (Livingstone et al., 1992, 2003). Failure of antioxidant defenses to detoxify excess ROS production can lead to significant oxidative damage including enzyme inactivation, protein degradation, DNA damage and lipid peroxidation (Barata et al., 2005). Lipid peroxidation is considered to be a major mechanism, by which oxyradicals can cause tissue damage, leading to impaired cellular function and alterations in physicochemical properties of cell membranes, which in turn disrupt vital functions (Rikans and Hornbrook, 1997). Lipid peroxides are known to decompose and produce a variety of substances, the most important of which is

Table 1. Therapeutic classes and international environmental detection of the tested pharmaceuticals.

Drug	Therapeutic class	Presence in surface water	References
Erythromycin	Human and veterinary antibiotic	positive	In US streams, Po and Lambro Rivers in Northern Italy, Germany and Spain (Hernando et al., 2006; Costanzo et al., 2005;Castiglioni et al., 2004; Calamari et al., 2003; Kolpin et al., 2002)
Fluoxetine	Antidepressant: selective serotonin reuptake inhibitor(SSRI)	positive	In US streams, Canada and Netherlands (Johnson et al., 2007; Lang et al., 2006; Brooks et al., 2003; Kolpin et al., 2002; Weston et al., 2001)
Naproxen	Non steroidal anti-inflammatory (NSAID)	positive	In USA, Italy and Spain (Hernando et al., 2006; Isidori et al., 2005; Boyd et al., 2003; Tixier et al., 2003)
Gemfibrozil	Lipid regulator	positive	Italy, France, Greece and Brazil (Isidori et al., 2007; Andreozzi et al., 2003; Stumpf et al., 1999)

Table 2. Concentrations of the tested drugs for each species during the acute toxicity experiment.

Tested organism	Erythromycin (mgl^{-1})	Fluoxetine (μgl^{-1})	Naproxen (mgl^{-1})	Gemfibrozil (mgl^{-1})
Chlorella vulgaris	8,10,12,16, 20	30,36,40,45,50	38,40,42,44,46	50,56,60,65,70
Ankestrodesmus falcatus	8,10,12,16, 20	30,36,40,45,50	38,40,42,44,46	50,56,60,65,70
Paramecium caudatum	10,12,16, 18, 24	100,125,150,175,200	30,34,36,40,45	55,60,66,70,75
Brachionus calyciflorus	20, 24,28, 30, 32	180,200,230,250,270	50,55,60,65,70	65,74,78,85,90
Daphnia longispina	20, 24,28, 30, 32	750,800,830,850,875	70,75,82,90,95	65,70,74,80,85

malondialdehyde (MDA), which is regarded as a good biomarker of the degree of oxidative stress in animals (Sohal, 1997). In *Daphnia* species, recent studies have reported antioxidant enzyme responses including CAT and SOD to UV radiation and varying oxygen concentration (Vega and Pizarro, 2000; Borgeraas and Hessen, 2000, 2002).

The aim of this investigation is to focus on 4 therapeutic classes of environmental concern which include antibiotics, antidepressant, non-steroidal anti-inflammatory and lipid regulators. This screening will only predict traditional environmental toxicity estimates over four trophic levels. They include algae (*Chlorella vulgaris* and *Ankestrodesmus falcatus*), protozoa (*Paramecium caudatum*), rotifers (*Brachionus calyciflorus*) and cladocerans (*Daphnia longispina*). The toxicity assessment is based on survival and biochemical responses (the variation in whole body antioxidant enzyme activities and lipid peroxidation of the investigated organisms) in order to obtain more information on the risk involved with the emission of drugs into the environment.

MATERIALS AND METHODS

Test drugs and concentrations

Tested pharmaceuticals, their therapeutic classes and presence in surface water are indicated in Table 1. Stock solutions were prepared by dissolving test compounds in dimethyl sulphoxide (DMSO), from which test solutions were prepared with appropriate dilution medium for each test species. DMSO concentration was 0.01% v/v and water was used for the dilution. Range-finding tests were previously performed for acute evaluation to approximate the appropriate toxic concentration for each organism. The used doses of the tested drugs for each species are given in Table 2.

Algal cultures

Cultures of test organisms were obtained from our laboratory at Girls College, Ain Shams University. To prevent contamination, the algae species were only cultured one at a time and 10–20 ml of the culture was transferred to a new media on a weekly basis. Occurrence of contamination with other algal species was assessed monthly using a Nikon compound microscope at 250 magnification and contaminated cultures were discarded. Half strength Bristol's medium (Bold, 1949) was used for culturing and testing. Media were autoclaved prior to use.

Culture of the studied zooplankton species

One species of freshwater ciliates was selected namely *Paramecium caudatum*. Organisms belonging to the selected species were obtained initially from the surface scum of shallow waters in the littoral zone of Ismailia Canal (Egypt). *Paramecium* individuals were cultured in mineral water supplemented with boiled

rice and wheat grains. The mineral water used as culture medium had total hardness (as $CaCO_3$) of 103 mg $l^{-1}$and pH 7.6. The ciliate cultures were maintained at 25°C, oxygen saturation O45%, and photoperiod of 16:8 h light: dark. Mineralized water with the previous conditions was also used for culturing the isolated species of both Brachionus and Daphnia.

Daphnia organisms and *Brachionus calyciflorus* were isolated separately from the Nile water and reared in 6 L glass jars. Cultures were maintained at 25±1°C and Photoperiod of 16L: 8D was maintained. The culture water was renewed twice a week. Daphnids were fed with yeast and algae (*Selenastrum capricornutum*) daily.

Growth rate

The growth rate, r was calculated for the number of organisms in each treatment and controls with the formula:

$$r = \frac{\ln N_{final} - \ln N_{start}}{T}$$

Where, N_{final}, number of rotifers in each cup at the end of the experiment; N_{start}, number of rotifers in each cup at the start of the test; T, time of exposure. The mean r values were subsequently calculated for each individual type of experiment.

Test procedure

The tests were carried out in 250 ml beakers filled with 100 ml of growth medium and autoclaved. Hardness was 80–100 mg l^{-1} as $CaCO_3$ and the dissolved oxygen content was at least 90% saturation at the beginning of the test. All bioassays were conducted under static conditions and included at least a negative and a reference toxicant control.

Algae, from a culture 5–7-day old (10^5–10^6 cells ml^{-1}, enumerated by cell counts with a Sedgwick– Rafter counting chamber), were then added to each cooled test flask to give a cell count of 10^4 cells ml^{-1}. Three replicates were used for each concentration after an initial range-finding test was conducted. The tests were run in a growth chamber under continuous cool white fluorescent light at 4300-7200 lux at a temperature of 25°C. For assays with algal cultures test beakers were shaken to homogenize the cultures and then 2-10 ml samples were counted using a counting chamber. Tests on *Paramecium* were performed in 24 well plates, 30 individuals per well (1.0 ml of test solution), 3 replicates per concentration, 5 concentrations and a negative control. Tests on *Brachionus* and *Daphnia* were carried out in 250 ml glass beakers, 30 organisms per beaker, 3 replicates per concentration, 5 con-centrations and a negative control (U.S. EPA, 1993). Test duration was 72 h, the test parameter considered was mortality and the concentration found to kill 50% organisms was indicated as LC_{50}.

Biochemical analysis

Enzymatic activities and lipid peroxides

Individuals (beaker wet mass 0.08–0.12 g) were homogenized at 48°C in 1: 4 wet weight /buffer volume ratio in 100 mM phosphate buffer, pH 7.4, containing 100 mM KCl and 1 mM EDTA. Homogenates were centrifuged at 10,000 g for 10 min and the supernatants were immediately used as enzyme sources for CAT and SOD activities, and lipid peroxide analysis. Biochemical measurements were carried out using a JENWAY Spectrophotometer model No. 6105 UV/VIS at 25 ±0.58°C. Assays were run at least in duplicate.

Lipid peroxidation

Lipid peroxidation was measured by the thiobarbituric reactive species (TBARS) assay, which measures the production of malondialdehyde (MDA) that reacts with thiobarbituric acid (Ohkawa et al., 1979).

Catalase (CAT) activity

CAT activity was measured by the decrease in absorbance at 240 nm due to H_2O_2 consumption (extinction coefficient; 40 M^{-1} cm^{-1})) according to Aebi (1974). The reaction volume was 1 ml, which contained 50 mM phosphate buffer, pH 6.5, 50 mM H_2O_2 (Ni et al., 1990). The reaction was started by the addition of the sample.

Superoxide dismutase (SOD) activity

SOD activity was determined by an indirect method involving the inhibition of cytochrome C reduction. In this method, SOD competes with cytochrome C for O_2^- generated by hypoxanthine and xanthine oxidase action. The reduction of cytochrome C by O_2^- was monitored by the absorbance increase at 550 nm **(**Mc Cord and Fridovich, 1969). The reaction solution contained 50 mM phosphate buffer, pH 7.8, 0.1 mM EDTA, 50 AM hypoxanthine, 5.6 mU xanthine oxidase and 10 AM cytochrome C (Livingstone et al., 2001). The results of this enzymatic assay are given in units of SOD per milligram of protein (U mg^{-1}), where 1 U of SOD is defined as the amount of sample causing 50% inhibition of cytochrome c reduction. Proteins were measured by the method of Lowry et al. (1951), using serum albumin as standard

Statistical analyses

Bivariate Pearson correlation analysis was also performed (by using SPSS Ver. 13 Program) between exposure treatments of the studied compounds, enzyme activities and lipid peroxidation to identify patterns of response of the studied oxidative stress indicators. Significant differences were established at $p<0.05$.

RESULTS

Toxicity to aquatic organisms

Acute toxicity data of the tested drugs expressed as median lethal concentrations (LC_{50}) to different aquatic organisms are presented in Table 3. LC_{50} values for all tested drugs were achieved after 24 h for both algal species and 48 h for *paramecium, Brachionus* and *Daphnia* except for Naproxen, where LC50 value of *Paramecium* was achieved after 24 h. The sensitivity of the algal species *A. falcatus* to all tested drugs was higher than *C. vulgaris*. Among zooplankton species, the protozoan *P. caudatum* was more sensitive followed by *D. longispina*, while *B. calyciflorus* was the most tolerant among the investigated organisms.

During the acute experiment, the antibiotic erythromycin was bioactive at low concentrations for both *A. falcatus* and *D. longispina*, where some cases of decaying in the cell contents and cell wall rupture were

Table 3. Acute toxicity values of the tested drugs on the investigated organisms (LC_{50}).

Tested organism	Erythromycin (mgl^{-1})		Fluoxetine (μgl^{-1})		Naproxen (mgl^{-1})		Gemfibrozil (mgl^{-1})	
	Doses	Time (h)	Doses	Time (h)	Doses	Time (h)	Doses	Time(h)
Chlorella vulgaris	12	24	40	24	42	24	60	24
Ankestrodesmus falcatus	10	24	36	24	40	24	56	24
Paramecium caudatum	16	48	150	48	36	24	66	48
Brachionus calyciflorus	28	48	230	48	60	48	78	48
Daphnia longispina	24	48	830	48	82	48	74	48

recorded. Average LC_{50} values for *A. falcatus* was 10 mg l^{-1}, followed by *C. vulgaris*, *P. caudatum* and *D. longispina* recording 12, 16 and 24 mg l^{-1}, respectively. Average LC_{50} of 28 mg l^{-1} was estimated for *B. calyciflorus* after 48 h**,** recording the highest value among the other tested organisms. The effect of Fluoxetine exposure on the growth rate was unique for all tested organisms therefore, it will be mentioned in details later on this paper. The average LC_{50} values of the tested organisms according to their sensitivity to fluoxetine were: 36, 40, 150, 230 and 830 µg $l^{-1}$for *A. falcatus, C. vulgaris, P. caudatum, B. calyciflorus* and *D. longispina*, respectively. During the present acute experiment *Daphnia* seemed to tolerate well the low concentrations of fluoxetine as well as *Brachionus*. On the other hand, cell decaying and rupture was observed at the higher concentrations for all species. Fluoxetine was found to be toxic for both algal species even at low concentrations, where it caused cell rupture and cell deformations in all used concentrations. In the group exposed to Naproxen, *P. caudatum* was the most sensitive species recording the lowest average LC_{50} value of 36 mg l^{-1} among the tested species observed after 24 h. On the contrary, *D. longispina* was the most tolerant species with average LC_{50} of 82 mg l^{-1}recorded after 48 h. The average LC_{50} values of *A. falcatus, C. vulgaris* and *B. calyciflorus* were 42, 40 and 60 mg l^{-1}, respectively. All tested organisms suffered from cell rupture at high doses of Naproxen. The lipid regulator, gemfibrozil had the least effect on the tested aquatic organisms among the tested drugs. Although mortalities were reported at higher concentrations, cell deformations were minimal at lower concentrations. The average LC_{50} values ranged between a minimum value of 56 mg l^{-1} recorded for *A. falcatus* and 78 mg l^{-1} for *B. calyciflorus.*

Biochemical activities

Enzyme activity responses and lipid peroxidation of each tested species with various drug exposures are presented in Figure 1. Results of antioxidant enzymes activity (SOD and CAT) revealed similar behaviors. Both enzymes showed reduction in their activities in all tested species after being exposed to each of the tested pharmaceuticals compared to their activities in the control group. The variations in SOD activity were narrow among the different drug treated groups. The lowest significant inhibition of SOD activity value was 5 µ mg^{-1} protein, observed in *A. falcatus* exposed to erythromycin and *C. vulgaris* exposed to fluoxetine, compared to 10 and 11 µm g^{-1} protein recorded for both species in the control group, respectively (p<0.05).

CAT enzyme activity values showed a remarkable significant reduction in all species exposed to erythromycin. *A. falcatus* recorded the greatest reduction in CAT activity from 250 µm g^{-1} protein in the control group to 50 µm g^{-1} protein after erythromycin exposure. The lowest significant reduction in CAT activity compared to the control was observed in gemfibrozil group and naproxen group particularly for *B. calyciflorus* and *D. longispina*.

Lipid peroxidation measured as TBARs levels tends to increase in all species under exposure to the four studied drugs compared to the control. *B. calyciflorus* and *D. longispina* showed greater significant elevation in their lipid peroxidation levels (1.6 and 1.4 µ mol mg^{-1} protein, respectively) after exposure to erythromycin compared to control values (0.7 and 0.6 µmol mg^{-1} protein, respectively). These two species showed significant increase in their lipid peroxidation levels after exposure to all tested drugs more than all other tested species (p<0.05). Fluoxetine was the second drug to cause elevation in TBARs levels in the tested organisms. *B. calyciflorus* recorded the highest significant elevation in TBARs levels among species exposed to fluoxetine with a value of 1.4 µmol mg^{-1} protein compared to 0.7 recorded in the control group (p<0.05).

Growth rate

The average growth rate of each species after 72 h of exposure compared to that of the control group is presented in Figure 2. The results revealed dramatic significant inhibition in growth rates of all species in the erythromycin treated group (p<0.05). Erythromycin was found to inhibit growth rate of *A. falcatus* to 0.2 compared to 1.02 recorded for the same species in the control group. Naproxen equally and significantly inhibited the

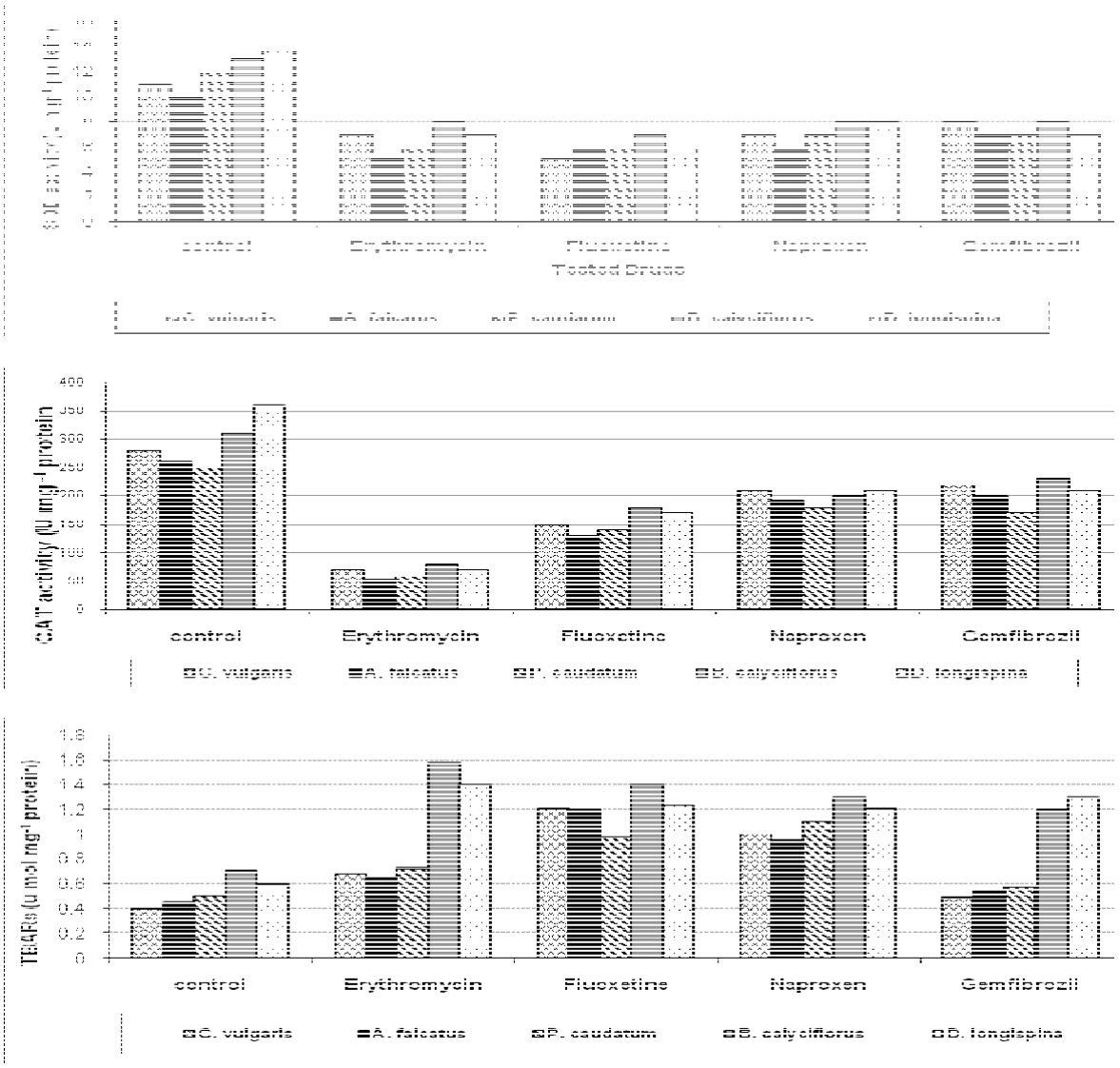

Figure 1. Levels of lipid peroxidation expressed as TBARS (µ mol mg^{-1} protein), antioxidant enzymes activities: SOD (U mg^{-1} protein) and CAT (µmol mg^{-1} protein) in the studied organisms exposed to the tested drugs after 24 h for algal species and' 48 h for the zooplankton species.

growth rate of both *A. falcatus* and *P. caudatum* with a value of 0.3 compared to 1.01 for both species in control group (p<0.05). Other species were less affected with naproxen with growth rate values of 0.4, 0.5 and 0.6 for *C. vulgaris, B. calyciflorus* and *D. longispina,* respectively. Gemfibrozil significantly inhibited the growth rate of the tested species in the following order: 0.4 (*A. falcatus*), 0.5 (*C. vulgaris* and *P. caudatum*) and 0.7 (*B. calyciflorus* and *D. longispina*) with p<0.05.

Fluoxetine was found to exhibit a two directional effect depending on the tested species and drug concentration. Therefore the growth rate of all tested species at different drug doses is shown in Figure 3. For all algal species, fluoxetine was found to significantly inhibit the growth rate even at low concentrations until it reached 0.25 for both species by the end of the acute experiment (p<0.01). Fluoxetine slightly inhibited the growth rate of *P. caudatum* at low concentrations but caused a great drop in LC_{50} and higher concentrations. For both *B. calyciflorus* and *D. longispina*, fluoxetine significantly enhanced their growth rates at low concentrations (p<0.01), then drops occurred in their growth rates by reaching LC_{50} values of both species and the higher doses.

DISCUSSION

The toxic effects of the pharmaceuticals erythromycin, fluoxetine, naproxen and gemfibrozil were assessed on organisms of the aquatic food web, using standard

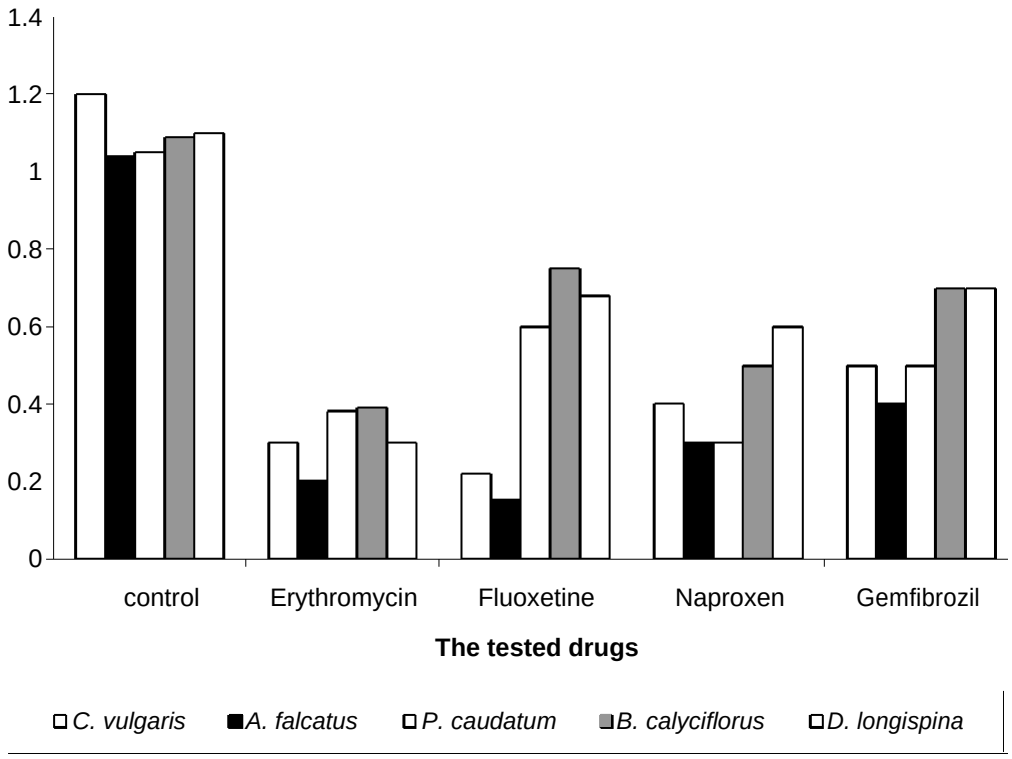

Figure 2. Growth rate values of the plankton organisms after 72 h of exposure to the tested drugs.

bioassays to enlarge the eco-toxicological information about the risk of these drugs when they reach the environment. Detectable quantities of the previous drugs have been found in surface waters (Table 1), soils and sediments (Hernando et al., 2006) but only few studies have investigated their toxic effects on aquatic and terrestrial organisms (Boxall et al., 2004). During the present study, algae were found to be more sensitive than other tested species to all tested drugs. The antibiotic erythromycin seriously affected the tested organisms even at the low concentrations causing remarkable drop in their growth rates compared to the control of each species. The average LC_{50} for the algal specie *A. falcatus* was 10 mg l^{-1}. This result coincides with Isidori et al. (2005) who showed that algae, rotifers and crustaceans were particularly affected by erythromycin with acute ECs_{50} in the range of 10-30 mg L^{-1} for all the organisms tested except the bacterial species. They also suggest that long term effects cannot be excluded at environmental concentrations (0.016-0.020 mg L^{-1}). The few literature data regarding erythromycin report that its toxicity increased with time and not with concentration (Migliore et al., 1997; Pomati et al., 2004; Isidori et al., 2005; Kim et al., 2007). From this fact arises the risk of all drug residues in the aquatic environments.

Among the issues raised by environmental detection of fluoxetine are concerns over potential sub-lethal effects on aquatic organisms, including behavioral responses (Brooks et al., 2003). Considering the potential for environmental SSRIs to act as they do in humans, to alter or increase serotonin concentrations, the potential disruptive effects of chronic exposure must be considered. Serotonin is likely to be one of the most potent and ubiquitous neuromodulators in vertebrates (Azmitia, 1999). Because of the critical nature of the functions regulated by serotonin, there is a potential for environmental SSRIs to alter appetite, the immune system, and reproduction as well as other behavioral functions (Mossner and Lesch, 1998; Meguid et al., 2000; Fong, 2001).The acute exposure to fluoxetine during the present experiment exerted dramatic effect on both tested algae with average LC_{50} values of 36 and 40 µg $l^{-1}$for *A. falcatus* and *C. vulgaris*, respectively. Johnson et al. (2007) concluded that fluoxetine would be considered very toxic to algae. Brooks et al. (2003) observed that fluoxetine adversely reduced growth of a green algae, *Pseudokirchneriella subcapitata* and the mechanism by which fluoxetine exerts its toxicity on algae has not been understood yet. However, Munoz-Bellido et al. (2000) identified that fluoxetine has antibacterial properties, potentially interfering with efflux pumps.

Our data revealed that fluoxetine slightly decreased the growth rate and activity of the ciliate *P. caudatum* at low concentrations. De Lange et al. (2006) concluded that low concentrations (10–100 ng L^{-1}) of fluoxetine and Ibuprofen reduce the activity of *Gammarus pulex*.

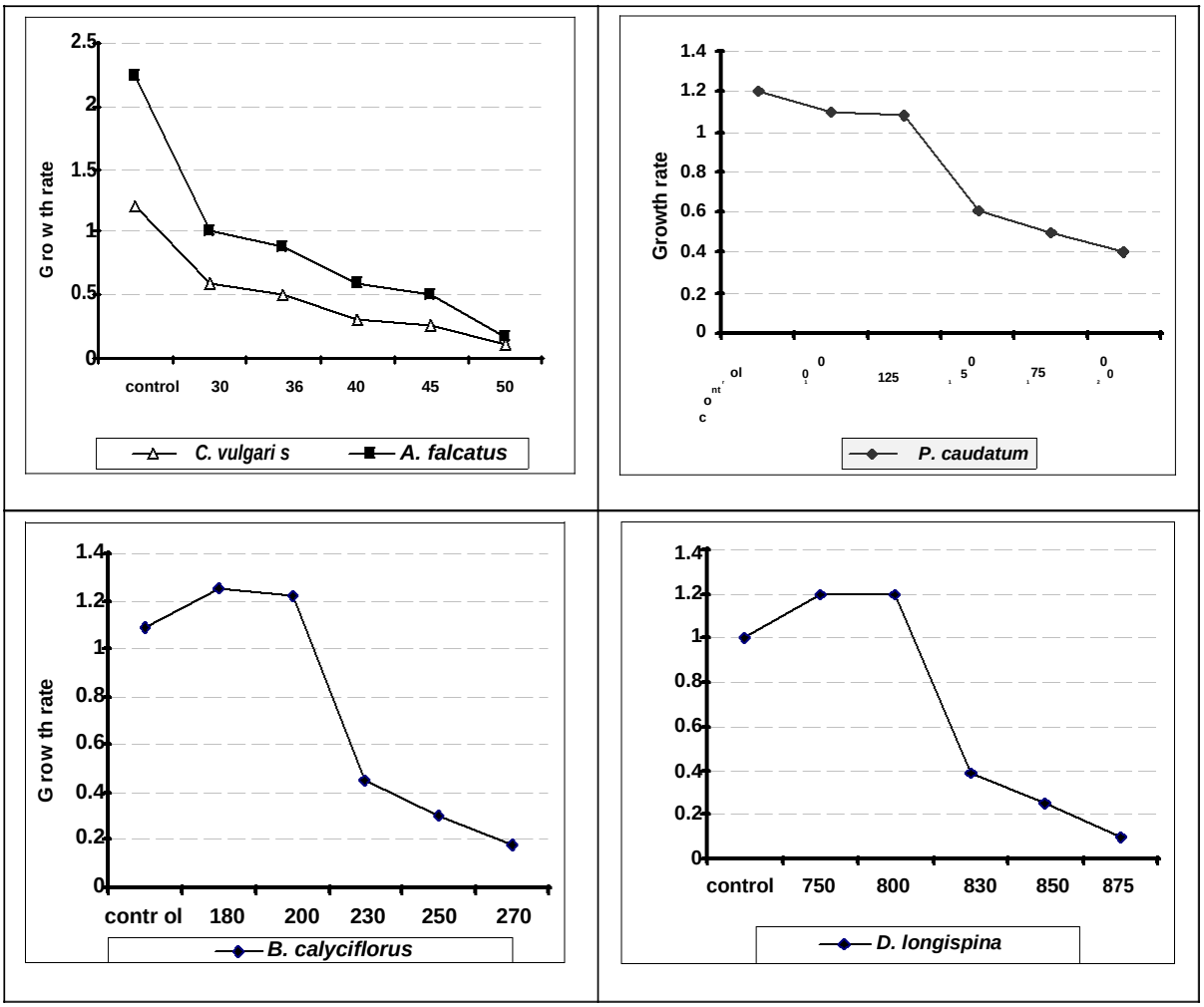

Figure 3. Effects of different dose of Fluoxetine (µg l^{-1}) on growth rate values of the tested organisms after 24 h of exposure for algae and 48 h for zooplankton.

Environmental concentrations of those compounds in surface waters are high enough to reduce *P. caudatum* activity. Weston et al. (2001) detected fluoxetine in municipal effluents from 0.32 to 0.54 mg l^{-1} and Kolpin et al. (2002) reported maximum fluoxetine levels of 0.012 mg l^{-1} in surface waters. The observed effect of fluoxetine may be related to the presence of the amino acid serotonin in the aquatic organisms. Since fluoxetine is a selective serotonin reuptake inhibitor, use of fluoxetine increases the serotonin concentration (De Lange et al., 2006). Serotonin is involved in a wide array of physiological roles, such as reproduction in molluscs, behavior and reflexes in molluscs, and release of neurohormones in crustaceans (Daughton and Ternes, 1999). On the contrary, fluoxetine enhanced the growth rates for both *B. calyciflorus* and *D. longispina* at low concentrations during the present experiment. This result agrees with Brooks et al. (2003) who observed an increase in *Ceriodaphnia. dubia* fecundity with 56 mg/l fluoxetine treatment. Flaherty et al. (2001) observed a comparable reproductive stimulation when *Daphnia magna* were exposed to 36 mg l^{-1} fluoxetine for 30 days. Similarly, Fong et al. (1998) observed fluoxetine to induce mussel spawning. We can explain the enhancement in growth rate and activity of both *B. calyciflorus* and *D. longispina* at low concentrations of fluoxetine by the fact that in invertebrates, serotonin may stimulate ecdysteroids, ecdysone, and juvenile hormone, which are responsible for controlling oogenesis and vitellogenesis (Nation, 2002). The role of serotonin in reproduction, and therefore the potential for SSRIs to disrupt normal serotonin function, varies across family groups. Serotonin and SSRIs potentiate spawning and oocyte maturation in some bivalves and crustaceans (Fong, 2001). On the other hand, during the present experiment fluoxetine caused cell rupture and cell deformations in all used concentrations for algae and at higher concentrations for zooplankton species. The same phenomenon was

reported by Brooks et al. (2003), when fish physiological and reproductive responses were evaluated, lower fluoxetine exposure levels of 0.1 and 0.5 mg l^{-1} affected female Japanese medaka plasma estradiol levels and the number of developmental abnormalities were elevated at all exposure levels.

Naproxen is a common non-steroidal anti-inflammatory drug (NSAID) used as analgesic, antipyretic and anti-inflammatory. During the present study, *P. caudatum* was the most sensitive species to naproxen exposure recording the lowest average LC_{50} value of 36 mg l^{-1} among the tested species observed after 24 h. On the contrary, *D. longispina* was the most tolerant species with average LC_{50} of 82 mg l^{-1}recorded after 48 h. All tested organisms suffered from cell rupture at high doses of naproxen. Isidori et al. (2005) observed that naproxen was bioactive at low concentrations mainly for the primary consumers *B. calyciflorus* and *Ceriodaphnia. Dubia*, while algae showed toxicity values two order of magnitude lower than rotifers and crustaceans. Naproxen was detected widely in surface water of Brazil, Canada, and Switzerland (Stumpf et al., 1999; Boyd et al., 2003; Tixier et al., 2003). The environmental long-term effects of continuous low-level exposure to naproxen, as well as other drugs, are not well studied even if the detection of the drugs in sewage, river and stream water samples was in small concentrations. Isidori et al. (2005) indicated that byproducts of naproxen were more toxic to the non-target aquatic organisms than parent compounds.

Gemfibrozil is one of the common used fibrates (lipid regulator) which are a class of medicines that lower blood triglyceride levels. Fibrates act by reducing the liver's production of very low density lipoprotein (VLDL) (the triglyceride-carrying particle that circulates in the blood) and by speeding up the removal of triglycerides from the blood. Most fibrates administered are absorbed (90%) and excreted unmodified. Gemfibrozil had the least effect on the tested aquatic organisms among the tested drugs during the present study. Although mortalities were reported at higher concentrations, cell deformations were minimal at lower concentrations. The average LC_{50} values ranged between a minimum value of 56 mg l^{-1} recorded for *A. falcatus* and 78 mg l^{-1} for *B. calyciflorus.* Isidori et al. (2007) demonstrated that acute exposure to fibrates and their derivatives do not represent a potential hazard for the aquatic environment considering the low yields of byproducts obtained. Our data agree with the study of Nunes et al. (2006) who evaluated the oxidative stress of *Artemia partenogenetica* after acute exposure to fibrates at concentrations significantly higher than levels found in the environment. The authors showed that the acute chemical insult to this class of pharmaceuticals was not considerable. Chronic data about the inhibition of the reproduction for *B. calyciflorus* and *C. dubia* were report ed (Isidori et al., 2007). They suggested that no toxic adverse effects occur at fibrate concentrations detected in the environment. On the other hand, the harmfulness of the drugs investigated cannot be excluded when considering the DNA damage found for some products. In addition, possible mixture toxicity, synergistic and additive effects, bioaccumulation and bio-magnification of these drugs should be evaluated even if this class of pharmaceuticals is going to be replaced by statins in lowering blood triglyceride levels (Studer et al., 2005).

During the present acute experiment, all the tested aquatic organisms suffered from oxidative stress in different degrees as a result of drug exposure. The degree of stress was dependent on the concentration of each drug. The symptoms of the oxidative stress were obvious and recognized by cell wall rupture dialysis in cell contents and deformation in the body shape. Oxidative symptoms were also confirmed through biochemical analysis by inhibition of the antioxidant enzymes activity and elevated levels of lipid peroxidation. Since induction of antioxidants at the beginning of exposure represents a cellular defense mechanism to counteract toxicity of reactive oxygen species (ROS), inhibition of these enzymes activities take place in case of intense production of ROS under sever exposure conditions (Pandey et al., 2003). Therefore, biochemical activities have been extensively used in several field studies to assess the extent of pollution in rivers, lakes and coastal waters (Goksoyr, 1995). SOD and CAT, considers among the major antioxidant enzymes may be useful biomarkers (Lackner, 1998). It has been suggested that the functional decline and increase in mortality rates with high drug concentrations observed in most species, is the result of accumulated molecular damage from ROS (Sohal, 2002; Finch, 1990) and that decline of antioxidant defenses may be one of the physiological mechanisms promoting oxidative damage and hence senescence of organisms.

Our results show reduction in the antioxidant enzymes SOD and CAT activities in all tested species after being exposed to each of the tested pharmaceuticals compared to their activities in the control group. The SOD–CAT system provides the first defense against oxygen toxicity (Barata et al., 2005). SOD catalyzes the dismutation of the superoxide anion radical to water and hydrogen peroxide, which is detoxified by the CAT activity. Usually, a simultaneous induction response in the activities of SOD and CAT is observed when exposed to pollutants (Dimitrova et al., 1994). The low levels of CAT could be attributed to high production of superoxide anion radical, which has been reported to inhibit CAT activity in case of excess production (Kono and Fridovich, 1982). An additional reason for the low antioxidant enzymes activities in the present study was that the enzymes activities were measured by the end of the acute experiment, which means high stress on all tested species after exposure to high drugs concentrations. In this study, it was observed that SOD and CAT responded to exogenous chemical sources of ROS. In *Daphnia* species, recent studies have reported antioxidant enzyme responses including CAT, SOD, GST and oxidative tissue damage (lipid

peroxidation) to drug exposure (Alonzo et al., 2006). Lipid peroxidation is considered to be a major mechanism by which oxyradicals can cause tissue damage leading to impaired cellular function and alterations in physico-chemical properties of cell membranes, which in turn disrupt vital functions (Rikans and Hornbrook, 1997). Our results indicate that acute drug exposure caused a significant oxidative stress in all tested species, detected by the increase in TBARs. In our experimental *B. calyciflorus* and *D. longispina* showed greater lipid peroxidation levels (1.6 and 1.4 µ mol mg^{-1} protein, respectively) after exposure to erythromycin compared to control values (0.7 and 0.6 µ mol mg^{-1} protein, respectively). These two species showed increase in their lipid peroxidation levels after exposure to all tested drugs more than all other tested species. Fluoxetine was the second drug to cause elevation in TBARs levels in the tested organisms. *B. calyciflorus* recorded the highest elevation in TBARs levels among species exposed to fluoxetine with a value of 1.4 µ mol mg^{-1} protein compared to 0.7 recorded in the control group. It is well known that different organisms are equipped with enzyme systems that protect the cell from oxidative damage (Alonzo et al., 2006). However, mortality was not reduced, suggesting that the large effect of ROS results from drug exposure (Dutra et al., 2007).

The results presented in this study suggest that the drugs investigated have toxic effects on the aquatic organisms tested. The algae were found to be the most sensitive species. We concluded that there is a potential risk for non-target organisms associated with low levels of pharmaceuticals in surface waters. Among the classes of pharmaceuticals investigated, antibiotics were shown to be the most harmful for the aquatic environment. Chronic responses of non-target biota, many of which are unidentified, may result from nanomolar or potentially even picomolar exposure (Fong, 1998). Pharmaceuticals can reach surface waters and although they can be degraded in the environment by biotic or abiotic process, it is assumed that drugs could act as persistent compounds simply because of their continual infusion into aquatic media (Isidori et al., 2007). Therefore, safe ways of discarding drug residues should be developed in order to protect the aquatic fauna. More drug toxicity tests must be carried out on different levels of aquatic organisms to be able to predict their impact on our aquatic environment.

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