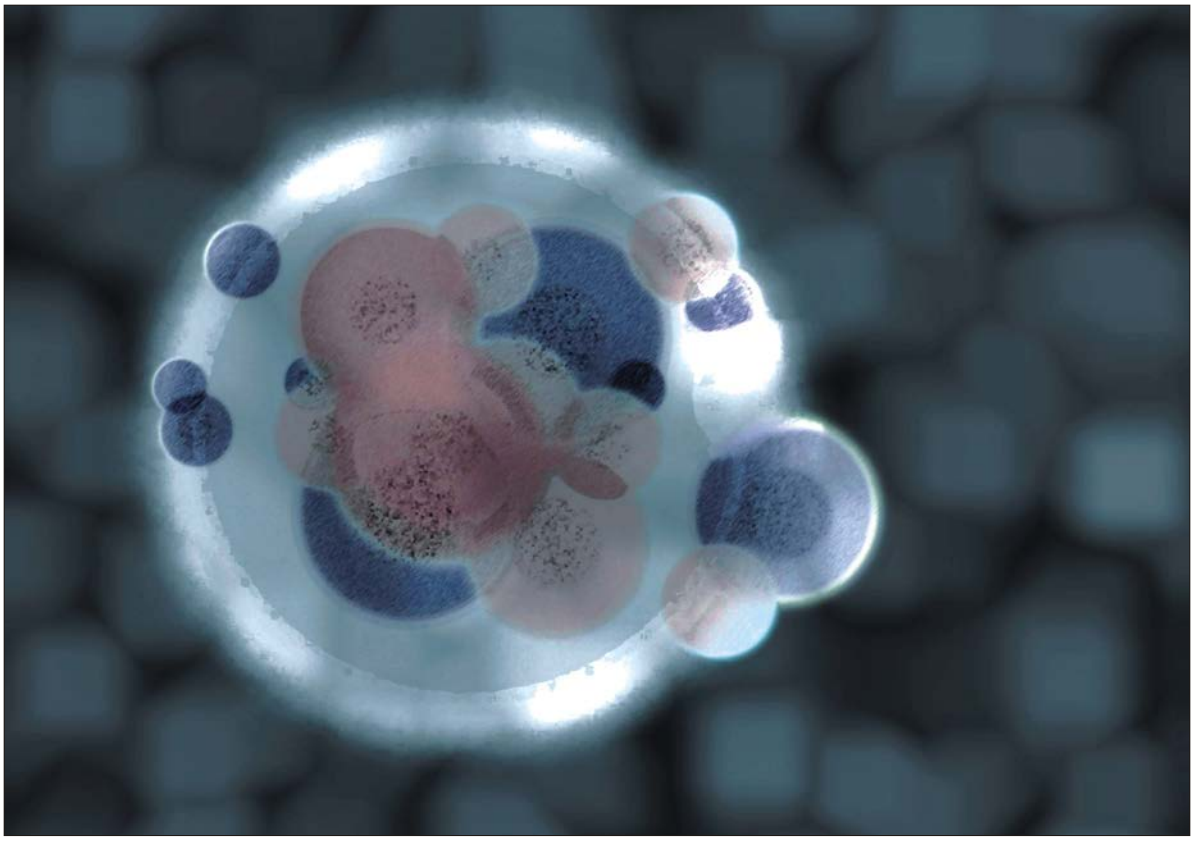

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 8 (1): 13-23, 2008
ISSN 1727-3048

Oxidative Stress of Plankton Community and Some Isolated Species During Paracetamol Toxicity Test

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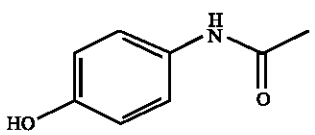
Abstract: The present research was carried out on 96 h to assess the possible effect of different doses of Paracetamol on both phytoplankton and zooplankton communities, as well as on two isolated algae *Chlorella ellipsoidea*, *Oscillatoria* sp. and a protozoan species *Vorticella convallaria*. The effect of the drug on some of the plankton biochemical activities such as (Arylesterase activity, lipid peroxidation and total thiol concentrations) was also studied. After 48 h, LC_{50} of zooplankton was recorded at 2 mg L^{-1}. The LC_{50} of the protozoans was recorded at 2 mg L^{-1} after 48 h of exposure, while the LC_{50} of rotifers was observed at 8 mg L^{-1} after 48 h of exposure. The total phytoplankton community during the experiment achieved LC_{50} at the concentration of 4 mg of paracetamol/l after only 24 h of exposure, while a 100% reduction was recorded at the highest concentration after 96 h of exposure. In conclusion, paracetamol as one of the commonly used anti-inflammatory has been found to have a drastic effect on the plankton organisms especially at long time exposure. In order to protect our fauna and flora, Pharmaceuticals in general should be monitored and detected in our water body.

Key words: Plankton, paracetamol, Lipid peroxidation, enzymes activity

INTRODUCTION

Potential risks associated with releases of pharmaceuticals into the environment have become an increasingly important issue for environmental regulators and the pharmaceuticals industry (Crane *et al*., 2006). Paracetamol is one of the pharmaceuticals that are extensively and increasingly being used in human and veterinary medicine, paracetamol or acetaminophen (brand names Tylenol in US; Panadol in UK and KSA; Tachipirina; Efferalgan in Italy and Paramol in Egypt), is a common analgesic and antipyretic drug that is used for the relief of fever, headaches and other minor aches and pains (Dart *et al*., 2006). Chemical structure of paracetamol is:



They are also considered as emerging environmental contaminants, since these chemicals are designed to have a specific mode of action and many of them for some persistence in the body (Fent *et al*., 2006). These features among others make pharmaceuticals to be evaluated for potential effects on aquatic flora and fauna. Accordingly, many environmental analyses have been performed in various countries, which are sunmarized by various studies (Halling-Sorensen *et al*., 1998; Daughton and Ternes, 1999; Kümmerer, 2004). These monitoring studies demonstrate that drug residues still exist in treated wastewater and surface water.

Aquatic organisms are particularly important targets, as they are exposed via wastewater residues over their whole life. In spite of the sizeable amounts of human drugs released to the environment, concise regulations for ecological risk assessment are largely missing. Among the pharmaceuticals, diclofenac and paracetamol are of the analgesics with highest rates of consumption (up to 100 t $year^{-1}$ in Germany; Zwiener and Frimmel, 2003).

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Diclofenac has been detected in wastewater effluents in several countries in concentrations ranging from 0.25 to 49 mg L^{-1} (Carballa *et al*., 2004; Ferrari *et al*., 2003; Stumpf *et al*., 1999; Ternes, 1998). Due to this incomplete degradation (50-75%), analgesic drugs can enter receiving waters and has been found in surface, ground water or even in drinking water (Buser *et al*., 1998; Farre *et al*., 2001; Quintana and Reemtsma, 2005). In 24% of samples from US streams, up to 10 μg L^{-1} (median 0.11 g L^{-1}) acetaminophen (paracetamol) has been found (Kolpin *et al*., 2002).

Pharmaceuticals are excreted after application in their native form or as metabolites and enter aquatic systems via different ways. They can reach wastewater treatment planet (WWTP) by discharge via urinary or faecal excretion or by improperly dispose of unused or expired drugs in toilets (Fent *et al*., 2006). Other minor sources are hospital and pharmaceutical industries. Pharmaceuticals not readily degraded in the Sewage Treatment Plant (STP) are being discharged in treated effluents resulting in the contamination of rivers, lakes, estuaries and rarely, groundwater and drinking water (Holm *et al*., 1995). Twenty different drugs and four corresponding metabolites including anti-inflammatory drugs, lipid regulators, betablockers and Carbamazepine were found to be ubiquitously present in streams and river water (Ternes, 1998; Crane *et al*., 2006).

The plankton organisms were found to be sensitive to different pharmaceuticals and considered excellent indicators in the toxicity tests (Ferari *et al*., 2003). The effect of pharmaceuticals as well as environmental stress on different biochemical activities of the living organisms were reported in several studies. Malanga *et al*. (1999) noticed increase in enzyme activity in photosynthetic organisms under oxidative stress. Thiols were found to play important role in triggering cellular control against drug damage, it was capable of protecting *Cholerella vulgaris* from the increase in lipid peroxidation resulting from oxidative stress and protects photosynthetic organisms in vivo against free radical generation (Malanga *et al*., 1999). Increase in enzyme activity as an adaptive response to oxidative stress has been reported to occur in bacteria such as *Escherichia coli* (Privalle and Fridovich, 1987) and in *Cholerella. vulgaris* (Malanga *et al*., 1999).

Most chronic aquatic toxicity data for human pharmaceuticals, other than synthetic steroids, are available for algae (Holten-Lützhøft *et al*., 1999; Halling-Sørensen, 2000; Boxall *et al*., 2003; Pro *et al*., 2003; Webb, 2004), probably because these are the briefest and therefore least expensive chronic toxicity tests to run. Although most pharmaceuticals are designed to target specific metabolic pathways in humans and domestic animals, they can have numerous often unknown effects on metabolic systems of nontarget organisms, especially on invertebrates (Daughton and Ternes, 1999; Hirsch *et al*., 1999; Dietrich *et al*., 2002; Belfroid and Leonards, 1996; Schulte-Oehlmann *et al*., 2004). Cladocerans, particularly daphnia have long been used for toxicological assessment of a diverse range of chemicals and drugs (Wu *et al*., 2006). Acetylsalicylic acid affected reproduction in *Daphnia magna* and *Daphnia longispina* at concentrations of 1.8 mg L^{-1} (Marques *et al*., 2004). Traditional chronic toxicity studies with Diclofenac were reported in invertebrates (Ferrari *et al*., 2003, 2004).

The aim of the present study is to assess the possible effect of different doses of Paracetamol as a widely used anti-inflammatory and analgesic drug on both phytoplankton community and zooplankton community, two isolated algae *Chlorella ellipsoidea* and *Oscillatoria* sp. as well as on *Vorticella convallaria*. The effect of the drug on some of their biochemical activities was examined, Such as quantitative estimation of enzymatic antioxidants level in the plankton organisms. Example of enzymatic antioxidants is Arylesterase activity. In addition, the lipid peroxidation products, for example TBA-RS was determined to evaluate the degree of lipid peroxidation and hence degree of cell injury. The thiol group was estimated as an example for thiol-containing antioxidants, which plays vital role in protection of the cells from oxidative stress.

MATERIALS AND METHODS

Test substance: Paracetamol was purchased in commercial form as paracetamol tablets (500 mg). Solutions of paracetamol were prepared by dissolving a known quantity of the compound in specified volume of water and stirred vigorously and left over night. These solutions were added to flasks containing the plankton community with specific ratios in order to obtain different concentrations of paracetamol (2, 4, 8, 12 and 15 mg L^{-1}).

Sampling: Water samples were collected by a Ruttener water sampler from Ismaelia Canal near from the Cairo Intake Treatment Station and were transferred to the laboratory directly.

Biochemical analysis

Total thiol content: Total thiol content was assayed in acid-soluble extracts as described by Tzietze (1969). The homogenate was prepared in trichloroacetic acid (3%, w:v) and after a brief centrifugation the supernatant was diluted 10-fold in 100 mM phosphate buffer (pH 7.5). Thiol

content was determined by spectrophotometer by measuring absorbance at 412 nm in the presence of 0.5 mM 5.5%-dithiobis (2-nitrobenzoic acid) (DTNB), 0.5 μ mL^{-1} glutathione reductase and 0.2 m M NADPH.

Thiobarbituric acid-reactive substances (TBARs): To 0.5 mL of the water sample, in a centrifuge tube, 2.5 mL 10% trichloroacetic acid were added and the mixture was placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 3000 rpm for 10 min and then 2 mL of the supernatant were added to 1 mL of 0.67% TBA solution in a test tube. The mixture was placed in a boiling water bath for 15 min, then cooled under tap water and the absorbance was measured by spectrophotometer at 532 nm against reagent blank.

Determination of arylesterase activity: Reaction mixture containing 1 mM phenylacetate, 1 mM $CaCl_2$ and 20 mM Tris-HCl buffer at pH 8.0 and at 25°C was prepared 0. 5 mL of water samples was added to a quartz cuvette containing 3 mL of reaction mixture. The initial rate of hydrolysis (first 90 sec) was recorded at 270 nm using JENWAY spectrophotometer model No. 6105 UV/VIS. The increase in absorbance due to phenol generated was recorded against reagent blank contains reaction mixture.

Biological measurements

Bioassays: These tests are based on the growth and inhibition of phytoplankton community, zooplankton community, *Chlorella ellipsoidea* (Greneck), *Oscellatoria* sp. (Vaucher) and *Vorticella convallaria*. Algal species strains were obtained from algae laboratory of the Girls College, Ain Shams University.

The experiment was carried out on 96 h in four sets of flasks. The first set was the plankton community; the second one was the isolated *Chlorella ellipsoidea*, while the third set was the *Oscillatoria* sp. and the last set was *Vorticella convallaria*. Each set consists of 24 flasks, arranged in 4 groups. Each group includes 6 flasks representing control and different doses of Paracetamol (2, 4, 8, 12 and 15 mg L^{-1}). After time interval of 24 h one group of each of the three sets was sampled.

For *Oscillatoria* sp. the flasks were inoculated with 80×10^6 individuals/l, while *Chlorella ellipsoidea* was inoculated with 101×10^6 cells L^{-1}. For *Vorticella convallaria*, 20 individuals were transferred to each flask of 250 mL capacity.

The cultures were kept at 20±0.5°C and a 14 h day/10 h night cycle (light intensity: 1200 lux), parameters were as follows: pH 7.20-7.35 (adjusted with HCl or NaOH solutions), DO: 7.2-8.4 mg L^{-1}; hardness (as $CaCO_3$): 82.0-91.2 mg L^{-1} and alkalinity: 56.1-58.0 mg L^{-1}; EC: 230 mS cm. Test duration was 96 h, the test parameter considered was mortality and the concentration found to kill 50% organisms was indicated as LC50.

The tests were carried out in triplicate, fresh sub samples were taken for the biochemical analysis and chlorophyll α measuring at the beginning of the experiment without Paracetamol (Control) and after 96 h of exposure to the drug, while the plankton sub samples were fixed with Legol Iodine (20 g iodine + 200 mL dist. Water + 20 mL glacial acetic acid). Ultermöhl technique was applied for phytoplankton enumeration (EPA, 1979).

Zooplankton examination: Zooplankton samples were preserved with 4% formalin solution and allowed to settle down. Excess water was siphoned and a sub-sample of 1 mL was transferred into a counting cell (Rafter Sedwick Cell) and examined under a binocular compound microscope (Motic).

Culture of *Vorticella convallaria* and toxicity test: Individuals of *Vorticella convallaria* were isolated from the Ismaelia Canal water and cultured in natural media according to Foissner *et al.* (1999). *Vorticella* sp. were fed by drops of fluid of boiled wheat.

Determination of chlorophyll a: Measurements of chlorophyll a were carried out spectrophotometerically by filtering known volumes of the water samples through glass microfiber filter paper (Wahtmann GF/C) according to APHA (1992).

Statistical analysis: Significant changes in growth of aquatic organisms were evaluated using Multi-way analysis of variance (ANOVA) by SPSS program Version 13. Organisms count data were square-root transformed to meet ANOVA assumptions of residual normality and homogeneous variance. Inhibitory concentrations resulting in a decrease in growth by 50% (IC50) were determined using linear and nonlinear regression performed with methods described in Stephenson *et al.* (2000) and Haanstra *et al.* (1985).

The previous analyses were performed between plankton, different drug doses and time of the experiment to determine the possible relations and significance. Correlation coefficient was applied to find the relationship between the biochemical analysis and chlorophyll a at the end of experiment.

RESULTS

Results of biochemical analysis

TBARs: The results revealed an obvious increase in the TBARs values in the four investigated groups (plankton

Table 1: Effect of different paracetamol doses on TBARs (μmol L^{-1}), Arylesterase activity (U mL^{-1}) and total thiol (μmol L^{-1}) in plankton community and isolated species

Paracetamol concentrations (mg)	Concentrations of TBARs	Enzyme activity	Thiol concentrations
Plankton community			
Control	0.186	43.99	170.59
2	0.210	37.23	155.88
4	0.492	15.38	50.00
8	0.914	14.15	23.53
12	1.960	15.38	23.53
15	2.810	12.92	11.76
***Oscillatoria* sp.**			
Control	0.351	42.45	100.00
2	0.412	37.53	85.29
4	0.976	32.30	51.49
8	1.510	24.60	51.41
12	1.550	19.90	47.10
15	1.740	5.54	19.21
Chlorella ellipsoidea			
Control	0.291	27.10	136.80
2	0.303	19.70	122.10
4	0.855	23.70	95.59
8	0.896	10.20	60.29
12	1.130	9.30	54.94
15	1.310	6.80	54.41
Vorticella convallaria			
Control	0.392	26.70	135.40
2	0.382	25.70	132.10
4	0.875	24.60	110.20
8	0.996	11.50	75.30
12	1.240	9.50	54.30
15	1.350	7.30	52.90

community, *Oscillatoria* sp. *Chlorella ellipsoidea* and *Vorticella convallaria*) compared to the control of each group. For the plankton community group value of TBARs at the control (drug free) was 0.186 μmol L^{-1}, while after 96 h of exposure it reached 2.81 μmol L^{-1} at concentration of 15 mg Paracetamol. For the *Oscillatoria* sp. group, values of the TBARs were 0.351 μ mol L^{-1} for the control and 1.74 μmol L^{-1} for the highest Paracetamol concentration after 96 h of exposure. The values for *Chlorella ellipsoidea* group changed from 0.291 μmol L^{-1} for the control to 1.31 μmol L^{-1} at 15 mg of Paracetamol by the end of the experiment. TBARs values for *Vorticella* culture increased from 0.392 μmol L^{-1} at control group to 1.35 μmol L^{-1} after drug exposure for 96 h (Table 1).

Arylesterase activity: Results of Arylesterase activity of the four studied groups revealed gradual decrease with increasing the Paracetamol concentrations (Table 1). For the plankton community the enzyme activity ranged between 43.99 U mL^{-1} for the control and 12.92 U mL^{-1} for the highest drug concentration. The Enzyme activity of *Oscillatoria* sp group decreased from 42.45 U mL^{-1} to 5.54 U mL^{-1} after 96 h of exposure to 15 mg of Paracetamol. For *Chlorella ellipsoidea* group, the activity declined from 27.1 to 6.8 U mL^{-1}. Values of *Vorticella* enzyme activity decreased from 26.7 U mL^{-1} at the control group to 7.3 U mL^{-1} after 96 h of exposures to 15 mg L^{-1} paracetamol.

Total thiol: A gradual reduction was observed in the values of total thiol in the four studied groups, following the same trend of the Arylesterase activity. For The community group the values of thiol varied from 170.59 to 11.76 μmol L^{-1}. For *Oscillatoria* sp. group the values declined from 100 to 19.21 μmol L^{-1}. For *Chlorella ellipsoidea* group, the results varied from 136.8 to 54.41 μmol L^{-1} after 96 h of exposure. Concentrations of the thiol group of *Vorticella* individuals reduced from 135.3 μmol L^{-1} for the control group to 52.0 μmol L^{-1} after exposure to high concentration of the drug (Table 1).

Phytoplankton results

Phytoplankton community: The phytoplankton community was represented by a total of 45 species belonging to 3 groups namely, Cyanophyceae, Chlorophyceae and Bacillariophyceae. They formed 58.69, 23.19 and 18.12% of the total phytoplankton, respectively.

All the previous groups were not affected by the first concentration 2 mg L^{-1} after 24 h of exposure recording the same density as the control, while they attained the same behavior showing gradual decreases in their abundances by increasing the paracetamol concentrations (Fig. 1). The remarkable observation was the decaying of the cell contents in members of the different groups after 72 h of exposure to the drug. For the group Bacillariophyceae, the population density reduced by 50% at 12 mg L^{-1} after 24 h of exposure, while Chlorophyceae suffered from dramatic decrease in its abundance reached 73% of its population at 4 mg L^{-1} after 24 h of exposure. Cyanophyceae was the most tolerant group during the experiment reaching the LC_{50} after 72 h at 4 mg L^{-1}. It is clearly noticed that the community was reduced by almost 50% at the concentration of 4 mg of paracetamol L^{-1} after only 24 h of exposure, while a 100% reduction was recorded at the highest concentration after 96 h of exposure.

During the experiment Cyanophyceae was represented by 10 species. The most leading species of the blue-green algae were *Microcystis aeuriginosa* (Kützing), *Merismopedia tenuissima* (Lemmermann) and *Phormidium tenue* (Menehini) Gomont. Among the previous species, *Microcystis aeuriginosa* (Kützing) was the most resistant species. Chlorophyceae was represented by 18 species. The most dominant species of

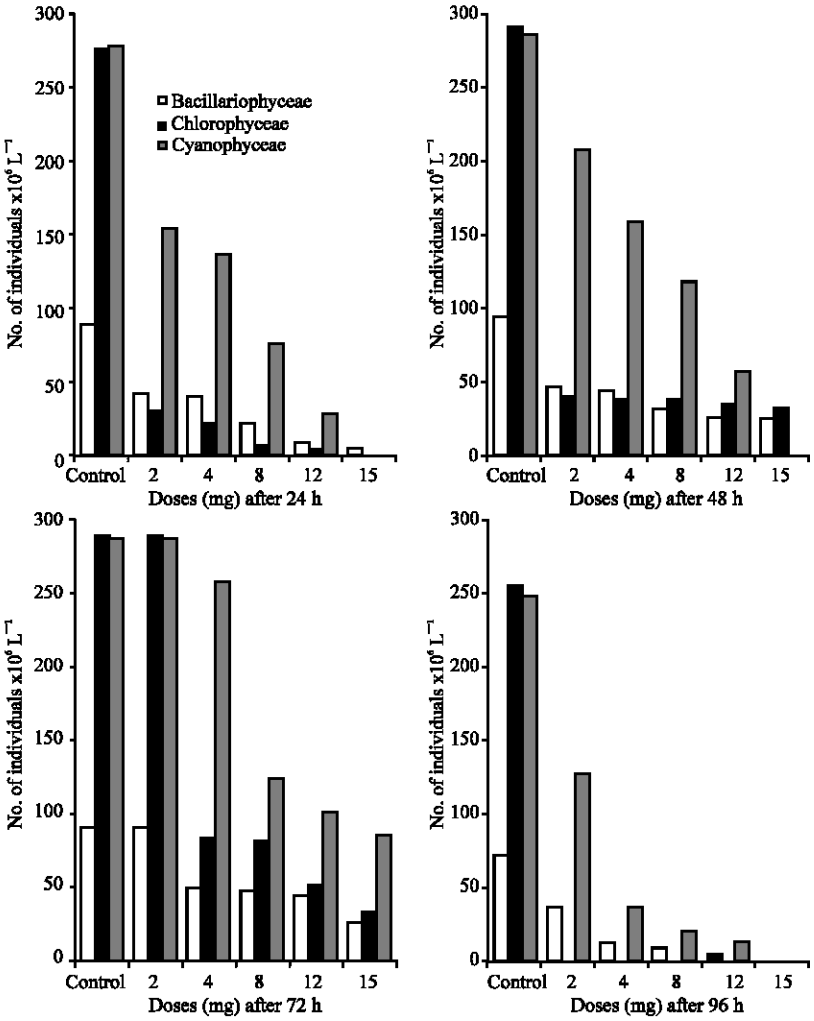

Fig. 1: Effect of paracetamol on different phytoplankton groups (No. of individuals×10^6 L^{-1})

this group were *Pediastrum simplex* (Meyen) Lemmermann, *Coelastrum reticulatum* (Dangeard) Senn and *Coelastrum microporum* (Naegeli). The most common species of this group was *Pediastrum simplex* (Meyen) Lemmermann. On the other hand, the most frequent species of Bacillariophyceae were *Melosira granulata* var. *angustissima* (Müller), *Synedra ulna* (Nitzsch) Ehrenberg and *Nitzschia acicularis* W. Smith. The most tolerant species of diatoms were *Synedra ulna* and *Nitzschia acicularis*.

Chlorophyll a: During the present work results of chlorophyll a were greatly affected by exposure to different concentrations of paracetamol. In fact values of chlorophyll were indirectly correlated to paracetamol concentrations, since the lowest chlorophyll values were observed at the highest concentrations of paracetamol at the three investigated groups (Table 2).

Table 2: Effect of different concentrations of paracetamol on chlorophyll a (μg L^{-1}) in plankton community and isolated algae

Groups	Paracetamol concentrations (mg)	Chlorophyll a	Control
Plankton community	2	88.20	90.10
	4	67.50	
	8	54.30	
	12	48.70	
	15	36.40	
Oscillatoria sp.	2	83.80	85.70
	4	68.10	
	8	53.30	
	12	44.65	
	15	38.00	
Chlorella ellipsoidea	2	16.47	19.80
	4	18.25	
	8	15.11	
	12	14.43	
	15	12.87	

Effect of paracetamol on *Chlorella ellipsoidea* (Greneck) and *Oscillatoria* sp. (Vaucher): The effect of the paracetamol on cultures of both *Chlorella ellipsoidea*

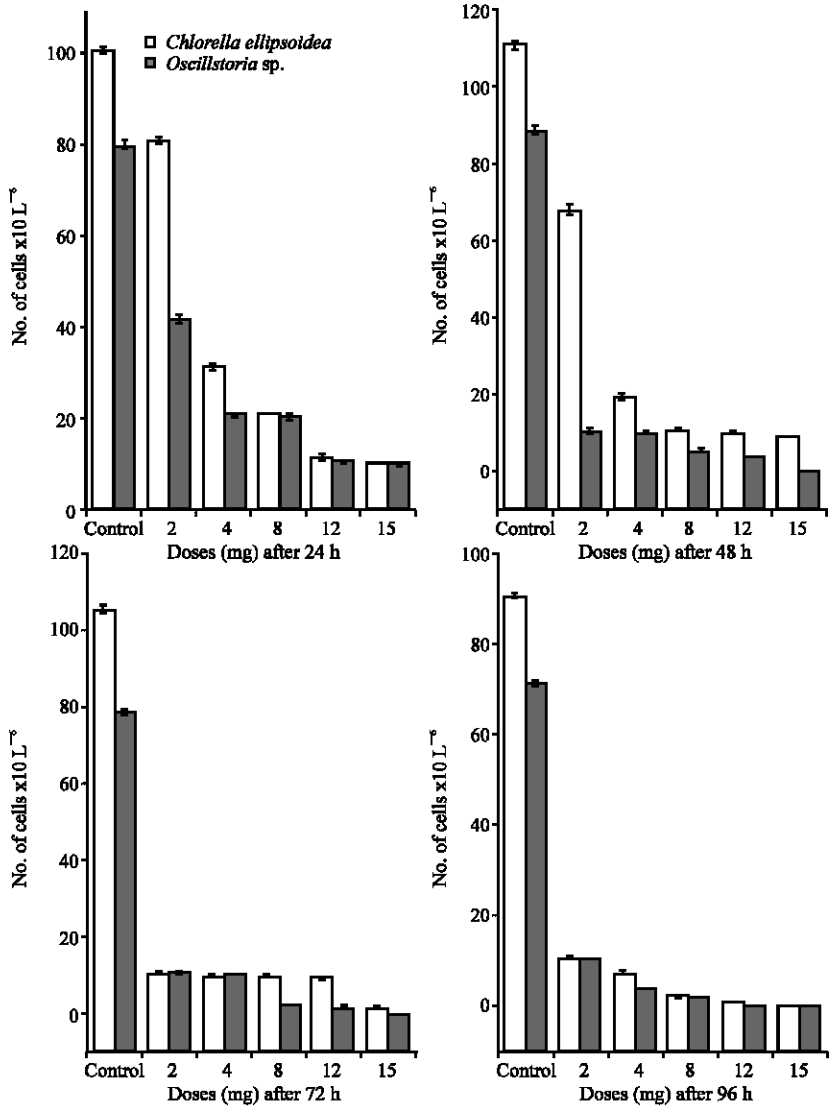

Fig. 2: Effect of paracetamol on different phytoplankton groups (No. of cell $\times 10^6$ L^{-1})

and *Oscillatoria* sp. (Fig. 2) was remarkable, since individuals of both cultures suffered from 70 and 50% reduction in their abundance at 4 mg L^{-1} of the paracetamol after 24 h of exposure, respectively. After 48 h the results revealed higher resistance of *Chlorella ellipsoidea* than *Oscillatoria* sp. which underwent 100% reduction in its density at the highest concentrations. The complete reduction of *Chlorella ellipsoidea* was recorded at 15 mg L^{-1} after 96 h of exposure, but it must be mentioned that both algae suffered from decaying in their cell contents after 72 h of exposure at all concentrations.

Effect of Paracetamol on zooplankton community: During the present experiment zooplankton community was represented by two main groups, namely Rotifera and Protozoa. They contributed 58.3 and 41.7% of the total zooplankton at zero time of the experiment respectively. The total density of zooplankton at the control flasks at zero time was 12000 organisms L^{-1}. After 24 h of exposure to Paracetamol, the first two concentrations (2 and 4 mg) did not affect the density of zooplankton community, then a slight gradual decrease was observed at the other three concentrations (8, 12 and 15 mg of paracetamol L^{-1}).

After 48 h, LC_{50} of zooplankton was recorded at the first two concentrations (Fig. 3). The LC_{50} of the protozoans was recorded at 2 mg L^{-1} after 48 h of exposure, while the LC_{50} of rotifers was observed at 8 mg L^{-1} after 48 h of exposure. The reduction in the community reached 75% at the flasks with 12 mg of Paracetamol and then decreased again to 67.7% of the total community at concentration of 15 mg.

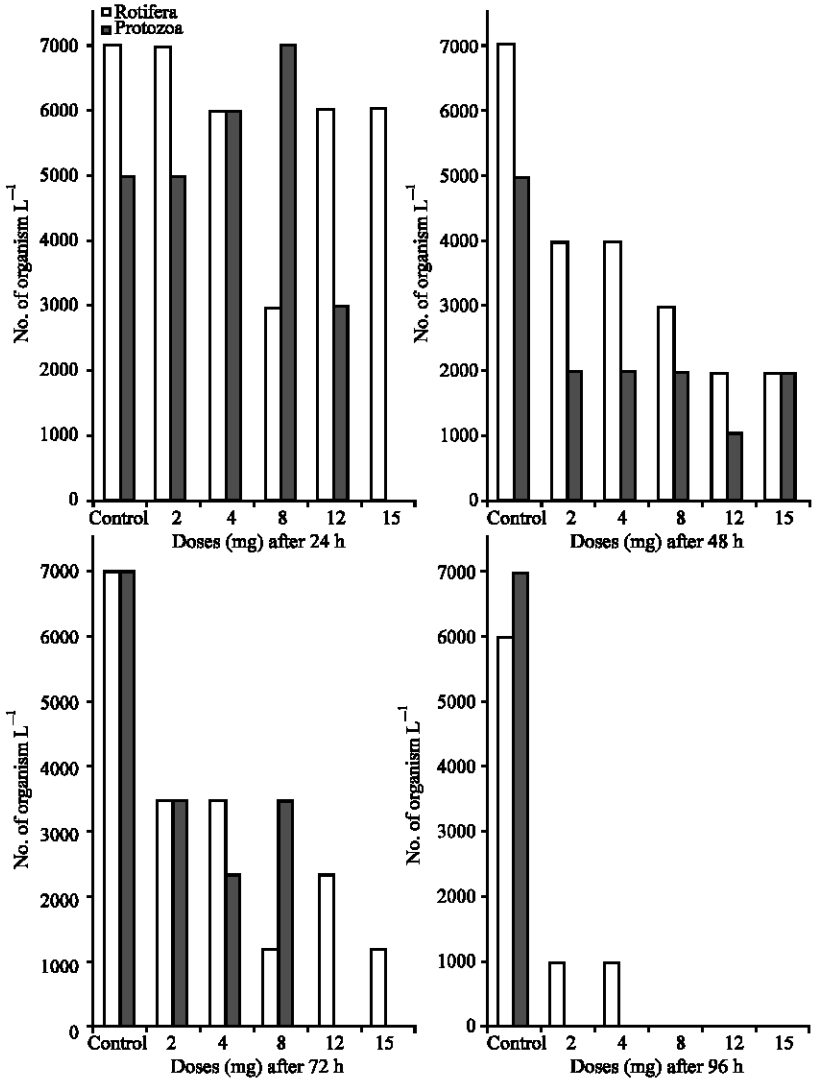

Fig. 3: Effect of different paracetamol doses on abundance of zooplankton community (No. of organisms L^{-1})

After 72 h, the gradual decrease in zooplankton abundance continued till it reached a reduction value of 91.7% for zooplankton community represented only by Rotifera (most of the organisms were ruptured or suffered from decaying), while the protozoans disappeared completely at the last two concentrations (12 and 15 mg).

After 96 h, decayed individuals of rotifers were recorded at the low concentrations and then disappeared completely at the higher concentrations. So after 96 h of exposure to high concentrations of the drug, zooplankton community suffered from dramatic reduction with 100% value.

During the experiment Rotifera was represented by four main species, *Brachionus angularis*, *Polyarthra vulgaris*, *Philodina roseola* and *Trichocerca cylindrica*. Among the previous species, *Trichocerca cylindrica* was the most resistant species. While, the protozoans community were represented by five species, *Vorticella convallaria*, *Difflugia globulosa*, *Codonella cratera* and *Strombilidium gyrans*. Among those species, *Difflugia globulosa* was the most tolerant species.

Effect of paracetamol on *Vorticella convallaria* culture: During the experiment individuals of *Vorticella* culture showed a gradual increase in its density in the control group reaching a maximum of 124 organisms L^{-1} after 72 h (Fig. 4). The density decreased a little after 96 h reaching 120 organism L. *Vorticella* culture was greatly affected by exposure to Paracetamol doses, its density reduced to the half (LC_{50}) after 24 h at 4 mg paracetamol L. By increasing the exposure period LC_{50} was recorded at 2 mg L^{-1} after 48 h. *Vorticella* individuals were completely ruptured at doses of 8, 12 and 15 mg L^{-1} after 96 h of exposure.

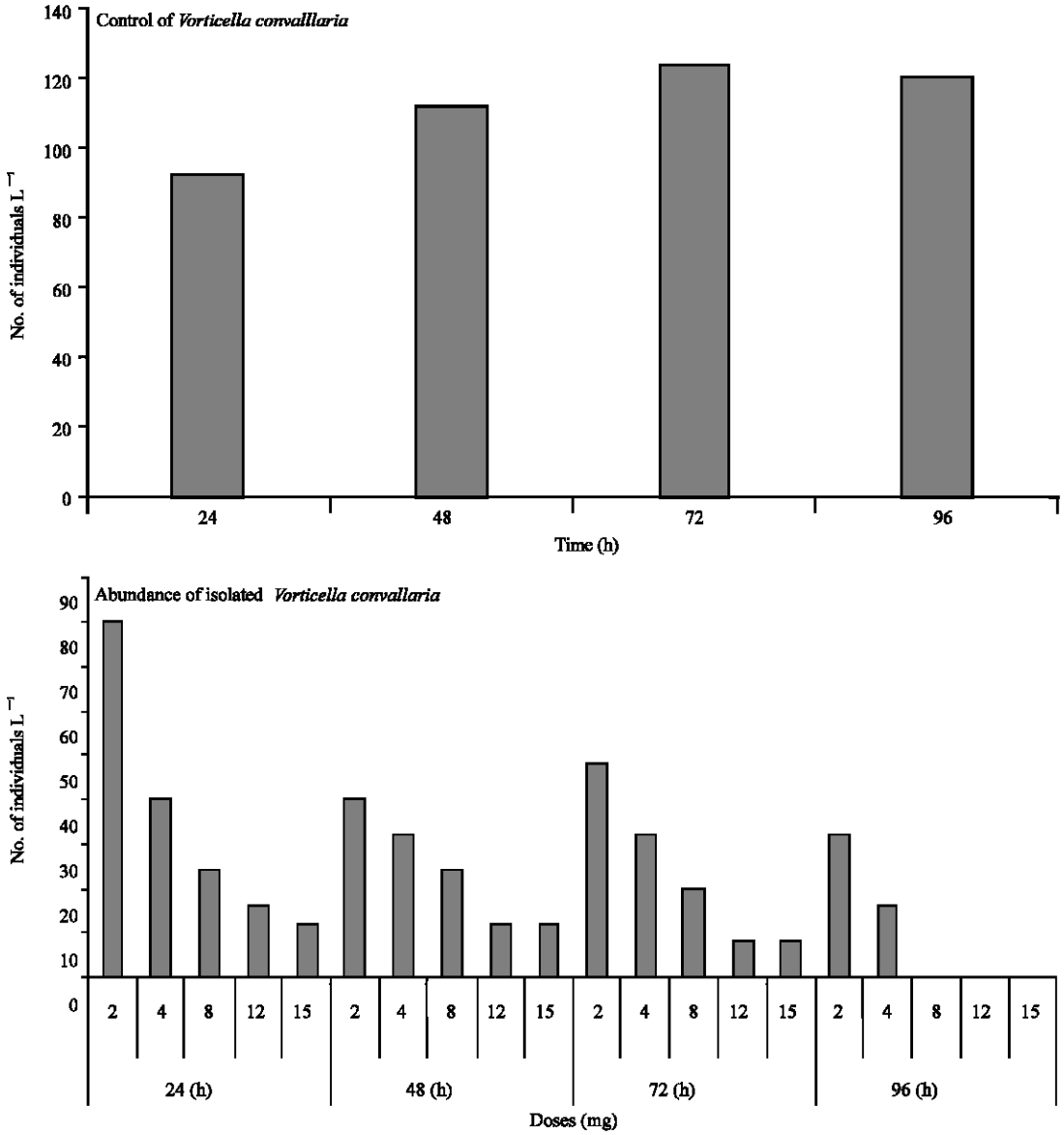

Fig. 4: Effect of different paracetamol doses on abundance of isolated *Vorticella convallaria* (No. of individuals L^{-1})

DISCUSSION

Although most pharmaceuticals are designed to target specific metabolic pathways in humans and domestic animals, they can have numerous often unknown effects on metabolic systems of non target organisms, especially on invertebrates (Daughton and Ternes, 1999; Hirsch *et al*., 1999; Dietrich *et al*., 2002). Certain receptors in lower animals resemble those in humans, others however, are different or lacking, which means that dissimilar modes of actions may occur in lower animals (Fent *et al*., 2006). Analgesics and non-steroidal anti-inflammatory drugs (NSAID) are the most consumed category of drugs especially paracetamol, they also commonly found in the aquatic environment (Crane *et al*., 2006). Paracetamol however, has recently been shown to have degradation products that are 58 and 25 times more toxic than itself (Bedner and MacCrehan, 2006).

During the present study paracetamol concentrations were found to affect both zooplankton and phytoplankton negatively. The common notice was that individuals of both groups suffered from decaying and deformation in the higher concentrations especially by increasing time of exposure to the paracetamol. This result coincides with Pascoe *et al*. (2003), who suggested that invertebrates as well as fish are at risk from long-term, low level drug

exposure, despite low acute toxicity e.g., the 96 h LC_{50} for *Gammarus pulex* is 1.7 mg L^{-1} (Watts *et al.*, 2001). They also added that there is possibility that pharmaceuticals could be lethal only at very high environmentally unrealistic concentrations but damaging at very low concentrations over long exposure times. The protozoan species were found to be more sensitive than rotifers; this fact was confirmed by the low LC_{50} recorded by individuals of *Vorticella* culture (4 mg L^{-1} after 24 h and 2 mg L^{-1} after 48 h). So the longer the exposure, the more dangerous the effect on these microorganisms is. The dramatic decrease in the population densities of both zooplankton and phytoplankton during the present experiment coincided with Kolpin *et al.* (2002) reported decreased diversity of both phytoplankton and zooplankton communities, also unexpected high lethality occurred in fish exposed to high and medium pharmaceutical concentrations. Lethality was observed also in plants in addition to decreased growth (Richards *et al.*, 2004).

The results of the biochemical activities were also good indicators for the effect of paracetamol on the investigated organisms. The negative relations between paracetamol doses and the concentrations of the thiol group as well as the enzyme activity in one hand and the positive relation with TBARs on the other hand explains the decaying in the cell contents of the plankton organisms. The decaying and deformation observed in plankton individuals at the higher concentrations of paracetamol during the present study can be attributed to the stress that these plankton organisms suffered from due to drug exposure. Roginsky and Barsukova (2001) found that in photosynthetic organisms, environmental stress can create oxidative stress through increased production of Reactive Oxygen Species (ROS), which may cause Lipid peroxidation, consequently increasing TBARs values.

Chlorophyll a has the same trend of the total plankton community as well as *Oscillatoria* sp. reordering significant inverse relation with TBARs concentrations (R = -0.91 and -0.98, respectively). On the other hand, chlorophyll a was directly proportional to enzyme activity and Thiol concentration of the plankton community (R = 0.9 and 0.95, respectively) and for *Oscillatoria* sp. (R = 0.95 and 0.93). At culture of *Chlorella ellipsoidea*, chlorophyll a attained a direct relation with thiol concentration and enzyme activity (R = 0.86 and 0.97, respectively) and had no relation with TBARs.

In stress conditions invertebrates, plants and micro algae may respond by increasing antioxidant defense, notably enzymes, however excessive ROS cause a decrease of the enzymes activities (Li and Hu, 2005). They also added that drug exposure affect the activities of enzymes in aquatic organisms. The previous fact explains the decrease in the Arylesterase activities of both the plankton community and the isolated species by increasing drug concentrations and time of exposure during the present study. The low concentrations of the thiol recorded in the four investigated groups at higher doses of paracetamol indicate the stress that those organisms suffered from. Malanga *et al.* (1999) explained that Cellular thiols play a key role in protection against oxidative damage arising from a number of stress conditions.

In a similar study Henschel *et al.* (1997) concluded that Paracetamol had clear effects on cell cultures and *Daphnia* sp with EC_{50} values between 19 and 50 mg L^{-1}. For *Daphnia* sp. a significant increase in toxicity with increasing exposure time was observed (24 h EC_{50}: 293 mg L^{-1}; 48 h EC_{50}: 50 mg L^{-1}). They explained that the most important side effect of paracetamol is the impairment of the human liver function (the process causing damage to the liver is based on the binding of reactive paracetamol metabolites to liver cell proteins).

With normal doses, these metabolites are trapped by glutathione. The observed toxicity on the cell contents of plankton organisms is similar to the disorders in liver function in humans, or binding to the cell proteins in the case of cell cultures. Li and Hu (2005) claimed that many kinds of pharmaceuticals may cause rapid change in the plasma membrane leading to leakage of ions in the protoplast. So the decaying in the plankton cells during the present study can be explained by the oxidative stress caused by high doses of the paracetamol leading to depletion of the cellular thiol and increasing of ROS. The ROS is unstable form, which bind with the cell protein to be stable leading to disorders in the cell including decrease in the enzyme activities and increase in the lipid peroxidation (TBARs).

Statistically, multiple ANOVA showed a high significant effect of experiment duration and drug doses on the following parameters, Rotifera, Protozoa, phytoplankton community ($p<0.01$).

In conclusion, paracetamol as one of the commonly used anti-inflammatory has been found to have a drastic effect on the plankton organisms especially at long time exposure. In order to protect our fauna and flora, Pharmaceuticals in general should be monitored and detected in our water body, since there are almost no data available about this important problem in Egypt.

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