Effects of Three Commonly Used Pharmaceutical products on Biochemical parameters of the Micro-alga *Pseudokirchneriella subcapitata* (Under Laboratory Conditions)

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ABSTRACT

Despite the great importance of pharmaceutical products in our daily life, they have negative effects on the environment; where it may threatens many non-target organisms. The present study provides an integrated framework of contribution to understanding the toxicity of three commonly used pharmaceutical products (amoxicillin, naproxen and tramadol) on the micro-green alga *Pseudokirchneriella subcapitata*. The impact of pharmaceutical pollution on aquatic organisms can be evaluated by assessing its physiological response. The alga was exposed to sub-lethal concentrations (LC_{10}, LC_{25} and LC_{50}) of each tested pharmaceutical to determine its impact on growth behavior, pigments, total lipids, protein and carbohydrate contents. The obtained results clarified that, exposure to the pharmaceuticals by their sub-lethal concentrations mostly resulted in a decrease in cell density up to 66.25% by amoxicillin and 37.24% by tramadol within LC_{50}, although exposure to low naproxen concentration promoted cell density (up to 21.7%) after 24h. Amoxicillin (67.74 - 97.44%) followed by tramadol (59.67- 93.55%) significantly reduced the content of the pigment after 96h but in a different manner, however except within LC_{50}, naproxen induced both carotenoids (4.55-11.61%) and chlorophyll-a (1.54 - 9.23%) during the first 24h. The tested pharmaceuticals also reducing protein, carbohydrates and total lipid content of *P.subcapitata* at the end of the experiment by ratios up to 96.7, 86.2 and 74.9% respectively. Due to the dramatic effects of pharmaceuticals on the aquatic non-target organisms all new pharmaceutical products must undergo an environmental risk assessment test during and after the development of the product to obtain marketing approval.

Keywords: Pharmaceuticals, *P.subcapitata*, protein, lipid, carbohydrates, pigments.

INTRODUCTION

Recently and in a striking way, the world market is packed with an infinite number of pharmaceutical products, including hormones, analgesics, antibiotics, antidepressants, antihistamines, stimulants, anti-inflammatory and various antiseptics additives, which designed to produce therapeutic effects in order to improve the life quality for both humans and animals (Gottschall *et al*., 2012; Miazek and Brozek-Pluska, 2019). Consequently, a considerable amount of these products seep through sewage systems due to the non-efficient removal by wastewater treatment, besides the contribution of the non-consumed drugs that disposed of in erroneous ways by pharmaceutical manufacturers (Daughton and Ruhoy, 2009; Zhang *et al*., 2019), which eventually reach the aquatic environment. In addition, the antibiotics and growth promoters that inserted in the feeding operations of large animals (Kummerer, 2009), also causing an increase in the concentration of some pharmaceutical active compounds in the aquatic environment on a global scale (Kosma *et al*., 2017). Worthwhile, the therapeutic groups of the pharmaceuticals products reached the aquatic environment are mostly active even in low concentrations range (pico-nmol.L^{-1}). Previous resear-chers detected the important differences between pesticides and pharmaceutical products (Relyea and Hoverman, 2006; Mosleh and Mofeed, 2014; Mosleh *et al*., 2014a), where pesticides used only if there is observed pest outbreaks during the growing season (Mosleh *et al*., 2005; Mosleh *et al*., 2014b), contrariwise, with increasing the global population, pharmaceutical products enter the aquatic environment incessantly and with large quantities.

Pharmaceutical products even at low concentrations have potential adverse effects on aquatic living organisms (Daughton and Ternes, 1999). Some pharmaceuticals such as ibuprofen, amoxicillin, mefenamic acid, oxytetracycline, and paracetamol were detected in water at levels that are not negligible for aquatic organisms (Isidori *et al*., 2005). However, until now there are no suffice strategies for environmental risk assessment of pharmaceuticals in order to protect aquatic organisms. Each compound of pharmaceuticals designed to do special functions in the cell; hence they can interact with specific receptors in the biochemical pathways (Tarfiei *et al*., 2018). Many of these pathways and receptors in the target cell be similar to that in the non-target aquatic organisms, therefore harmful effects could be expected (Boxall, 2004), especially where most pharmaceutical compounds resist any change in its chemical structure and it remains stable and active after being released into the environment (Kolpin *et al*., 2004; Bound and Voulvoulis, 2004). Therefore, pharmaceuticals can affect non-targeted organisms in the aquatic environment even by its low concentrations.

The widely distributed microalgae are the primary food supplier in the aquatic ecosystem, representing the first step of the food chain (Lai *et al*., 2009; Mosleh and Mofeed, 2014), hence any threat affecting them will be reflected on the other aquatic organisms of the higher trophic levels and by extension, the human being. Many researchers recommended microalgae as sensitive bioindicators, for both long and short-term

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monitoring for ecotoxicological assessments of the aquatic ecosystem because the nonlinear responses of biological communities can reflect any environmental disturbances (Guo *et al.*, 2015). Therefore, aquatic microalgae considered as a good pointer for aquatic monitoring programs to track any changes in water quality, hence it can follow up the impacts of the inserted pharmaceutical pollutants on the aquatic organisms, even with low concentrations, by evaluating the biochemical responses of the algal cells (Zhang *et al.*, 2019).

Three pharmaceutical compounds belonging to the most consumed products in daily life were selected, namely amoxicillin (antibiotic), tramadol (analgesic) and naproxen (anti-inflammatory). The broad-spectrum antibiotic; amoxicillin is effective against both gram-negative and gram-positive bacteria, where it has a high absorption rate (Miazek and Brozek-Pluska, 2019). Due to its pharmacological properties, it is widely used not only in human treatments but also in veterinary medicine for curing and prevention of skin, urinary and gastrointestinal bacterial infections (Isidori *et al.*, 2005). While in 1976 naproxen was approved for medicinal use, where it belongs to one of the most important worldwide pharmaceutical groups and identified as a nonsteroidal anti-inflammatory and antipyretic drug, hence it can be widely used in the treatment of numerous widespread diseases, such as rheumatoid arthritis and other rheumatic, beside musculoskeletal disorders, menstrual cramps, acute gout, and fever (Daughton and Ruhoy, 2009). Tramadol, which used to moderately severe pain, is

available as a generic medication with many trade names and it is generally safe at recommended doses but higher doses may lead to toxicity. Generally, pharmaceutical compounds have detrimental hassled unavoidable effects on aquatic microbial communities and hence on the whole aquatic ecosystem, which consequently negatively influence human health (Gottschall *et al.*, 2012). Therefore, the overall aim of this study was to contribute in the understanding the toxicity of the three commonly used pharmaceutical products (amoxicillin, naproxen and tramadol) on the micro-green alga, *Pseudokirchneriella subcapitata*, to investigate how those products impact on its growth beside its influence on the production of protein, carbohydrate, total lipid, as well as carotenoids and chlorophyll which would basically, upgrade our information about the expected modern environmental hazard on the aquatic ecosystems and develop a strategy to control it.

MATERIALS AND METHOD

Tested pharmaceutical compounds

The three pharmaceutical compounds (tramadol, naproxen, and amoxicillin) used in this study with purity $\geq$ 99% (Sigma). The stock solutions of the three pharmaceutical products were prepared using de-ionized water and stock solutions were sterile-filtered using glass filter paper and stored in the dark at 5 ^{o}C until used (Table 1).

Table (1): Chemical structure and drug class of the tested pharmaceuticals.

Tested Pharmaceuticals	Drug CLASS	Molecular Formula	Chemical Structure Depiction
Amoxicillin	Antibiotic	$C_{16}H_{19}N_3O_5S$	
Naproxen	Anti-inflammatory	$C_{14}H_{13}NaO_3$	
Tramadol	Analgesic	$C_{16}H_{25}NO_2$	
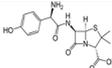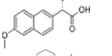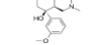
Algal strain and culture medium

The unicellular green alga *Pseudokirchneriella subcapitata* (Korshikov) F. Hinda, which formerly named *Selenastrum capricornutum* Printz, was obtained from Microbiology Lab of Faculty of Fish Resources, Suez University, Egypt. The unicellular green alga *P. subcapitata* strain has been maintained under the laboratory conditions using the OECD growth medium, at 23±1°C and under continuous illumination (60-80 µmol.m^{-2}S^{-1}) provided by white fluorescent lamps (Fora 50W) with continuous aeration and shaking (by orbital shaker, 130 rpm). *P. subcapitata* cells were harvested during their exponential growth phase and treated with different concentrations of the three pharmaceuticals.

Quantification of the pharmaceuticals residues

After incubation of *P. subcapiotata* with the pharmaceuticals, an aliquot (10 ml) media of each concentration was collected and then directly analyzed by high-performance liquid chromatography (HPLC), using a reversed phase column (Kromasil, C18, 100 A, 5 mm, 250 mm, 3 mm, CIL-Cluzeau). The pharmacyeuticals were eluted isocratically using methanol and water solvent (35:65%) for amoxicillin, methanol: acetonitrile: water and triethylamine (20:28: 52%) for naproxen or orthophosphoric acid, trieth-ylamine, acetonitrile and methanol in a complex gradi-ent mode for tramadol. The UV absorbance 273, 270 and 340 nm for amoxicillin, naproxen, and tramadol, respectively, with a Diodearray detector their concentrations were detected (Javier *et al.*, 2009).

The rate of recovery was detected by the addition of 1 ml methanol containing 1 mg ml^{-1} pure of three pharmaceuticals compounds (Olarinmoye *et al.*, 2016). The extraction of three pharmaceuticals compounds were carried out as described above and the average rate recovery was 92.874, 90.58 and 93.35% based on three replicates for amoxicillin, tramadol and naproxen respectively.

Determination of sub-lethal concentrations

The tests were conducted to determine the sub-lethal concentrations (LC_{10}, LC_{25} and LC_{50}) of the tested pharmaceutical compounds against *P. subcapitata* by using different concentrations of the tested pharmaceutical compounds (0.1, 0.4, 0.8,1.6, 3.2, 6.4, 12.8 and 25 mg. L^{-1}) after 24, 48 and 96 hours in 100 ml conical flasks. Three replicates without amoxicillin, tramadol and naproxen were used as control. The LC_{10}, LC_{25} and LC_{50} values were determined graphically according to Finney (1971).

Determination of algal growth

After different time of exposure to the sub-lethal concentrations (LC_{10}, LC_{25} and LC_{50}) the growth of *P. subcapitata* was determined as cell number by using Rafter Counting Chamber.

Estimation of carotenoids and chlorophyll a&b

By treating *P. subcapitata* with the sub-lethal concentrations of each pharmaceutical compounds and extraction by using acetone (80%) after different exposure time (24, 48 and 96 hrs), chlorophyll a and b were determined according to Wellburn (1994), meanwhile the total Carotenoids were calculated according to Holm (1954).

Determination of total lipid

Lipid was extracted and quantified from freeze-dried algal biomass according to Lewis *et al.*, (2000) during the entire period of the experiment.

Determination of protein content

With the sub-lethal concentrations of the tested pharmaceutical compounds, *Pseudokirchneriella subcapitata* suspensions were incubated for 24, 48 and 96 hrs in 20 ml of medium supplied; under the conditions described previously, then after algal collection, the extracts were analyzed for protein determination according to Bradford method (Bradford, 1976).

Determination of carbohydrate

After incubation of *P. subcapitata* with the sub-lethal concentrations of pharmaceuticals for 24, 48 and 96 hrs, the cultures were centrifuged at 2000 xg for 5 min. Then, 100 mg of algal pellets were used for quantification of carbohydrate content as described by Dubois *et al.*, (1956).

Statistical analysis

All the recorded data were determined from the triplicates and the results are expressed as a mean ± standard deviation (SD). All statistical analyses were performed with Sigma Stat 2.03 (SSCP Inc.) for Windows.

RESULTS

Sub-lethal concentrations of each pharmaceutical product

The response of *P. subcapitata* to all concentration of the tested pharmaceuticals over the exposure time (24, 48 and 96 hrs), based on the LC_{10}, LC_{25} and LC_{50} values clarified that naproxen was the least toxic compound, while regardless of response variable or exposure duration, amoxicillin was the most toxic compound (Table 2). The influence of tramadol on *P. subcapitata* revealed, LC_{50} value is 2.34 ± 0.09 mg.L^{-1} after 96 hrs of exposure. Meanwhile LC_{50} value for amoxicillin gave 0.35 ±0.01, 0.38 ±0.02 and 0.47± 0.02 mg.L^{-1} after 24, 48 and 96 hrs, respectively.

Residues of pharmaceutical products in both algal biomass and medium

Residues of naproxen, amoxicillin and tramadol were quantified in both medium and *P. subcapitata* cells (Table 3) by using the HPLC. The present study revealed that amoxicillin is the most frequently detected pharmaceuticals in *P. subcapitata* within LC_{50}, with residue fluctuated between 0.08±0.01 to 0.16±0.01 µg.g^{-1}, followed by the tramadol, which ranged between 0.14±0.02 and 0.35±0.0.1 µg g^{-1}. The naproxen was also detected in *P. subcapitata* samples at residue levels from 0.22±0.03 to 0.42 ±0.10 µg.g^{-1}.

The residues of the three pharmaceutical products were also detected in the media after different time of exposure. The pharmaceuticals residues in the media showed a relative decrease during the interred period of investigation. Indeed, after 2 days with the lowest initial concentration, 57.1 % of the amoxicillin remained in the media, while after 4 days of exposure the residues of amoxicillin were 43.11, 49.38 and 57.1 % of initial concentrations for LC_{10}, LC_{25} and LC_{50} respectively.

Influence of pharmaceuticals on the growth

The addition of the tested pharmaceuticals by its sub-lethal concentrations (LC_{10}, LC_{25} and LC_{50}), mostly resulted in a decrease in cell density of *P. subcapitata*, although the apparent disparity in responses to each product. A glance on figure (1-a, b and c) revealed that, the maximum growth suppressions during the entire period of investigation was recorded with the antibiotic amoxicillin, giving its maximum inhibition within LC_{50} after 96 hrs (0.54X10^6 cell.ml^{-1}) when compared to the control which reached 6.31 X10^6 cell.ml^{-1}at the same time. Although tramadol also reduced cell density, it has less influence than amoxicillin, where it gave 1.82X10^6 cell.ml^{-1} within the high concentration at the end of the experiment. Inspection of figure (1) reflects the less influence of naproxen on cell density of *P. subcapitata* during the investigation period. Paradoxically, it is worth mentioning that, exposure to low naproxen concentrations (LC_{10} and LC_{25}) promoted the cell density over the control during the first 48h. However, at the end of the experiment (after 96 hrs), all treatments showed growth inhibition trend.

Table (2): Toxicity of three pharmaceutical products (mg. L^{-1}) on *Pseudokirchneriella subcapitata* after 24, 48 and 96 h of exposure.

| Pharmaceutical Compounds	Time (hr)	Toxicity (mg. L^{-1})*		
		LC$_{10}$	LC$_{25}$	LC$_{50}$
Amoxicillin	24	1.67 ± 0.08	0.81 ± 0.05	0.35 ± 0.01
	48	1.54 ± 0.05	0.93 ± 0.02	0.38 ± 0.02
	96	1.67 ± 0.03	0.93 ± 0.10	0.47 ± 0.02
Tramadol	24	10.2 ± 0.04	6.03 ± 0.95	2.10 ± 0.08
	48	9.6 ± 0.12	5.12 ± 0.65	2.02 ± 0.05
	96	11.2 ± 0.89	6.51 ± 0.69	2.34 ± 0.09
Naproxen	24	25.01 ± 1.25	13.33 ± 1.02	4.85 ± 0.08
	48	24.30 ± 1.35	13.21 ± 0.95	4.61 ± 0.06
	96	26.22 ± 1.85	14.61 ± 0.101	5.99 ± 0.54
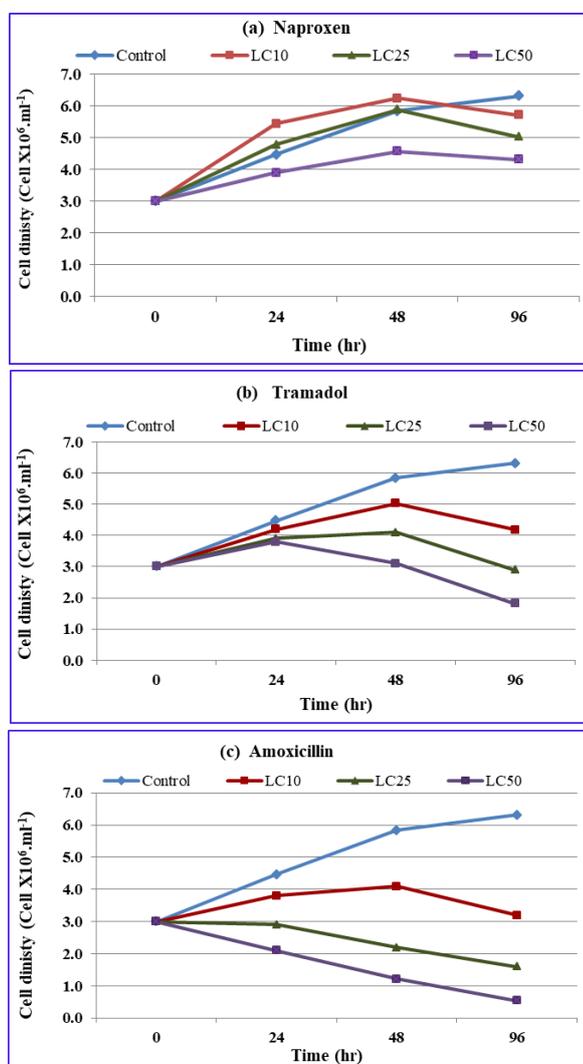
Figure (1): Growth of *P. subcapitata* after 24, 48 and 96 hrs exposure to (a) Naproxen, (b) Tramadol and (c) Amoxicillin.

Impact of pharmaceuticals on pigments content

The result illustrated in figure (2- a, b and c) clarify that, the tested pharmaceuticals had more or less the same trend of influence on the *P. subcapitata* pigments (chlorophyll a, chlorophyll b and carotenoids). Among those pigments, amoxicillin followed by tramadol significantly reduced the pigment content along the period of the experiment but in a different manner. It is noticeable that, the decline in pigment content increased with concentration. The data pertaining to naproxen clarified that except within LC$_{50}$, naproxen induced both carotenoids and chlorophyll content during the first 48 h. However, it tended to reduce all pigments content during the rest of the experiment but with less effect than amoxicillin and tramadol (Figure 2).

Impact of pharmaceuticals on protein, lipid and carbohydrate contents

The recorded results showed that, the three tested pharmaceuticals (amoxicillin, tramadol and naproxen) significantly reduce protein content after 48 and 96 h; while a slight inhibition effect was recorded after the first 24 h. It is of interest to mention that, the amoxicillin dramatically inhibit protein production within all concentrations during the period of investigation, giving its maximum (96.7%) within LC$_{50}$ after 96 h (Figure 3). As it was illustrated in figure 4, total carbohydrates suffer from the same inhibition trend. It is worth to mention that, the lowest inhibition of carbohydrate production by *P. subcapitata* was by tramadol, where its inhibition ranged between 17.4 % and 57.9%, obtaining the maximum inhibition within LC$_{50}$ after 96 h (Fig. 4). Contrarily, amoxicillin was responsible for the maximum reduction in carbohydrate production with concentration and time (from 30 to 86.2%).

Concerning lipid, control of *P. subcapitata* showed stable total lipid content during the 96 hrs of the experiment (Figure 5). Total lipid content with amoxicillin and tramadol exhibited the same trend of decrease depending on both the concentration and the time of exposure, where it shows the maximum

decrease when alga exposed to the LC_{50} of amoxicillin for 24, 48 and 96 hrs (51.6, 59.1 and 74.9%). Meanwhile, naproxen enhanced total lipid productivity by 6.1, 14.1 and 4.7% within LC_{10}, LC_{25} and LC_{50} respectively after the first 24 hrs and only by 3% after 48 within LC_{10}.

Table (3): Residues of Amoxicillin, Tramadol and Naproxen in the medium and in *P.subcapitata* after 24, 48 and 96 h of exposure.

Pharmaceutical Compounds	Initial conc.(mg.L^{-1})	Residues (mg.L^{-1}) in medium/hr*			Residues (µg. g^{-1}) in algae/hr*		
		24	48	96	24	48	96
Amoxicillin	LC_{10} (1.67)	1.52 ± 0.12	1.32 ± 0.23	0.95 ± 0.05	0.42 ± 0.10	0.54 ± 0.24	0.65 ± 0.023
	LC_{25} (0.81)	0.75 ± 0.21	0.62 ± 0.09	0.41 ± 0.04	0.21 ± 0.05	0.28 ± 0.01	0.38 ± 0.012
	LC_{50} (0.35)	0.33 ± 0.01	0.20 ± 0.02	0.16 ± 0.05	0.08 ± 0.01	0.12 ± 0.03	0.16 ± 0.01
Tramadol	LC_{10} (10.20)	9.25 ± 0.5	8.60 ± 0.24	5.36 ± 0.21	3.65 ± 0.08	4.58 ± 0.3	5.01 ± 0.021
	LC_{25} (6.03)	5.65 ± 0.41	4.23 ± 0.21	3.1 ± 0.25	2.56 ± 0.25	2.95 ± 0.14	3.12 ± 0.14
	LC_{50} (2.10)	2.01 ± 0.02	1.45 ± 0.03	0.95 ± 0.06	0.14 ± 0.02	0.20 ± 0.01	0.35 ± 0.01
Naproxen	LC_{10} (25.01)	23.23 ± 1.59	19.1 ± 0.92	16.6 ± 0.59	6.05 ± 0.56	7.25 ± 0.42	9.25 ± 0.54
	LC_{25} (13.33)	12.6 ± 1.05	10.1 ± 0.82	8.35 ± 0.25	2.95 ± 0.12	3.25 ± 0.09	4.25 ± 0.12
	LC_{50} (4.85)	4.85 ± 0.06	3.32 ± 0.91	2.01 ± 0.10	0.22 ± 0.03	0.26 ± 0.01	0.42 ± 0.10

*Data presented are means ± standard deviation.

DISCUSSION

Recently, it was noted that there are many pharmaceutical products reached water bodies due to receiving untreated or even insufficiently treated wastewater as a result of an increase in the consumption of these compounds in our daily life. The influence of three tested pharmaceuticals on the performance of *P. subcapitata* has been assessed by determining cell density, chlorophyll a, chlorophyll b, carotenoids, protein, lipid and carbohydrates contents. It is noticeable in the present study that, even by the sub-lethal concentrations of amoxicillin, tramadol and naproxen, regarded to be toxic or at least suppressor for *P. subcapitata*. The impacts of LC_{50} of the tested pharmaceuticals were compatible with those reported by using sulfamethoxazole, erythromycin and ciprofloxacin on microalgae and aquatic plants (Tang *et al.*, 1997; Teisseire *et al.*, 1999; Liu *et al.*, 2011). The determined residues of amoxicillin, tramadol and naproxen in the media and algal biomass clarified that, the decrease in the concentration of pharmaceuticals in the media may be attributed to degradation, transmission, adsorption and absorption (Musleh et al., 2005; Mofeed and Mosleh, 2013; Mofeed and El-Bilawy, 2020), but in our experiment, adsorption and transmission can be excluded since no sediments were present in the experimental system. Tramadol, amoxicillin and naproxen do not readily degrade (Mudgal et al., 2013), so the decrease in their concentration attributed to absorption by the algal cell, especially where it accompanied with increasing their residue in the algal biomass. However, the rat of decrease was

slower with high concentrations than the lower, due to the toxic effects of the high concentrations on *P. subcapitata*.

The obtained results showed that, the exposure to the sub-lethal concentrations of each pharmaceutical can reduce the growth and pigment productivity of *P. subcapitata*, which indicates an inhibitory effect of those pharmacologically active compounds by disturbing the metabolic and photosynthetic activities. In this line, Olivier et al., (2003) reported that the chlorophenol and triclosan compounds can be accumulated in the green algae and have strong growth inhibitory effects, causing damage to the integrity of cell walls, interferes with fatty acid synthesis and cell division dysfunction. In another study on the green algae *Raphidocelis subcapitata* exposed to antineoplastic pharmaceuticals, Thrupp (2016) demonstrated growth inhibition and disturbance in its bioassay test. Moreover, Guo et al., (2015), indicated that some pharmaceuticals such as amphotericin, erythromycin, ciprofloxacin, and sulfamethoxazole inhibited the photosynthetic electron transport of the photosynthetic algae and caused disruption of cell growth. The result which agreed with the obtained result and also in accordance with Gentili and Fick (2017) who found that when algae exposed to 0.05 mg.L^{-1} metconazole, Chlorophyll a, Chlorophyll b, and total Chlorophyll contents decreased compared to the control by 16.5, 22.4 and 40.4% respectively. These decreases attributed to the influence of the pharmaceuticals which induced hindrance in pigment biosynthetic pathway, besides the degradation in the existing pigment causing
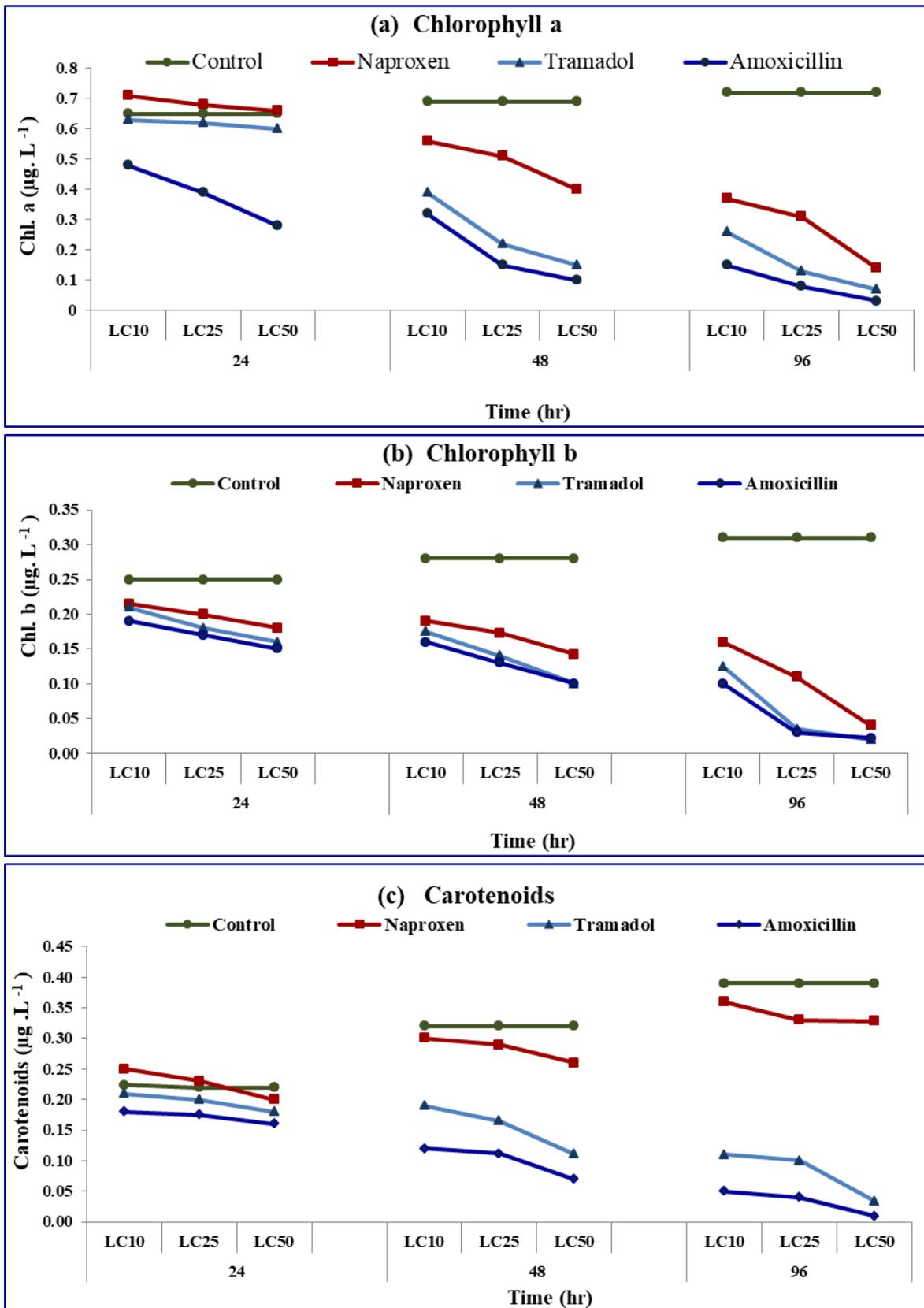
Figure (2): Effect of Naproxen, Tramadol, and Amoxicillin on (a) Chlorophyll a, (b) Chlorophyll b and (c) Carotenoids contents of *P. subcapitata* after 24, 48 and 96 hrs of exposure.
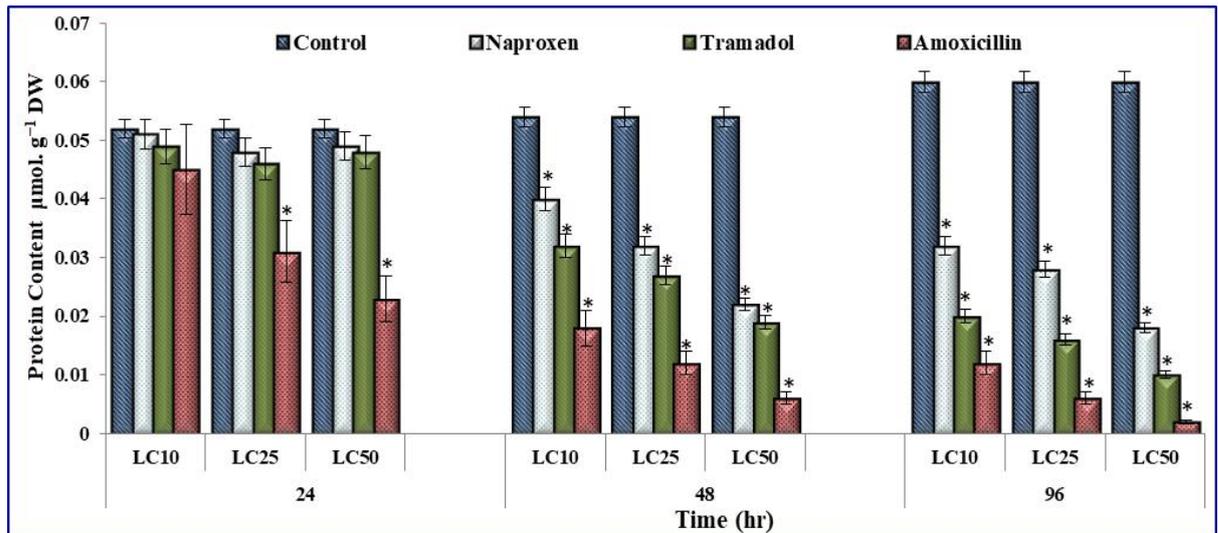
Figure (3): Protein content of *P. subcapitata* after 24, 48 and 96 hrs of exposure to Naproxen, Tramadol, and Amoxicillin.
*Significantly different at *P* ≤0.05 in a Student Newman–Keuls test.
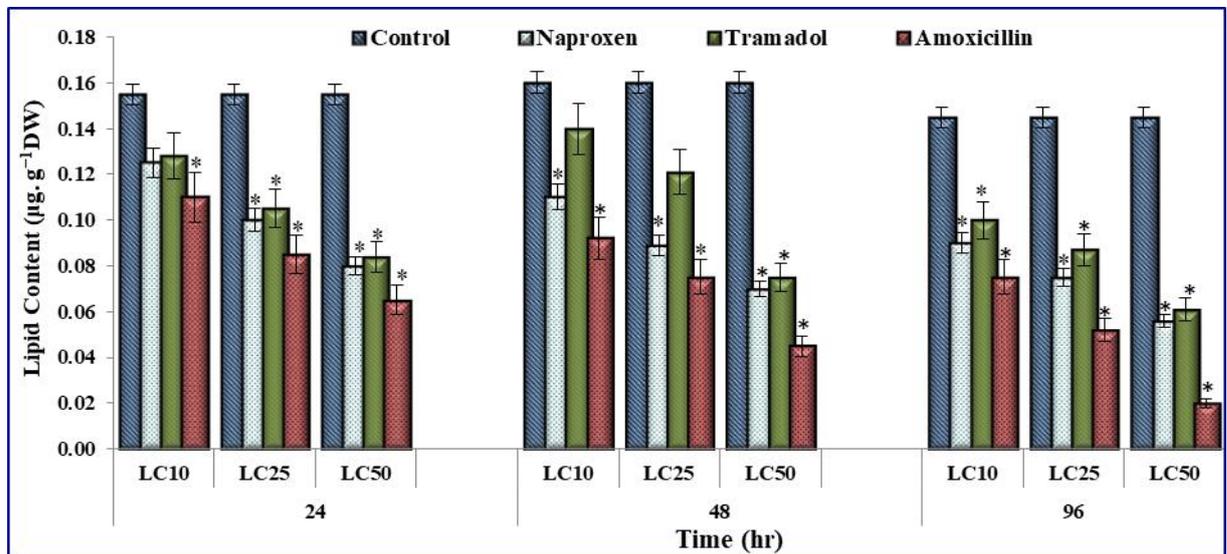
Figure (4): Carbohydrate content of *P. subcapitata* after 24, 48 and 96 hrs of exposure to Naproxen, Tramadol, and Amoxicillin.
*Significantly different at *P* ≤0.05 in a Student Newman–Keuls test.
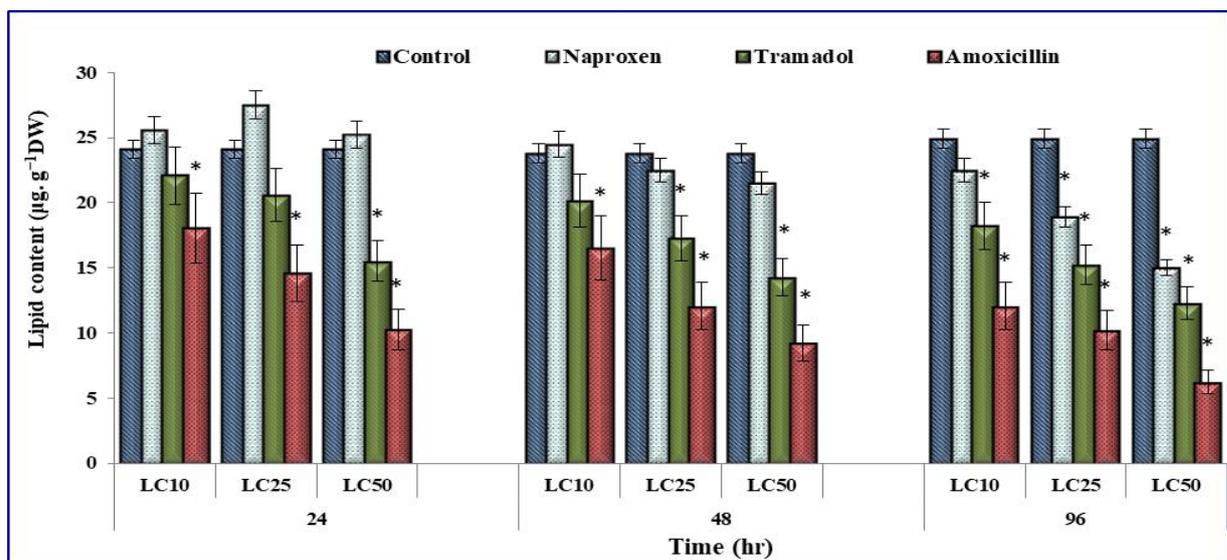
Figure (5): Total lipids content of *P. subcapitata* after 24, 48 and 96 hrs of exposure to Naproxen, Tramadol, and Amoxicillin.
*Significantly different from control at *P* ≤0.05 in a Student Newman–Keuls test.

a reduction in photosynthesis capacity. In another study by Wong (2000), who demonstrated the influence of glyohosate on the algal growth, chlorophyll and carotenoids of *Scenedesmus quadricauda*, and explained that exposure to 0.78 µmol.L^{-1} of glyphosate lead to growth inhibited by 84.7%, while chlorophyll suppressed by 65.7%. In contrary, we found that during the first 48 hrs of exposure to the low concentration of naproxen (LC$_{10}$), growth was promoted and the inhibition of cell growth increased with increasing naproxen concentrations. The increase in the beginning which followed by decrease with concentrations is an attempt by the cell to adapt with the used pharmaceuticals through establishing antioxidant and antitoxic strategies that failed with increasing concentrations.

Miazek and Brozek-Pluska (2019) reported that, exposure to pharmaceuticals and personal care products disturb enzymes and intracellular structure of the microalgal cells. The obtained results showed that, protein synthesis in *P. subcapitata* was dramatically reduced by the three tested pharmaceuticals (amoxicillin, tramadol and naproxen) after 48 and 96 hrs, however the slight suppression was recorded after 24 hrs, which indicates the toxicity of these compounds. While, the maximum inhibition, during the experimental period, was by amoxicillin within LC$_{50}$ after 96 hrs. The phenomena which explained by Brain *et al.*, (2008), who reported that, antibiotics can hinder the synthesis of protein by irreversibly binding to the subunits of ribosomes, which might disrupt transcription and translation (Sharma and Dubey, 2010). Unfortunately, the inspection of the literature shows that there is limited information about the influence of pharmaceutical compounds on lipids and carbohydrates in microalgae, which gives this study importance as one of the pioneer studies on this point. The obtained results elucidated that, naproxen, tramadol and amoxicillin inhibited both total lipid and carbohydrates production during the entire period of the experiment. However, the impact of tramadol on carbohydrate productivity by *P. subcapitata*, was lower than (17.4-57.9 %) amoxicillin, and naproxen. Amoxicillin was responsible for the maximum reduction (30 - 86.2 %) in lipids production with both concentration and time. Miazek and Brozek-Pluska (2019) indicated that, pharmaceutical products can discourage carbohydrates composition in the cells by disturbing the enzyme activity and inevitably the cell metabolism. In this connection, lipid content exhibited decrease with amoxicillin and tramadol depended on the concentration and the time of exposure, giving its maximum decrease when alga exposed to LC$_{50}$ of the pharmaceuticals. Meanwhile, naproxen enhanced total lipid productivity (14.1-4.7%) after the first 24 h and after 48 hrs (3%) only within LC$_{10}$, meanwhile, it declined with higher concentrations. The results which conformed with Zhang *et al.*, (2019) who mentioned that, the detailed mechanisms about the impact of pharmaceuticals and industrial chemicals still unclear, where some of them had motivating impact on algae lipid accumulation within low concentrations, however higher concentrations inhibited the lipid production, and added that this inhibition could be due to the partially suppressing the relevant enzyme activities that led to blocking lipid synthesis.

CONCLUSION

Pharmaceutical products may have therapeutic benefits, but the uncontrolled increase in consumption does not only harm our bodies, but it negatively reflected on other non-target organisms. The current study provides evidence on the toxic effect of three commonly used pharmaceutical products (amoxicillin, naproxen and tramadol) on micro-green alga *Pseudokirchneriella subcapitata*. Where the tested pharmaceuticals not only influenced both growth and photosynthetic pigments, but also cause decrease in its protein, carbohydrates and total lipid content. Therefore, competent authorities should prevent or at least reduce the access of pharmaceutical products to water bodies by using advanced effective sewage treatment. Besides, implementation of strict legislation for all new pharmaceutical products to undergo environmental risk assessment tests during and after the development of the product to obtain marketing approval. This is in addition to highlighting the need for indispensable extensive studies to give an obvious picture of the effect of these pharmaceutical products on aquatic organisms.

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آثار ثلاثة منتجات صيدلانية شائعة الاستخدام على المعايير البيوكيميائية للطحالب الدقيقة
Pseudokirchneriella subcapitata
(تحت ظروف المختبر)

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الملخص العربــــي

على الرغم من الأهمية الكبيرة للمنتجات الصيدلانية في حياتنا اليومية ، إلا أن لها آثارًا سلبية على البيئة حيث قد يكون لها تأثيراً سلبياً على كائنات غير المستهدفة. لذا توفر هذه الدراسة إطارًا متكاملًا للمساهمة في فهم سمية ثلاثة منتجات صيدلانية شائعة الإستخدام (أموكسيسيلين ونابروكسين وترامادول) على الطحالب الخضراء الدقيقة "*Pseudokirchneriella subcapitata* " . بحيث يمكن تقييم تأثير التلوث بالمنتجات الصيدلانية الثلاثة على الكائنات المائية من خلال تقييم الإستجابة الفسيولوجية لـ *P.subcapitata* . حيث تم تعريض الطحلب لتركيزات شبه قاتلة (LC_{10} و LC_{25} و LC_{50}) لكل دواء لتحديد تأثيره على النمو والأصباغ والدهون الكلية و كذا البروتين والكربوهيدرات. أوضحت النتائج التي تم الحصول عليها أن التعرض للمستحضرات الصيدلانية بتركيزاتها شبه القاتلة أدى في الغالب إلى إنخفاض في كثافة النمو ، على الرغم من أن التعرض لتركيزات منخفضة من النابروكسين عزز النمو (حتى٪ 21.7) في التركيزات الصغيرة خلال 48 ساعة الأولى. في حين قلل أموكسيسيلين متبوعًا بالترامادول بشكل كبير من محتوى الأصباغ طوال فترة التجربة ولكن بطرق مختلفة . و من المثير للإهتمام انه بإستثناء تركيز $LC_5$0 ، تسبب النابروكسين في زيادة محتوى كل من الكاروتينات والكلوروفيل خلال 48 ساعة الأولى. من الجدير بالذكر ان الأدوية قيد الاختبار (أموكسيسيلين ، ترامادول ونابروكسين) لم تؤثر على كلاً من النمو و الأصباغ لطحلب *P.subcapitata* ، فقط ولكنها أدت أيضًا إلى تقليل البروتين والكربوهيدرات ومحتوى الدهون الكلي. لذا يجب على السلطات المختصة منع أو على الأقل تقليل وصول المنتجات الصيدلانية إلى المسطحات المائية بإستخدام طرق فعالة ومتطورة لمعالجة مياه الصرف الصحي.