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Response of *Chlorella vulgaris* to Some Selected Pharmaceutical Drugs

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ABSTRACT

The objective of this study was to assess the toxicological effects of commonly prescribed pharmaceuticals (Voltaren, Panadol, Selgon, and E.mox) on the microgreen alga *Chlorella vulgaris.* The algal culture was subjected to various concentrations of the test drugs (0.1, 3.2, 12.8, and 25 mg/L) and incubated for different durations (24, 48, and 96-hrs.). The findings revealed a general decline in algal growth response due to the pharmaceutical drugs, although variations were observed among individual drugs. Both the total carbohydrate and pigment levels exhibited a gradual reduction, displaying a dose- and time-dependent trend. Notably, *Chlorella vulgaris* treated with the pharmaceuticals at a dose of 25 mg/L exhibited lower total amino acid content compared to the control group. Among the drugs tested, E-mox at a dose of 25 mg/L caused the most significant increase in proline levels. As the drug dosage increased, the activity of three antioxidant enzymes (glutathione reductase, ascorbate peroxidase, and superoxide dismutase) was induced. Electron microscopy analysis of the algal cells grown at a dose of 25 mg/L and incubated for 96 hrs. revealed distinct morphological changes. Panadol treatment resulted in a cell wall surface covered with crowded nipples, while Selgon had no discernible effect on the cell wall. The addition of Voltaren led to a coated surface with dense nipples, whereas E-mox treatment caused irregular cell shapes, loss of distinctive morphology, and wear and fracture of the cell wall. These findings emphasize the importance of controlling such pollutants and implementing monitoring strategies, particularly in water bodies exposed to these pharmaceuticals, to safeguard ecosystem equilibrium and human well-being.

Keywords: Amino acids; Antioxidant enzymes; *Chlorella vulgaris*; Microgreen alga; Pharmaceuticals; Toxicological impacts.

INTRODUCTION

From ancient times to the present day, pharmaceuticals have been extensively utilized for their therapeutic properties in the treatment and prevention of diseases, benefiting both human and animal health and enhancing their quality of life (Miazek and Brozek-Pluska, 2019). However, a significant portion of these pharmaceutical products is released into the environment through sewage systems due to inadequate removal during wastewater treatment processes. Furthermore, the improper disposal of unused drugs by pharmaceutical manufacturers also contributes to this issue (Daughton and Ruhoy, 2009; Zhang *et al.*, 2019), ultimately leading to their presence in aquatic ecosystems. Moreover, the use of growth promoters and antibiotics in animal husbandry and veterinary practices (Kummerer, 2009) has been linked to the global rise in concentrations of pharmaceutically active compounds in aquatic environments (Kosma *et al.*, 2017).

It is important to aware that, the therapeutic groups of those products that have reached the aquatic environment are often remain active even at low concentration ranges (Pico-nmol/L). Additionally, the active ingredients of the pharmaceutical products are biologically potent chemicals intended to interact with receptors in humans, animals, or combat infectious organisms by targeting bacteria, fungi, and parasites (Boxall, 2004). Reports claim that pharmaceutical medications, even in small concentrations, have a detrimental impactt on the aquatic environment. However, there are currently no effective techniques for evaluating the potential hazards caused by medications in order to preserve aquatic life. Several commonly used therapeutic drugs were included in this study. Selgon, a non-narcotic centrally acting antitussive, has the chemical composition $C_{21}H_{25}N_3O_3S$ HCl (Sandra *et al.*, 2004). Panadol, an antipyretic containing paracetamol, has the chemical composition $C_8H_9NO_2$. Another drug included in the study is Voltaren, which is an anti-inflammatory drug with the chemical composition $C_{14}H_{10}C_lNNaO_2$. Additionally, the broad-spectrum antibiotic Amoxicillin (E-mox), known for its effectiveness against both Gram-negative and Gram-positive bacteria, was also selected (Boxall, 2004; Bobak *et al.*, 2022).

Algae are photosynthetic organisms that are very sensitive to pharmacological exposure (Guo *et al.*, 2015, Mofeed, 2020 and Yun *et al.*, 2020). Additionally, they provide excellent, quick, and affordable biomarker organisms for the analysis of environmental health assessments. As a result, aquatic monitoring programs are thought to use aquatic microalgae as a suitable bio-indicator to follow any changes in water quality. As a result, by examining the biochemical behavior of the algal cells, it is achievable to assess the effects of newly

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discovered pharmaceutical contaminants on aquatic species even at low concentrations (Mofeed and El-Bilawy 2020). Since algae carry out such essential ecological tasks as the production of oxygen, the circulation of nitrogen, and food supply, the harmful effects of pharmaceuticals on algae may not only result from their actions that inhibit algal growth, but also from their effects on the ecosystem as a whole (De-Larenzo and Fleming 2008 and Monika *et al.,* 2022). Microalgae are the principal food providers in aquatic ecosystems. They also constitute the initial link in the food chain, so any hazards to them will ultimately have an impact on aquatic organisms as well as humans at higher trophic levels (Mosleh and Mofeed 2014). Aquatic algae are consequently considered an excellent bio-indicator for aquatic monitoring systems to follow any changes in water quality. Therefore, the objective of this study was to examine the impact of four frequently employed pharmaceutical products (Panadol, Selgon, Voltaren, and E-mox) on the growth of the micro-green alga *Chlorella vulgaris*. Additionally, the influence of these products on the synthesis of protein, carbohydrate, lipid, and carotenoid compounds were also studied.

MATERIALS AND METHODS

Algal culture and growth conditions

Chlorella vulgaris was the most dominant unicellular green alga in fresh water samples and was obtained from Microbiology Lab. Faculty of Fish Resources, Suez University, Egypt. The unicellular green alga *C. vulgaris* was grown in 250 ml culture flasks. Each flask containing 100 ml of nutritive medium, Bold's Basal Media (Nichols and Bold 1965), pH was adjusted at 6.8 according to (Allen, 1952). After autoclaving at 121^{o}C for 20 min., the media were inoculated under aseptic conditions with 1 ml of algal suspension (1 ml = 5.2 X10^{4} cells) from the previous alga. All flasks were incubated for 10 days under continuous illumination at 6000 lux at 27 ^{o}C (Cheunbarn and Peerapornpidal 2010).

Tested pharmaceutical drugs

Four pharmaceutical compounds obtained from Sigma, with a purity of approximately 99%, were included in this study. These compounds include Panadol (paracetamol), Selgon (pipazethate hydrochloride), Voltaren (diclofenac sodium), and E-mox (amoxicillin). Panadol, an antipyretic, has a chemical composition of $C_8H_9NO_2$ (Paracetamol as its basic ingredient). Selgon, an antitussive, is characterized by the chemical formula of $C_{21}H_{25}N_3O_3S$-HCl (Pipazethate HCl). Voltaren, an anti-inflammatory medication, has a chemical composition of $C_{14}H_{10}C_lNNaO_2$ (Miazek and Brozek-Pluska, 2019). Lastly, E-mox, a broad-spectrum antibiotic effective against both gram-negative and gram-positive bacteria, has the chemical formula $C_{16}H_{19}N_3O_5S$ (National Center for Biotechnology Information, 2024).

Experiment design

In this experiment, the effects of four pharmaceutical compounds (Panadol, Selgon, Voltaren, and E-mox) on the green alga *C. vulgaris* were assessed under standard conditions. The concentrations of the tested compounds used were 0.1, 3.2, 12.8, and 25.0 mg/L. To prepare the algal inoculum, 100 ml of the stock cultures were centrifuged at 1000 rpm for 5 min. The supernatant was carefully removed, and the algal residue was re-suspended in sterilized distilled water. An equivalent inoculum of 1 ml of algal cells (5.2X10^{4} cells/ml) was added to each flask containing different concentrations of the pharmaceutical compounds. The flasks were then incubated for 24, 48, and 96 hrs. under continuous illumination at 6000 lux and a temperature of 27°C. A control culture without any drugs was also prepared. Three replicates were conducted for each drug concentration (Figure 1).

Determination of growth parameters

To assess the growth parameters of *C. vulgaris*, various measurements were conducted.

Cell density

Cell density, a crucial indicator of organism growth, was assessed by measuring the optical density (OD) at 560 nm (Cheunbarn and Peerapornpisal, 2010) using a spectrophotometer (Spectronic 20) at the end of each incubation period. This measurement provided valuable insights into the proliferation and density of the tested organism.

Determination of pigments

The determination of photosynthetic pigments including Chlorophyll a, Chlorophyll b, and Carotenoids was performed following the method adapted from Metzner *et al.* (1965). This method was utilized to accurately quantify the levels of these pigments in the samples under investigation.

Determination of Carbohydrates

The total carbohydrate content of *C. vulgaris* was determined using the extraction method described by Said and Naguib (1964). Subsequently, the extracted carbo-hydrates were quantitatively assessed as glucose-equivalent using

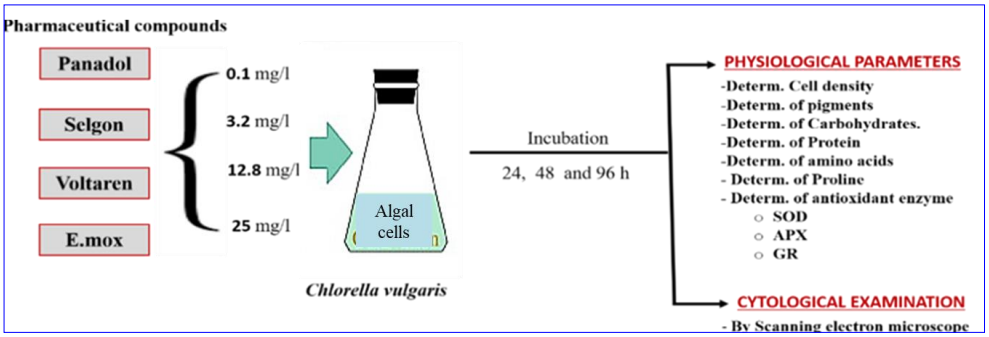

Figure (1):.Experimental design for assessing the impact of different pharmaceutical drugs on *Chlorella vulgaris*.

the modified Nelson method (Naguib, 1964) based on the original method developed by Nelson (1944). This approach ensured an accurate measurement of the carbohydrate content in the samples.

Nitrogen Estimation

Proline Analysis

The proline content was determined following the method outlined by Bates *et al.* (1973) and measured using spectrophotometry at an optical density of 520 nm. The proline content on a fresh weight basis was expressed as µmoles/g fwt using the following formula:

Proline content (µmoles/g fwt) =

$$\mu moles / g\ fwt = \frac{\mu g\ proline/mL \times mL\ toluene}{115.5} \times \frac{5}{g\ sample}$$

Where, 115.5 represent the molecular weight of proline. This calculation allowed for the accurate quantification of proline content in the samples.

Amino acids

The extraction of amino acids was conducted following the procedure outlined by Baily (1967), and their estimation was performed using the method described by Steven *et al.* (1989). Amino acid analysis was carried out using the amino acid analyzer (Eppendorf – Germany, LC 3000) with the following parameters: a flow rate of 0.2 ml/min, a buffer pressure range of 0–50 bar, a reagent pressure range of 0–150 bar, and a reaction temperature of 123 °C. This analytical approach allowed for the accurate determination of amino acid composition in the samples.

Protein Estimation

Protein content was estimated using the method established by Lowry *et al.* (1951). The protein extraction was quantified spectrophotometrically at an optical density of 750 nm, by comparing against a blank. This approach allowed for accurate determination of protein content in the samples.

Antioxidant Enzymes Analysis

The activity of antioxidant enzymes was assessed to evaluate the antioxidant defense system in the samples.

Superoxide Dismutase (SOD)

Superoxide dismutase activity was estimated following the procedure outlined by Beyer and Fridovich (1987). The activity of SOD was expressed in terms of units per milligram of protein. In this assay, one unit of enzyme activity was defined as the amount of enzyme required to inhibit approximately 50% of the reduction of nitroblue tetrazolium (NBT) under the specified assay conditions.

Ascorbate peroxidase (APX)

Ascorbate peroxidase activity was determined according to Nakano and Asada (1981). The activity of APX is expressed as unites µg/mg^{-1} protein). One unit is defined as micromoles of ascorbate oxidized per minute per milligram of protein.

Glutathione reductase (GR)

The activity of glutathione reductase was assessed according to the method outlined by Goldberg and Spooner (1983). The rate of change in absorbance at 430 nm per minute was directly proportional to the GR activity in the sample. In this assay, one unit of enzyme activity was defined as the amount of enzyme required to catalyze the conversion of one micromole of oxidized glutathione (GSSG) per minute.

Scanning Electron Microscopy (SEM) Analysis

For the SEM analysis, the algal cells under investigation were dehydrated by gradually increasing the ethanol concentrations (up to 96% ethanol). Subsequently, they were transferred to formaldehyde dimethyl acetal for 24 hrs. and 2 hrs, respectively. The samples were then subjected to critical point drying using CO_2. Prior to examination, the specimens were coated with a thin layer of palladium/gold. The SEM imaging was performed using a Philips XL20 SEM microscope, following the methodology described by Georg *et al.* (2015). This technique allowed for detailed visualization and analysis of the algal cell structures at high magnification.

Statistical Analysis

The significance of variation in the parameters was determined using analysis of variance (ANOVA), as described by Larson (2008). Additionally, Detrended Correspondence Analysis (DCA) ordinations were conducted using the CANOCO program version 7.1. This analysis aimed to explore the relationships between the pharmaceuticals and algal species. The axes in the DCA were constrained to optimize their relationship, providing valuable insights into the associations between the variables.

RESULTS

Evaluation of Pharmaceutical Drugs on growth parameters of *C. vulgaris*

Cell density

The effects of different concentrations (0.1, 3.2, 12.8, and 25 mg/L) of commonly used pharmaceutical drugs, namely Panadol (P), Selgon (S), Voltaren (V), and E-mox (E), on the growth of *C. vulgaris* were assessed. Figure 2 illustrates the growth suppression observed throughout the investigation period. Notably, the highest growth suppression of 88.36% was observed after a 96-hrs. exposure to the antibiotic E-mox at a concentration of 25 mg/L. Conversely, the lowest growth suppression of the tested alga, recorded at 14.16%, was observed at the lowest E-mox concentration of 0.1 mg/L. These findings provide insights into the impact of different pharmaceutical drugs on the growth of *C. vulgaris*. Both Voltaren and E-mox exhibited inhibitory effects on algal cell density. However, E-mox resulted in a more pronounced suppression of cell density, with a reduction of 87.22% observed at the same concentration and after the same incubation period. In contrast, the suppression of cell density by Voltaren appeared to be less significant. Interestingly, analysis of the results revealed that Selgon had the lowest impact on cell density, followed by Panadol, throughout the entire investigation period. Furthermore, the least severe suppression effects on cell density were observed when the algal cells were exposed to low doses of the tested drugs (0.1 mg/L) during the 24-hrs. incubation period. However, as the experiment approached the 96-hrs.. time frame, all treatments exhibited indications of growth inhibition. These findings highlight the varying effects of the pharmaceutical drugs on algal cell density and the importance of exposure duration in assessing their impact.

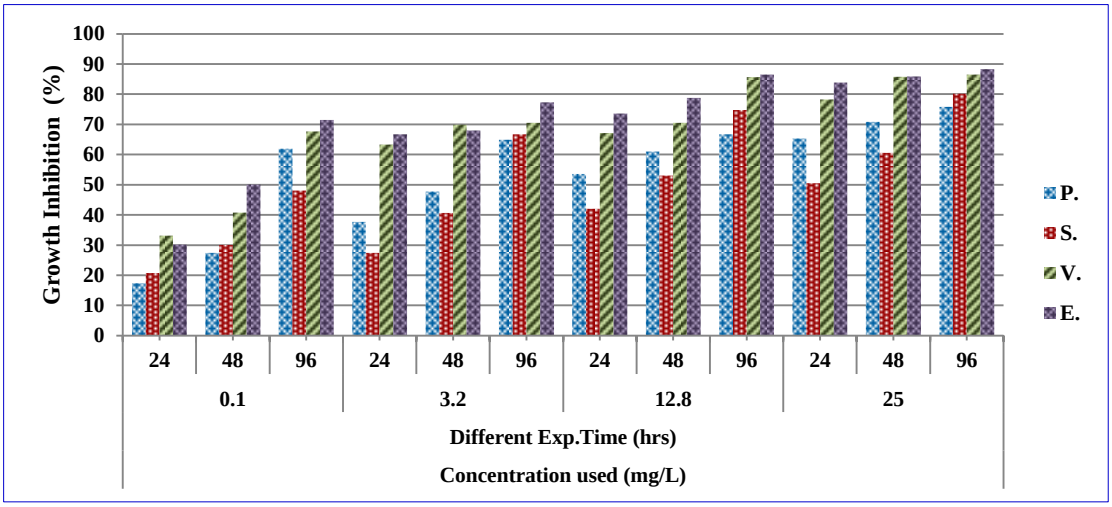

Figure (2): Comparative Assessment of Growth Inhibition Percentage in *Chlorella vulgaris* at different exposure times to four pharmaceutical drugs: Panadol (P), Selgon (S), Voltaren (V), and E-mox (E).

Pigment content

Regarding the order of changes in the total pigment content following application of the different tested drug doses during different incubation times, Table (1) clarifies that, applying different drug concentrations during different incubation periods led to a significant decrease in total pigments in the order E.mox > Voltaren > Selgon > Panadol. It was noted that for all of the tested medicines, such progressive decline was dose- and time-dependent.

Carbohydrate content

Regarding the total carbohydrates contents of *C. vulgaris*, Tables (2) reflects a progressive reduction in total carbohydrates in all treatments with a percentages of decrease reached (6.25, 9.72 ,16.7 and 23.6 %, respectively) in concentration 0.1 mg/L in Panadol, Selgon, Voltaren and E-mox and (10.4, 16.7, 23.6 and 30.6) in concentration 3.2 mg/L (14.93, 18.61, 30.51and 37.5%, respectively) and (20.1, 24.9, 33.9 and 44.16 %, respectively) in *Chlorella vulgaris* treated with Panadol, Selgon, Voltaren and E-mox respectively, if compared with their corresponding control. It is worth mentioning that, the effect of the previous selected drugs on total carbohydrates content was dose and time dependent. Also, it could be stated that, by comparing the data of total carbohydrates with those of polysaccharides, it could be observed that the pattern of changes in total carbohydrates was similar to that of polysaccharides which appeared to be the predominant fraction in the algal carbohydrates pool.

Proline content

The results for proline contents in *C. vulgaris*, as illustrated in Figure (3), demonstrated a steady increase in proline content as the pharmaceutical concentrations increased from 0.1 mg/L to 25 mg/L. Among the investigated algae, the highest percentage increase in proline content of C. vulgaris was observed with E. mox treatment at a concentration of 25 mg/L, which was 21.3 mg/g dry weight higher than the comparable control (4.00 mg/g dry weight). Conversely, the smallest growth proportion was estimated for Panadol at a concentration of 0.1 mg/L, resulting in a decrease of 5.4 mg/g dry weight compared to the control. Importantly, the increase in proline content exhibited a dose-dependent relationship.

Amino acids content

The studied microalga, *Chlorella vulgaris*, consisted of 18 amino acids, categorized into four families: Glutamate (Figure 4A), Aspartate (Figure4B), Trios (figure 4C), and Pyruvate (Figure 4D), with varying quantities. When the experimental algae were treated with the four pharmaceutical drugs at a concentration of 25 mg/L, the total amino acid contents of *C. vulgaris* generally decreased compared to the corresponding control. The antibiotic E-mox exhibited the highest percentage of decrease, followed by Voltaren, Selgon, and Panadol (57.8%, 46.1%, 32.5%, and 17.8% respectively). Notably, Panadol demonstrated the lowest percentage of inhibition, potentially attributed to the shikmic acid family being 22.6% lower than the control. Conversely, the antibiotic E-mox displayed the most significant reduction in total amino acid content, with a decrease of 57.8% compared to the control, potentially due to a drop in the shikmic acid family (52.3%) and other factors.

Total soluble protein

The data pertaining to total soluble proteins (Figure 5:A, B, C and D) show that, the protein content was decreased by the four tested pharmaceutical drugs at all concentrations and during the exposure times. However, it is noteworthy to highlight that a very minor inhibitory impact, particularly after 24 hrs., was observed with the four tested drugs. The decreases in the corresponding percentages for the drugs Panadol, Selgon, Voltaren, and E-mox were 8.6, 14.3, 28.6, and 37.1%, respectively. However, during all incubation times, both E-mox followed by Voltaren exhibited a significant reduction of protein synthesis at all concentrations (3.2, 12.8 and 25 mg/L), with their respective maximal suppressions

Table (1): Effect of different concentration (mg l^{-1}) of the tested pharmaceutical drugs on measurement pigment parameters including Chlorophyll and Carotenoids of *Chlorella vulgaris*.

Used Cocn. (mgL^{-1})	Drug used	Measuerment parameters														
		Chlorophyll a			Chlorophyll b			Total Chlorophyll			Carotenoids			Total pigment		
		Time exposure (hrs.)														
		24	48	96	24	48	96	24	48	96	24	48	96	24	48	96
Control		12.20	40.80	41.00	8.00	13.00	26.00	20.20	53.80	67.00	6.70	8.30	16.00	26.90	60.50	83.00
0.1	P	10.00	33.01	25.00	7.50	11.00	20.00	17.50	44.01	53.01	6.20	7.00	13.00	23.70	51.01	66.01
	S	9.50	25.00	19.50	6.30	10.00	18.00	15.80	35.00	37.50	5.10	6.00	11.00	20.90	41.00	48.50
	V	8.00	20.00	14.70	6.00	8.00	15.00	14.00	28.00	29.70	4.00	4.30	9.00	18.00	32.30	38.70
	E-mox	7.00	15.10	12.20	4.90	7.00	13.00	14.00	22.10	25.20	3.70	4.00	7.20	17.70	26.10	32.40
3.2	P	9.60	30.00	21.00	6.20	9.00	17.00	15.80	39.00	38.00	5.50	5.30	10.00	21.30	44.30	48.00
	S	7.80	23.50	14.50	5.90	8.70	16.20	13.70	32.20	31.00	4.50	4.50	8.00	18.20	36.70	39.00
	V	6.90	17.00	12.20	4.90	7.40	14.00	11.80	24.40	26.20	3.20	4.30	7.00	15.00	28.70	33.20
	E-mox	5.40	13.50	10.01	4.00	5.90	11.00	9.40	19.40	21.01	3.00	3.50	6.00	12. 4	22.90	27.10
12.8	P	7.00	24.10	18.20	5.00	7.50	14.00	12.00	31.60	30.20	4.20	3.70	6.90	16.20	35.30	37.10
	S	65.00	19.00	17.00	4.50	6.50	12.00	11.00	25.50	29.00	4.00	4.00	6.30	15.00	29.50	35.30
	V	4.50	15.00	9.30	3.20	4.20	8.00	7.70	19.20	17.30	2.20	3.20	4.60	9.90	22.40	21.90
	E-mox	4.00	10.00	8.80	2.50	3.00	5.00	6.50	13.00	13.50	1.90	2.10	3.20	8.40	16.20	16.70
25	P	5.50	18.00	16.20	4.50	6.90	11.20	10.00	24.90	27.40	4.00	2.90	5.10	14.00	27.80	32.50
	S	4.00	13.00	10.00	3.90	4.80	9.00	7.90	17.80	19.00	3.80	3.00	5.50	11.70	20.80	24.50
	V	3.20	10.30	5.01	3.00	3.50	5.00	6.20	10.01	13.80	2.00	2.50	2.90	8.20	12.51	16.70
	E-mox	3.02	5.20	3.00	1.90	1.70	2.00	4.92	6.90	5.00	1.50	1.30	0.90	6.42	8.20	5.90

† P, Panadol; S, Selgon; V, Voltaren; E-mox, Antibiotic.

Table (2): Effect of different concentrations (mg/L) of tested pharmaceutical drugs on measurement parameters of soluble sugars and total carbohydrates in *Chlorella vulgaris*.

Conc. Used (mg L^{-1})	Drug used†	Soluble sugars (mg/g DryWt.)		Total soluble sugars	Poly-saccharide (mg/L)	Total carbohydrates (mg/L)
		DRV††	Sucrose			
Control	0.0	29.00 ±0.13	35.00 ±0.82	64.00	80.00 ±1.20	144.0
0.1	P	28.50 ±0.13	51.50 ±0.82	80.00	65.00 ± 2.2	135.0
	S	20.80 ±0.17	49.20 ±0.21	70.00	60.00 ± 1.70	130.0
	V	20.00 ±0.7	50.00 ±0.33	70.00	50.00 ± 2.5	120.0
	E-mox	20.00 ±0.13	54.00 ±0.67	74.00	36.00 ±1.5	110.0
3.2	P	21.50 ±0.77	51.50 ±1.10	73.00	56.00 ±0.97	129.0
	S	29.62 ±0.3	38.38 ±0.17	68.00	52.00 ±0.22	120.0
	V	20.90 ±0.41	59.10 ±0.52	80.00	30.00 ±0.79	110.0
	E-mox	23.64 ±0.66	59.36 ±0.78	83.00	17.00 ±0.65	100.0
12.8	P	20.40 ±0.33	54.60 ±0.47	75.00	47.50 ±1.25	122.5
	S	31.78 ±0.36	40.22 ±0.24	72.00	45.20 ±0.67	117.2
	V	25.80 ±0.93	29.20 ±0.73	85.00	15.07 ±0.54	100.1
	E-mox	22.50 ±0.42	65.50 ±0.55	87.00	3.00 ± 0.09	90.0
25	P	16.10 ±0.32	59.90 ±0.81	76.00	39.07 ±0.17	115.1
	S	31.96 ±0.54	43.04 ±0.33	75.00	33.01 ±0.28	108.0
	V	30.13 ±0.66	35.87 ±0.61	66.00	29.09 ±0.37	95.1
	E-mox	10.00 ±0.21	60.00±0.45	70.00	10.41 ±0.25	80.4

†P, Panadol; S, Selgon; V, Voltaren; E-mox, Antibiotic; ††, Direct reducing value.

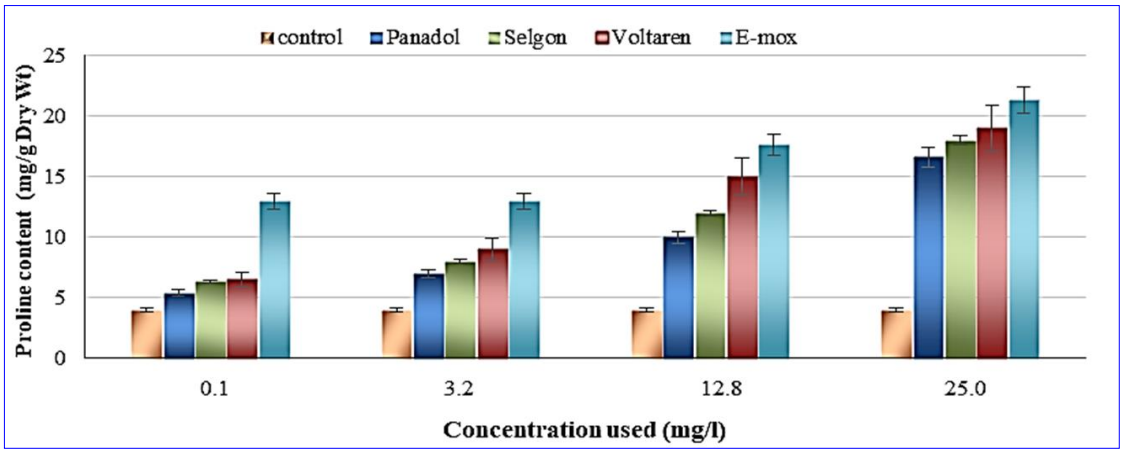

Figure (3): Impact of various concentrations (mg/L) of tested pharmaceutical drugs on proline content (mg/g Dry Weight) of *Chlorella vulgaris* following 96 hrs. of exposure.

being 59.6 and 50% at 25 mg/L, respectively. On the other hand, Panadol (35.8%) and Selgon (39.1%) recorded lower inhibition even with their high concentrations (25 mg/L) at the end of the experiment.

Antioxidant content

The impact of different concentrations of pharmaceutical drugs (Voltaren, Panadol, Selgon, and E-mox) on the activity of antioxidant enzymes, specifically glutathione reductase (GR), ascorbate peroxidase (APX), and superoxide dismutase (SOD), in *C. vulgaris* is shown in Figure (6: A, B, and C). It is evident that increasing drug concentrations resulted in enhanced activity of antioxidant enzymes. The most significant stimulations were observed after 96 hrs. of treatment with a high dose of 25 mg/L. Notably; the enzyme glutathione reductase (GR) exhibited the most substantial increase, reaching two and a half times the control value when exposed to the broad-spectrum antibiotic E-mox (Fig. 6D). Moreover, notable elevations in the activity of glutathione reductase were observed when exposed to the maximum concentration (25 mg/L) compared to 12.8 mg/L with all tested drugs. The enzyme's activity increased by 21.8%, 20.1%, and 11.66% with Voltaren, Selgon, and Panadol, respectively. Additionally, the investigation has shown that a high dose of 25 mg/L can induce the maximum production of ascorbate peroxidase (APX). Results for E-mox, Voltaren, Selgon, and Panadol were 26.4 $\mu g/mg^{-1}$ protein, 25.0 $\mu g/mg^{-1}$ protein, 23.4 $\mu g/mg^{-1}$ protein, and 19.3 µg/mg-1 protein, respectively. A comparable pattern was noted for superoxide dismutase (SOD), suggesting that exposure time and dose had an impact on the activity of both the APX and SOD enzymes.

In conclusion, the current investigation discovered that the amount and length of exposure to the examined pharmaceutical medicines affected the activity of the SOD and APX enzymes in *C. vulgaris*. These results emphasize the significance of taking dosage and exposure duration into account when assessing how pharmaceutical pollutants affect.

Ultrastructure of *Chlorella vulgaris*

The electron micrographs taken after a 96 – hrs. of

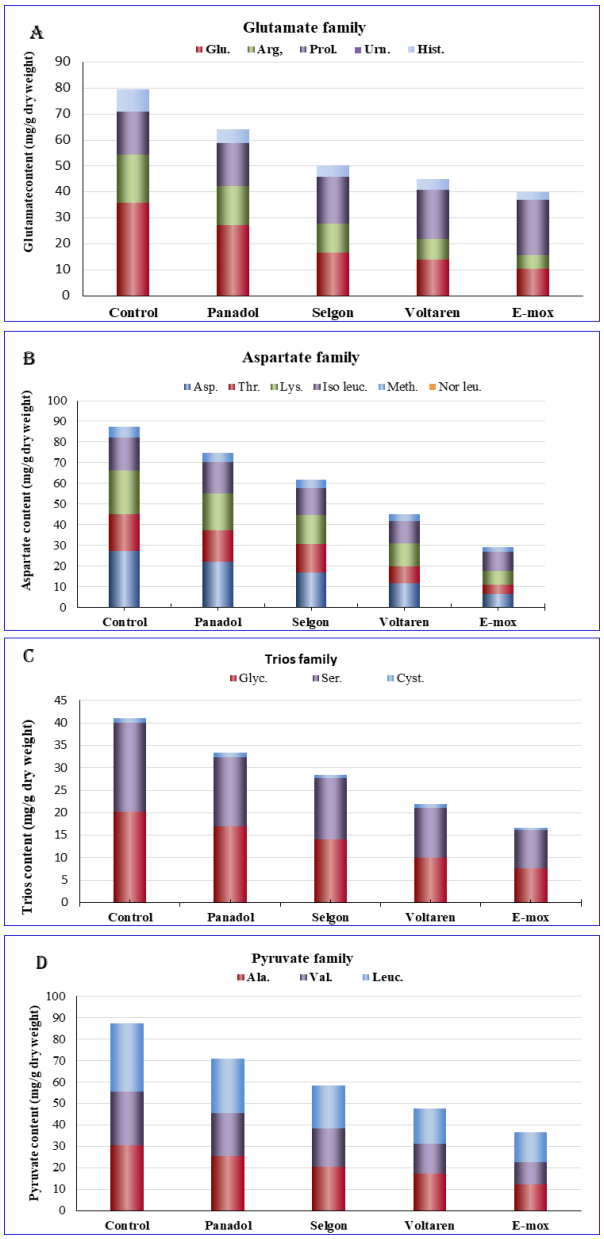

Figure (4): Impact of various concentrations (mg/L) of tested pharmaceutical drugs on Amino Acid Content (mg/g D.Wt.) in at different exposure time in *Chlorella vulgaris* .A, glutamate family; B, Aspartate family; C, Triose family; D, pyruvate family.

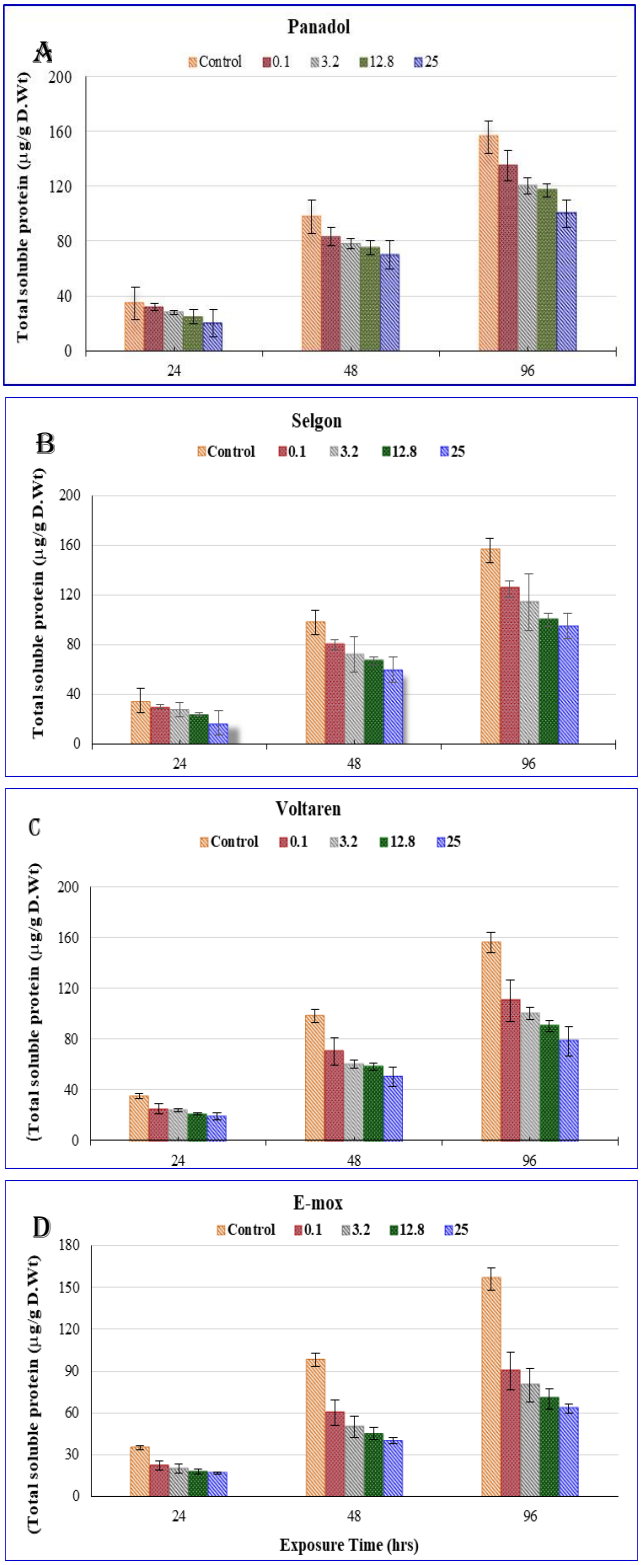

Figure (5): Impact of different pharmaceutical drugs at various concentrations (mg/L) and exposure times on Total Soluble Protein Content (µg/g Dry Weight) in *Chlorella vulgaris*. A, Panadol; B, Selgon; C, Voltaren; D, E-mox."

incubation of *C. vulgaris* in BBM medium enriched with the highest concentration of each pharmaceutical drugs, along with the control, offer important novel insights. This image (Fig. 7A) shows the rugose surface of the cell wall and the normal cell morphology of *C. vulgaris*. However, when exposed to a dosage of 25 mg/L of Panadol, the dist-

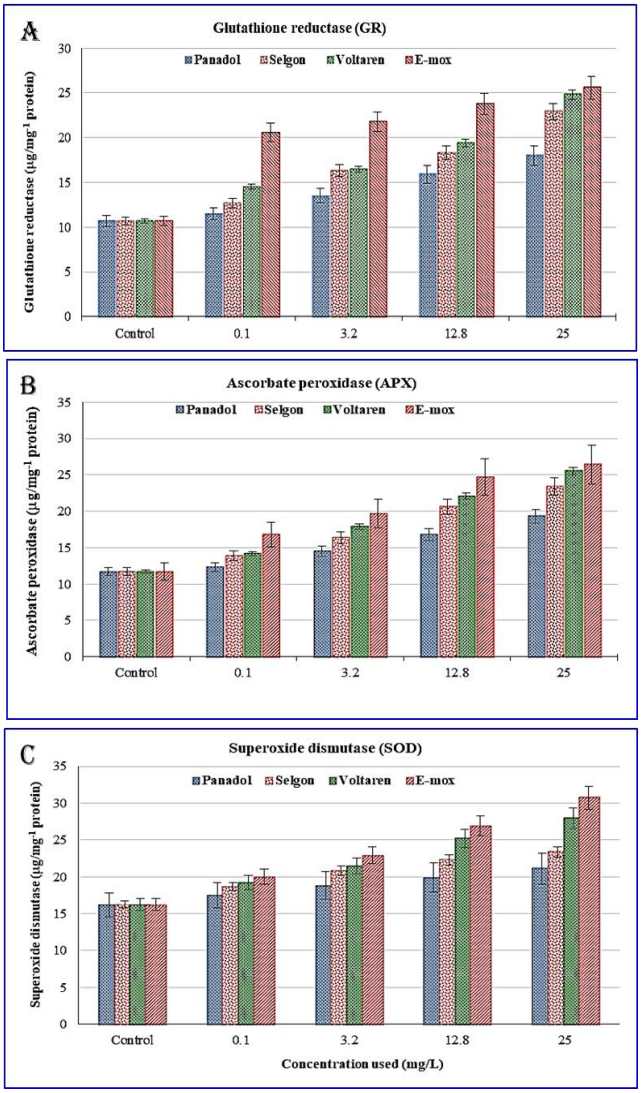

Figure (6): Impact of Different Pharmaceutical Drugs at Various concentrations (mg/L) and exposure times on the measured Antioxidant Content (µg/mg^{-1} protein) in *Chlorella vulgaris*. A, Glutathione reductase (GR); B, Ascorbate peroxidase; C, Superoxide dismutase.

inctive rugose surface of the cell wall disappeared, and the surface was instead covered in closely spaced nipple-like structures (Fig. 7B). In the second image (Fig. 7C), the cell subjected to Voltaren therapy exhibited a clear change in form, with a noticeable twist in the center of the cell body. Additionally, the cell wall lost its distinctive rugose surface, appearing smooth without any protrusions or projections. These micrographs provide compelling visual evidence of the morphological modifications induced by the tested pharmacological medicines on *C. vulgaris*. Meanwhile examining photomicrograph (7D), it is apparent that the application of the antitussive drug Selgon, which is commonly used to suppress or alleviate coughing, to the alga *C. vulgaris* did not provide significant alterations in the shape or appearance of the cell wall. However, there was a visible aggregation of cells. These cells showed a tendency to group together in large numbers, creating an asymmetrical unit composed of many cells. In addition, the cells grouped in big clusters and interacted with one another in a way that combined to form an irregular unit consisting of many cells.

Analysis of association among tested pharmaceutical drugs

Regarding the extent of association between the tested pharmaceutical drugs, cluster analysis was performed, and the results were visualized in Figure (8). The analysis revealed interesting findings: a, Panadol and Selgon were found to be related in a minor sub-group, indicating a close extent of influence between these two products. Another minor subgroup was observed, where Voltaren and E-mox were separated with a high dissimilarity factor, suggesting significant differences in their effects. These results provide valuable insights into the relationships and similarities among the tested pharmaceutical products.

DISCUSSION

By measuring the optical density of the algal suspensions at 560 nm, it was possible to assess the consequences of different doses of the tested drugs on *Chlorella vulgaris* growth. It was noticeable that the maximum inhibitory effect. The maximum inhibition was recorded with the broad spectrum antibiotic E-MOX, while Panadol had a lower effect. By measuring the optical density of the algal suspensions at 560 nm, it was possible to assess the consequences of different doses of the tested pharmaceutical drugs on *Chlorella vulgaris* growth. The obtained results coincide with Mofeed and El-Bilawy (2020) assertion that *Pseudokirchnerilla subcapitata* growth and pigment productivity were reduced when it was treated with sub-lethal doses of the drugs being examined (Amoxicillin, Naproxen, and Tramadol). This shows how drugs with pharmacological activity may inhibit specific metabolic and photosynthetic processes (Schmitt-Jansen *et al.*, 2007). Furthermore, according to Boxall (2004), exposure to antibiotics can harm algal growth in several ways. This might be carried out by blocking the production of proteins and enzymes required for the photosynthetic process (Liu and Xiong, 2011). According to a study conducted by Haig *et al.* (2016), pharmaceuticals have been demonstrated to suppress the growth of microalgae, even at extremely low concentrations.

Concerning the pigments, it is evident that they generally exhibit a decline when subjected to the tested pharmaceutical drugs at various concentrations and time points throughout the entire duration of the experiment. These findings align with the results reported by Copolovici *et al.* (2017), who observed a decrease in photosynthetic efficiency and chlorophyll content in *Phaseolus vulgaris* due to the phytotoxicity of diclofenac (DCF). Similarly, Liu and Xiong (2011) demonstrated that erythromycin, at a concentration of 0.41 µmol L^{-1}, resulted in a reduction in the chlorophyll content of *Pseudo-kirchneriella subcapitata*. Moreover, Gentili and Fick (2017) discovered that exposing algae to 0.05 mg/L of metconazole led to a decrease in chlorophyll a, b, and total chlorophyll content compared to the control group. These declines were attributed to the pharmaceuticals' influence, which hindered the pigment biosynthetic pathway and caused degradation of existing pigments, ultimately reducing photosynthetic efficiency.

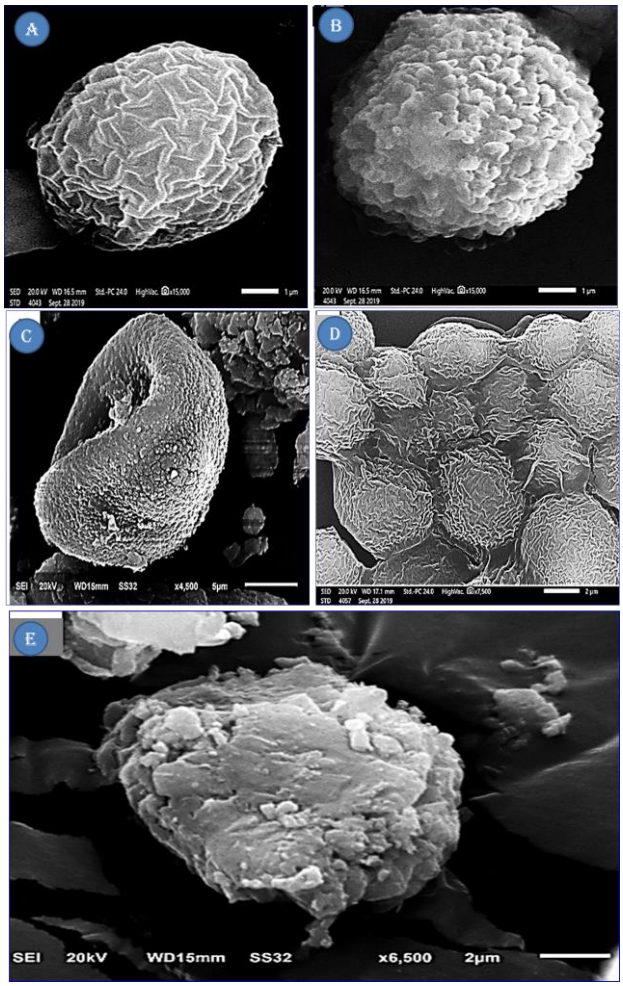

Figure (7): The electron micrograph of *Chlorella vulgaris* obtained after a 96-hrs. incubation period with BBM media, supplemented separately with 25.0 (mg/L) of different pharmaceutical drug. A, the vegetative cell of *Chlorella vulgaris* serves as the control in normal growth conditions (Bar, 1µm); B, the image shows that the *C. vulgaris* cell exposed Panadol (Bar, 1µm); C, the cells exposed to Voltaren (Bar, 5µm); D, cells exposed to Selgon (Bar, 2µm).

Pharmaceutical treatments can cause a decrease in chlorophyll, as demonstrated by Angum *et al.* (2011). This is a sign of photooxidation of pigment and chlorophyll breakdown by the enzyme chlorophyllase. Furthermore, when drugs are administered, it is possible for the photosynthetic electron transport system and chlorophyll pigments to be destroyed. This might cause ROS to be produced and fast diffusion across the cell membrane, which would ultimately end in cell death (Upad Hyaya *et al.*, 2007 and Mahipal *et al.*, 2023). As opposed to their respective controls, the total carbohydrate contents of *C. vulgaris* treated with varying concentrations of the four tested pharmaceuticals, Panadol, Selgon, Voltaren, and E. mox, showed a progressive decrease in all treatments after 96-hrs. of incubation. This might be explained by both an increase in total soluble carbs and a decrease in polysaccharide content. The pattern of changes in total carbohydrates was found to be comparable to that of polysaccharides, which were found to make up the majority of the pool of algal carbohydrates. These findings are consistent with the findings of Farooq et al. (2008), who reported that drugs have an impact on the chlorophyll contents, photosynthesis, and carbohydrates of *Chlorella*

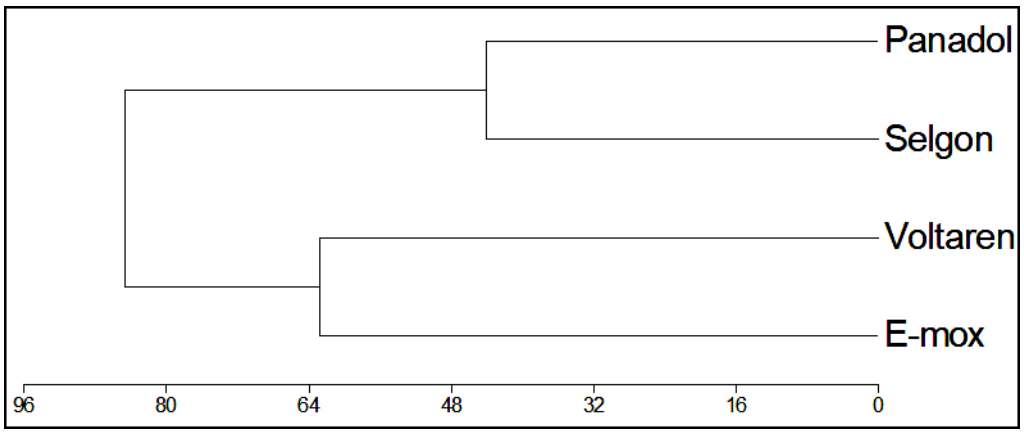

Figure (8): Dendrogram produced by the cluster analysis to shows the extent of convergence in the effect of the four tested pharmaceutical products (Panadol, Selgon, Voltaren and E-mox).

vulgaris. They are also consistent with the findings of Mofeed (2020), who reported that drugs have an impact on the microalga *Pseudokirchneriella subcapitata*.

Regarding proline contents, it is worth mentioning that as pharmaceutical concentrations increased, the proline content of the studied alga exhibited a steady increase. It is crucial to remember that this increase in proline was dose-dependent. These findings align with the research conducted by Szabados and Savoure (2010), who demonstrated that proline accumulation, serves as a crucial non-enzymatic antioxidant and is associated with various stress responses. Proline accumulation has been linked to stress tolerance, as evidenced by the study conducted by Sofia *et al.* (2023), which found that stress-tolerant plants generally exhibit higher levels of free proline compared to stress-sensitive plants. Furthermore, under stressful conditions, proline contents significantly increase, underscoring the role of this amino acid in mitigating oxidative stress. These results are consistent with our own findings, which demonstrated an elevation of proline levels under stress conditions. It has been revealed that the accumulation of appropriate solutes in plants aids in water uptake, protects cells against oxidative damage, and mitigates the detrimental effects of proline (Ashraf and Foolad, 2007).

In terms of amino acid composition, a decrease in the concentration of free amino acids is one of the earliest signs of stress in plants (Szabados and Savoure, 2010). Importantly, we found a correlation in our study between *C. vulgaris* amino acid levels and pharmaceutical-induced stress. As a result of osmotic adjustment to prevent negative effects, it is generally recognized that under stress, amino acid buildup rises (Rai, 2002). This conclusion supports our findings and suggests that the higher buildup of amino acids in stressed plants may be due to protein hydrolysis.

The obtained data analysis is also consistent with the findings of Schubert *et al.* (1995) and Hebat-Aalah *et al.* (2023), who reported that a significant rise in the concentration of free amino acids was caused by water stress. Finally, Zhang and colleagues (2019) demonstrated that varying antibiotic concentrations have a substantial impact on metabolic pathways associated with the metabolism of carbohydrates and amino acids, as well as those pertaining to the metabolism of glycine, serine, and threonine, the degradation of lysine, the metabolism of D-glutamine and D-glutamate, the degradation of valine, Lucien, and isoleucine.

With regard to the protein data, it can be shown that all four of the tested medications, at all doses and throughout the exposure periods, reduced the amount of protein. Mojiri *et al.* (2022) found that exposing algae to varying quantities of personal care items can negatively impact their growth and productivity. Additionally, the generation of reactive oxygen species may result in a decrease in the quantity of protein and chlorophyll present in the algae. Xia-Xiang (2020) investigated the toxicity of triclosan and carbamazepine to *Chlorococcum* sp. and found that triclosan breaks down proteins. Furthermore, as the impacts of pharmaceuticals and personal care items are greatest at low concentrations, Patel *et al.* (2019) claim that these compounds have a major environmental impact in trace amounts.

Antibiotics possess the capability to bind reversibly to ribosomal subunits, thereby disrupting transcription and translation processes and hindering protein synthesis (Brain *et al.*, 2008). This observation aligns with the results obtained from our investigation, which indicated that the antibiotic E-mox exhibited the highest degree of inhibition. Following a 96-hrs. incubation period, the data obtained revealed a significant enhancement in antioxidant enzyme activity as the dosage of the tested medications increased. Specifically, the treatment group administered a high dose of 25 mg/L displayed the most substantial increase in all antioxidant enzymes.

Finally, it can be concluded that the activity of the enzyme is dependent on both the dosage and duration of exposure. These findings are consistent with previous studies conducted by Alscher *et al.* (2002) and Anna *et al.* (2023), which reported that the extent of pharmaceutical-induced growth inhibition in plants and algae varies among species and influences the quantity and activity of antioxidant enzymes. Furthermore, it has been observed that various physical and chemical stresses, including drug exposure, impact the growth of microalgae. Such stresses can induce the synthesis of reactive oxygen species (ROS)

such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (Liu and Xiong, 2009). To counteract the oxidative damage caused by ROS, algae have developed a repertoire of intricate antioxidant defense mechanisms, including low-molecular-mass antioxidants and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and peroxidases (POD) (Guo *et al.*, 2007; Beatrycze, 2022).

The obtained data demonstrated a considerable increase in glutathione reductase (GR) activity in response to an increase in the tested drug concentrations. However, such an increase was greater under the therapy with 25 mg/L concentrations since they were 25.6, 24.8, 22.9, and 18 (g/mg protein) in E-mox, Voltaren, Selgon, and Panadol, respectively. Because of increased (GR) activity, the current work maintained the metabolic balance between GSH and ascorbate (ASH) levels and the breakdown of H_2O_2, which can be involved in the detoxification of pharmaceuticals. According to Sharma and Dietz (2009), glutathione reductase (NADPH) is used in the glutathione-ascorbate cycle to convert glutathione (GSSG), which is produced by peroxidases as APX, into GSH (Foyer and Noctor 2003). In the meantime, to cleanse cells of superoxide anions (O_2^-) that may have been created by the administered medications, SOD activity rose in a dose-dependent way (Foyer and Noctor, 2003). Furthermore, a high induction of SOD activity accelerates the spontaneous dismutation of superoxide anion radicals (O_2^-) into H_2O_2, which can lead to an accumulation of H_2O_2 and is also responsible for lipid peroxidation and chlorophyll degradation, according to Kapoor *et al.* (2014). The H_2O_2 generated by SOD activity is broken down into H_2O and O_2 via the ascorbate-glutathione cycle. The present investigation revealed that *C. vulgaris* exhibited increased APX activity, which is often associated with an adaptation mechanism to the increased level of ROS content brought on by drug use (Hasanuzzaman *et al.*, 2012). The role of APX is to detoxify hydrogen peroxide (H2O2) during stress by using ascorbate as a substrate in the glutathione-ascorbate cycle (Sharma and Dubey, 2005).

The results obtained after 96-hrs. of incubation in BBM medium supplemented with a high concentration of individual pharmaceutical drugs are consistent with the findings reported by Spain and Fun (2022). However, treatment with the antibiotic E-mox had a profound impact on the shape, regularity of borders, and cell wall structure of *C. vulgaris* cells. The cells became irregular and lost their distinctive form (Canelli *et al.*, 2021). Moreover, the cell wall exhibited cracks and fragmentation. When examining the extent of convergence among the tested pharmaceutical drugs, cluster analysis revealed that Panadol and Selgon were closely associated in a minor sub-group, indicating a similar level of influence. Conversely, Voltaren and E-mox formed another minor sub-group but were clearly separated due to their significant dissimilarity in effects.

CONCLUSION

The current study shows that the pharmaceutical medications (Panadol, Voltaren, Selgon, and E-mox) under investigation can have toxicological effects on the microgreen alga *C. vulgaris*, even at low concentrations, resulting in altered cellular structure and decreased algal development**.** Particular differences were seen for every medication: Selgon did not exhibit any obvious impacts on the *C. vulgaris* cell wall; Voltaren resulted in a dense coating of structures resembling nipples on the cell wall surface; and E-mox induced degradation and fracture of the cell wall along with abnormalities in cell shape. The study also showed that antioxidant enzyme activity rose with increasing drug doses, suggesting that algae are sensitive to pharmacological exposure. According to these results, aquatic monitoring programmes may be able to detect changes in water quality by using aquatic microalgae as bio-indicators. Controlling this kind of pollution, which has previously gotten little attention or research in earlier decades, must thus be given top priority. Moreover, the formulation of remediation plans is necessary, especially in water bodies that are contaminated by pharmaceutical drugs. Any variation in the population of microalgae will have a profound effect on the balance of the environment and ultimately impact several facets of human existence. Therefore, in order to maintain a healthy and sustainable ecosystem, it is essential to address and reduce any potential dangers related to pharmaceutical pollutants.

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تأثير بعض العقاقير الصيدلانية المختارة على الطحلب الأخضر الدقيق *Chlorella vulgaris*

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[3] قسم النبات، كلية العلوم، جامعة الزقازيق، الزقازيق، مصر

الملخص العربـــي

تهدف هذة الدراسة إلى تقييم التأثيرات السمية لبعض العقاقير الشائع إستخدامها وهى: بانادول، فولتارين، سيلجون، وإيموكس على الطحلب الأخضرالدقيق وحيدالخليه كلوريلا فولجارز. أظهرت النتائج أن تأثير التركيزات المختلفة من المستحضرات الدوائية بجرعات مختلفة على الطحلب الأخضر أدت أدت إلى إنخفاض كثافة النمو مع وجود تباينات واضحة فى كيفية تأثير كل دواء على النمو الطحلبى. كما اثبتت الدراسة إنخفاض المحتوي لكلا من الأصباغ والكربوهيدرات الكليه وهذا بشكل تدريجى معتمدا على تركيز الجرعه والوقت. و قد لوحظ أن معاملة الطحلب بتركيز 25مجم /لتر من المستحضرات الدوائية أدى إلى نقص فى الأحماض الأمينيه الكلية مقارنة بالمجموعة الضابطة.مع زيادة فى الحمض البرولين الأمينى. كما سجلت أعلى زيادة مع المضاد الحيوى (إيموكس) بتركيز 25 محم /لتر. كما زاد نشاط الإنزيمات المضادة للشوارد الأيضية (الجلوتاثيون المختزل , بيروكسيديز أسكوربات , فوق أكسيد د يسميوتاز.هذا بالاضافة الي ان التصوير الإلكترونى لطحلب الكلوريلا المعامل بالجرعة العالية 25مجم /لتر والمحضن لمدة 96 ساعة أظهر تشوهات في شكلا لخلية حيث انة في حالة تعريضة للبانادول أصبح سطح الجدار الخلوى فقد انتظامة و شكلة المميز.بينما فى حالة الفولتارين فظهر سطح الخلية مغطى بحلمات كثيفة بينما اصبحت الخلية ذاتها غير منتظمة وفقدت شكلها المميز بالإضافه إلى تلف الجدار وتصدعه فى حالة الإيموكس . وبناءً على ذلك،يجب أن نظهر اهتمامًا كبيرًا بالسيطرة على هذا النوع من الملوثات ، وأن نضع اسلوب استراتيجي للمتابعة ، خاصة في المسطحات المائية المعرضة لهذاالنوع من الملوثات، لحماية توازن النظام البيئي،وبالتالي حياة الإنسان.