2024, 21(2): 0289-0304
https://doi.org/10.21123/bsj.2023.8214
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

The Role of *Chlorella vulgaris* in Reducing Some Pharmaceutical Wastes Toxicity in Clam *Pseudodontopsis euphraticus*

Zahraa H. Obaid [1], ***Nuha F.Kadhim*** [2], ***Jasim Mohammed Salman**** [2]

[1]Environmental Research & Study Center, University of Babylon, Babylon, Iraq.
[2]Department of Biology, College of Science, University of Babylon, Babylon, Iraq.
*Corresponding Author.

Received 11/12/2022, Revised 27/01/2023, Accepted 29/01/2023, Published Online First 20/07/2023, Published 01/02/2024

Abstract

Applications of microalgae in environmental studies have recently increased. Current uses of immobilized microalga *Chlorella vulgaris* include reducing pharmaceutical substances such as amoxicillin AMX and potassium dichromate $K_2Cr_2O_7$ on freshwater clam *Pseudodontopsis euphraticus* as a biotic model. Recent research pointed out a change in biomarkers of oxidative stress in an evaluation of induced toxicity. Where clams were exposed to different concentrations100, 200, and 400 mg/L for 7 days and 20, 30, and 50 mg/L for 5 days of amoxicillin and potassium dichromate, respectively. The results showed that exposure to AMX and $K_2Cr_2O_7$ led to a significant change in the activity of antioxidant enzymes, with significant increases ($p<0.05$) in reactive oxygen species (ROS) production. The highest ROS value was 51.05 µg/mg under concentrations of 50 mg/L of $K_2Cr_2O_7$, and the highest recorded percentage of Superoxide Dismutase SOD, Catalase CAT, Malondialdehyde MDA, and Glutathione Reductase GSH, as: 33.40 U/m, 33.32KU/L, 23.22 µmol/l and 21.30µg/g respectively, in concentrations of 50 mg/L of $K_2Cr_2O_7$ non-treated. It was observed in this study that potassium dichromate was more effective than amoxicillin in causing toxicity. According to the current study, immobilized *C. vulgaris* was instrumental in decreasing chemicals toxicity, by relieving oxidative stress on *P. euphraticus* clam, as it recorded a significant decrease $p\leq 0.05$ in ROS values and oxidizing enzymes such as Superoxide Dismutase SOD, Catalase CAT, Malondialdehyde MDA, as well as ascorbic acid. AA, total protein and GPX in treated samples.

Keywords: Biochemical markers, *Chlorella vulgaris*, Freshwater clam, Immobilized algae, Pharmaceutical wastes.

Introduction

The use of microalgae in biotechnology has increased in recent times, frequent uses of immobilized algae are the nutrients, inorganic [1], organic pollutants removal from aquatic systems, culturing for metabolite production, and measurement of toxicity[2].In the last years, growing more attention has been paid to the presence of pharmaceutical substances in aquatic ecosystems, due to their potential to have detrimental impacts to non-target aquatic species[1,3]. There are probably entering freshwater systems by many pathways including effluents from wastewater treatment

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plants (WWTPs), chemical industrialization plants, and animal rearing and aquaculture [3].

Amoxicillin AMX has been classified as an emerging pollutant it causes great damage to aquatic organisms, such as changes in embryonic development and oxidative stress, and it has been discovered that AMX is capable of causing DNA damage and cytotoxic effects in common carp blood cells [4,5]. The most serious issue caused by antibiotic-contaminated water, is the rise of antibacterial drugs and genes for antibiotic resistance, which result in the annual deaths of 700,000 people per year [2, 6].

Chromium is a highly toxic inorganic pollutant that enters environment from a variety of natural and artificial sources, including medical facilities, textile manufacturers dye, and chrome electroplating. Chromium has been designated as a priority pollutant by numerous environmental and health organizations, when present in excess, it induces toxic effects on the cells such as genotoxicity and oxidative damage and can damage lipids, proteins, DNA, and cause carcinogenic and mutagenic effects in living beings[7, 8].

The utilization of biomarkers as early warning tools for contamination in an environment can be toxic and dangerous to aquatic life [9]. Chemical compounds can affect biological systems by forming radicals or high-energy molecules, which eventually reflect oxidative stress on organisms and lead to the production of ROS in aquatic organisms [10]. Thus, differences in the action of the enzymes that make up the antioxidant protective mechanism can be used as an early warning sign of toxic compound contamination.[11].

Bivalves are considered good bio-indicator organisms for determining the degree of contamination in freshwater and marine ecosystems[11-13]. This is due to several significant characteristics, including their wide dispersion, abundance, sedentary behaviour, physical size, and frequently, their ecological and/or economic value. As a result, various authors have studied responses of molluscs reacting to environmental pressures and contaminants [14-17]

It has been shown that *C. vulgaris* can adapt to antibiotic stress through its own physiological adaptation and its ability to degrade pollutants, it is therefore a good option for removing antibiotics from aqueous systems[18]. Algal immobilization technology has received increasing attention and has been used in many applications in the environmental field, such as treating wastewater by removing nutrients, pharmaceutical compounds, hazardous textile dyeing, and heavy metals [19-22].

The current study aimed to use immobilized alga as an eco-friendly method to a reduced the toxic effect of amoxicillin and potassium dichromate on some biomarkers in freshwater clam *Pseudodontopsis euphraticus*.

Materials and Methods

Tested Organisms

1-A freshwater clam, *P. euphraticus*, was selected for toxicity testing and collected from the Euphrates River in Al Hindiya District 32° 32' 29.9" N, 44° 13' 38.7" E, which is about 20 km east of Karbala city and approximately the same distance west of Hilla city ,Iraq .

2- The microalgal species used in this study was *Chlorella vulgaris* that belonged to green algae and most commonly used for wastewater treatment which have high growth rates and can grow under a wide range of culture conditions.This microalgal strain was obtained from the Environmental Research and Studies Center, University of Babylon, Iraq.

The *C. vulgaris* was identified by microscopic observation and incubated under controlled conditions of light intensity 286 µE/m²/s, light/dark period 16:8 hours and temperature 25±2 °C. All equipment and media were sterilized in an autoclave at 121 °C, 1.5 h for 15 min. Modified Chu-10 was used for the algal growth.

The method was followed by taking 50 ml of the algae culture in the stabilization phase and concentrate by centrifugation at 3000 rotation / minute for a period 15 minutes. Afterwards, an equal volume of 2% sodium genes solution was

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added to it and shaken well to homogenize the mixture (algae and genes) which then placed in a syringe or separating funnel. The contents of the medical syringe or the funnel are gradually distilled in the calcium chloride solution where the algae fall in the beads form and leave for 5-10 minutes to harden, then wash the beads from the calcium chloride solution with tap water and rinse thoroughly with distilled water by using a tea strainer [23].

Pharmaceutical Substances

Pharmaceutical substances were used in this work, including potassium dichromate $K_2Cr_2O_7$ and pure amoxicillin trihydrate ($C_{16}H_{19}N_3O_5S.3H_2O$) was obtained from the General Company for the Manufacture of Medicines and Medical Supplies Samarra, Iraq.

Experimental Design

Cultivation of fresh clams was performed in 24 plastic containers 18 cm x 21 cm x 31 cm, which were selected for the experiment. *P. euphraticus*. which ranged in shell length from 3.5–4.6cm, were collected from the Euphrates River and transported to the laboratory. The stocks were prepared for all macro, and microelements were dissolving the weight of the salt, Table. S1The components and concentration of modified Chu-10 medium and the concentration of each component (Bleakley and Hayes, 2017).

They were acclimated within standard conditions with dechlorinated water for 5 days and exposed to amoxicillin for 7 days at concentrations of 100, 200, and 300 mg/l, and they were also exposed to $K_2Cr_2O_7$ for 5 days at concentrations of 20, 30, and 50 mg/l. On the other hand, 5–15 beads of immobilized *C. vulgaris* were added to all containers of treatments with pharmaceutical substances. At the end of the exposure, the haemolymph was extracted, to study the variation of biochemical biomarkers [24].

Measuring Biomarkers

Method of EreI[25] was used to determine reactive oxygen species (ROS)activity , and determine super oxide dismutase (SOD) activity using the method described by Marklund&Marklund[26].Catalase (CAT) activity was determined according to Goth [27]. Malondialdehyde (MDA) tested by method of Buege and Aust[28].The Glutathione peroxidase GPx activity determined was completed by using the methodology adopted from Hafeman *et al*,[29]. GSH was determined according to Moron *et al*,[30] . Total protein was determined by Lowry *et al*., [31] moreover, ascorbic acid AA was determined by McCormick and Greene [32] by three replicates

Statistical Analysis

The results of statistical analyses study and the significance level were considered at $p<0.05$. Descriptive analyses included means and standard deviations. Variables were tested for normality distribution prior to analysis. To determine the significance of differences, analysis of variance (ANOVA) was used, and *p-values* less than 0.05 were considered significant. SPSS program was also used for the analysis.

Results

Results recorded the experimental toxicity effects of the amoxicillin (AMX) and potassium dichromate ($K_2Cr_2O_7$) toxicity effects experiments on *P. euphraticus* are shown in Figs.1,2. and Tables 1,2 respectively.

Exposing *P.euphraticus* to amoxicillin showed that the ROS mean values in the exposure experiments without the addition of immobilized alga ranged between 12.99 -22.52 µg/mg compared to the control which recorded 11.77 µg/mg ,while with *C. vulgaris*, ROS mean values decreased and ranged from 8.89-16.73g/mg compared to 10.91 µg/mg in the control group for 100-300mg/L AMX concentrations. In $K_2Cr_2O_7$ experiment, the ROS mean values recorded without *C. vulgaris* 37.50 - 51.05µg/mg compared to the control (which recorded 12.58 µg/mg) but with added *C.vulgaris*, ROS mean values were suppressed and ranged from

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33.88 -42.66µg/mg compared to 11.80µg/mg in the control group of 20-50 mg/L concentrations.

In amoxicillin, without adding immobilized alga, in the exposure experiments' CAT, mean values ranged from 25.44-30.53 KU/L, compared the control group of 24.14 KU/L, while with *C. vulgaris*, a pronounced elevation of CAT values was recorded which ranged from 17.56 -25.15 KU/L compared to 14.26 KU/L in the control group for 100-300mg/L AMX concentrations. In $K_2Cr_2O_7$, without *C. vulgaris*, higher CAT value was recorded (26.88 -33.32KU/L) compared to the control which recorded 23.60 KU/L ,but with adding immobilized *C. vulgaris*, CAT mean values were decreased and ranged from 27.16–25.76 KU/L compared to 22.82 KU/L in the control group of 20 -50 mg/L concentrations.

Superoxide Dismutase (SOD) mean values for AMX varied from 20.79 to 33.33 U/m in the exposure trials without the addition of immobilized *C. vulgaris*, compared to the control which recorded 17.51 U/m. while with *C. vulgaris*, SOD mean values were significantly decreased and ranged from 12.72 -21.62 U/m compared to 9.80 U/min the control group of 100-300 mg/L concentrations. In $K_2Cr_2O_7$, the SOD mean values without *C. vulgaris* were 28.36 -33.40 U/m as opposed to the control group 24.02 U/m values, but with the addition of *C. vulgaris*, the SOD mean values ranged from 23.36 - 31.41U/m while that of control group's was 20.35 of 20-50 mg/L concentrations.

In AMX, the GPX mean values in the exposure experiments without the addition of immobilized *C. vulgaris* ranged between 5.70 to 8.36 U/L compared to the control which recorded 18.48 U/L, While with *C. vulgaris*, GPX mean values was apparently not affected and ranged from 5.02-9.58 U/L compared to 13.96 U/L in the control group for 100-300 mg/L concentrations. However in case of $K_2Cr_2O_7$, The GPX mean values recorded without *C. vulgaris* were 4.35 to 6.34 U/L compared to the control which recorded 7.99 U/L but with added *C. vulgaris*, GPX mean values ranged from 3.57-7.34 U/L compared in to 6.28 U/L in the control group of 20-30mg/L concentrations.

The GSH values for AMX mean ranged from 7.55 - 10.37µg/g in the exposure experiments without adding immobilized *C. vulgaris* compared to the control which recorded 5.92 µg/g, while with *C. vulgaris*, GSH values slightly decreased and ranged from 6.54 -9.46 µg/g compared to 5.14 µg/g in the control group for 100 -300 mg/L concentrations. In $K_2Cr_2O_7$, The GSH mean values recorded (without *C. vulgaris*) 21.30 - 16.30 µg/g compared to the control which recorded 8.57 µg/g but on the addition of *C. vulgaris*, GSH mean values ranged from 14.52-22.47µg/g compared to 9.7µg/g in the control group 20- 30mg/L concentrations.

The malondialdehyde (MDA) mean values produced during the effect of AMX ranged from 17.69 -42.43 µmol/L in the exposure experiments without addition of immobilized *C. vulgaris*, compared to the control which recorded 13.28 µmol/l, while *C. vulgaris*, MDA mean values were highly decreased and recorded from 11.60 -23.97 µmol/l compared to 8.89 µmol/l in the control group for 100-300 mg/L concentrations. While in $K_2Cr_2O_7$ experiment, the MDA mean values recorded (without *C. vulgaris*) from 12.68 to 23.22 µmol/l compared to the control which showed 7.99 µmol/l but with added *C.vulgaris*, MDA mean values were largely decreased and ranged from 9.81-11.44µmol/l compared to 8.86µmol/l in the control group of 20-50 mg/L concentrations.

In AMX, the total protein (TP) mean values in the exposure experiments without the addition of immobilized *C. vulgaris* ranged from 9.65 to 11.14 mg/g compared to the control, which recorded 13.17 mg/g, while with *C. vulgaris*. TP mean values significantly increased and recorded ranged from 14.25 to 15.60mg/g, compared to 12.44 mg/g in the control group for 100-300mg/L. In $K_2Cr_2O_7$. The TP mean values recorded without *C. vulgaris* were from 10.86 to17.25mg/g compared to the control, which recorded 12.12mg/g but with *C. vulgaris*, TP mean values recorded ranged from 10.93 to 15.42 mg/g compared to 29.21 mg/g in the control group at a 20-30mg/L concentrations.

The Ascorbic acid AA mean values for AMX ranged from 13.41 to 14.48 µM in the exposure experiments without the addition of immobilized *C.*

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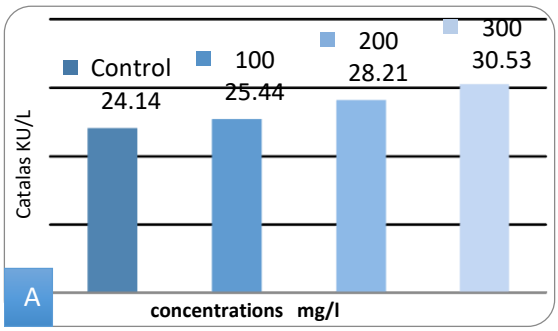
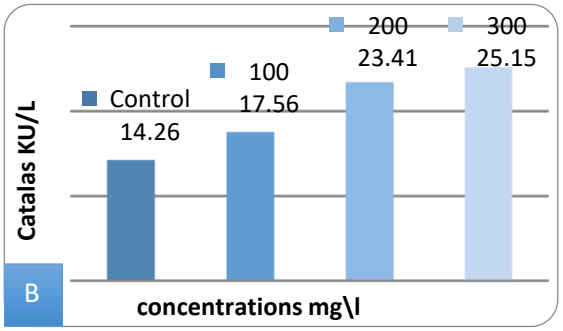
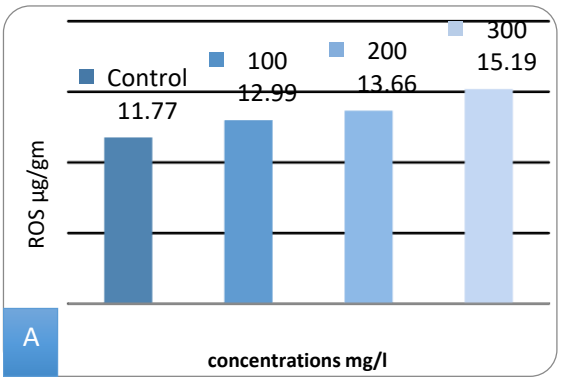
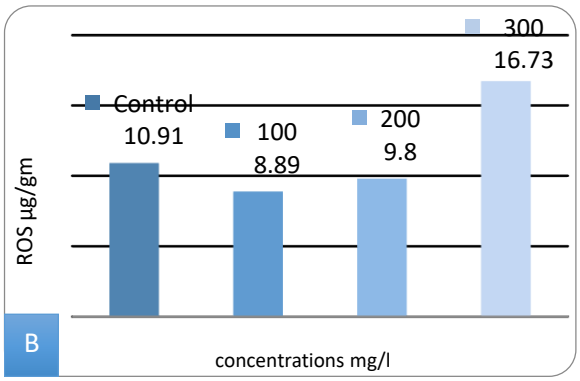
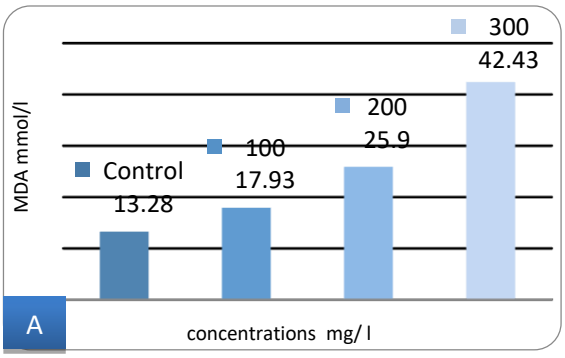
vulgaris compared to the control which recorded 11.59 µM, while with *C. vulgaris*, AA mean values were apparently unaffected and ranged from 10.31 - 13.40 µM compared to 11.47 µMin the control group for 100-300 mg/L concentrations. In $K_2Cr_2O_7$.The AA mean values recorded without *C. vulgaris* were 25.27-27.93µM compared to the control which recorded 25.36 µM but with adding *C. vulgaris*. AA mean values were apparently unaffected and ranged from 24.83-27.94 µM compared to 23.92 µMin the control group in a 20-50 mg/L concentrations.

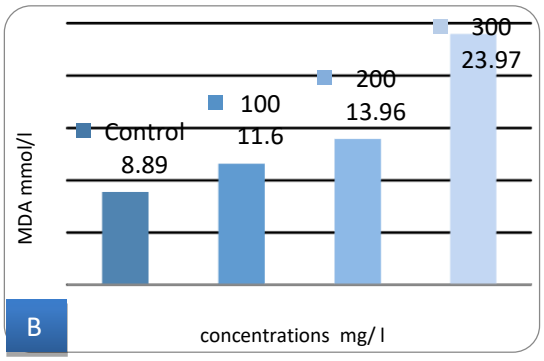


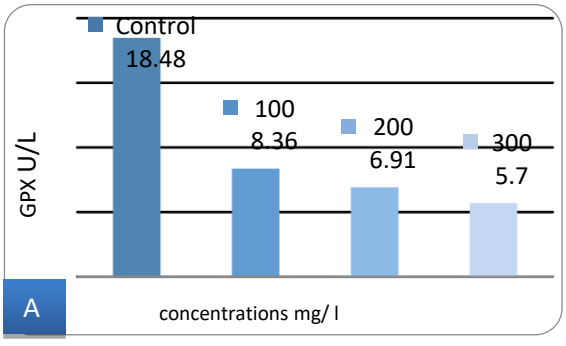


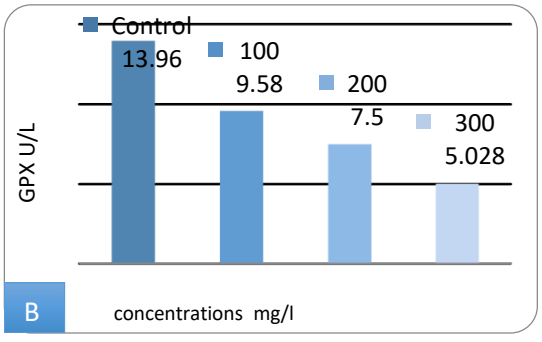












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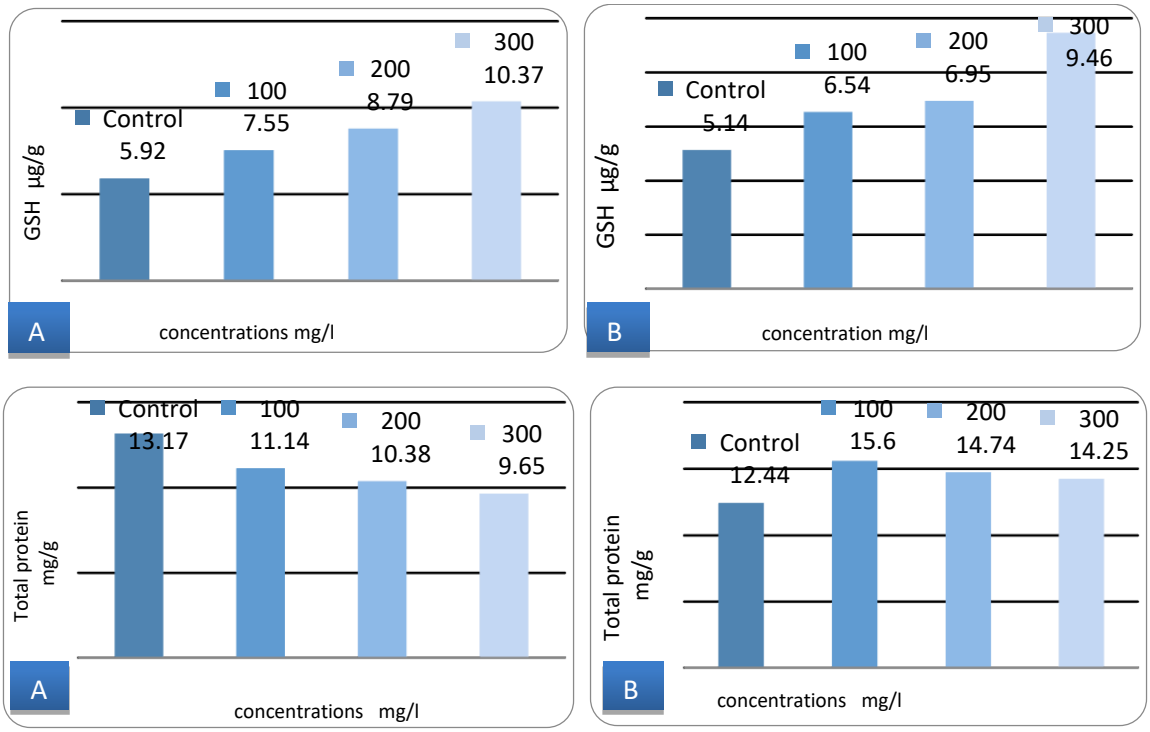
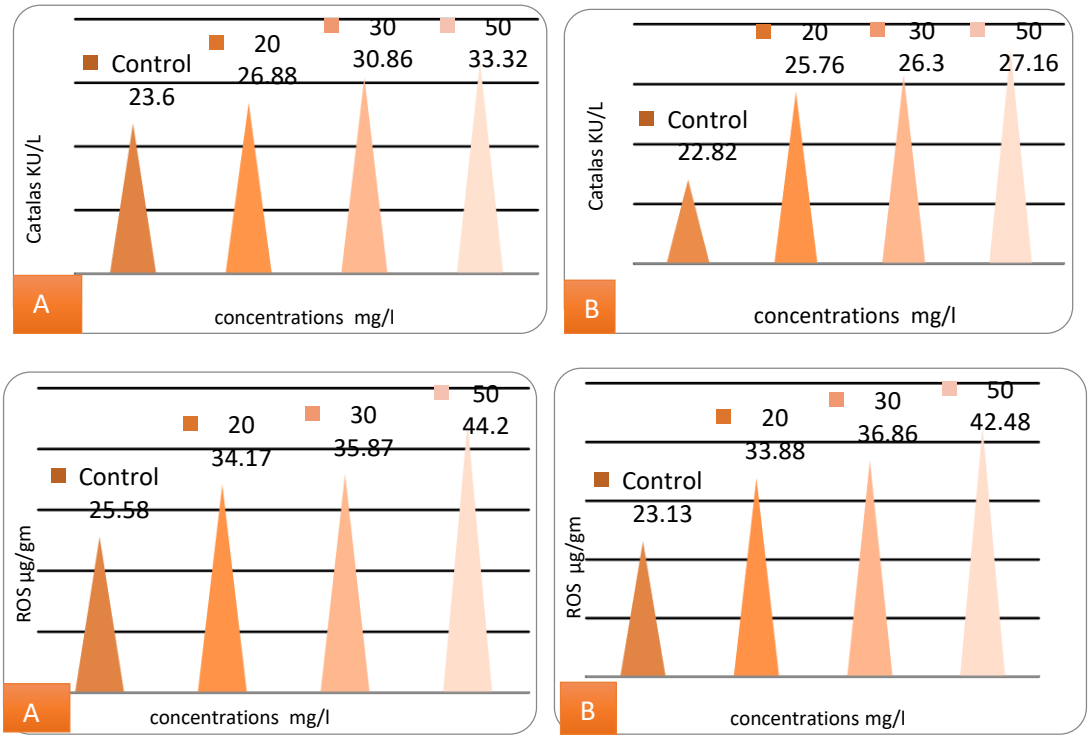


Figure 1. Effect of the antibiotic amoxicillin (AMX) on mean of biochemical markers SOD,CAT,ROS,MDA,GPX, GSH, Total Protein and Ascorbic Acid in mussel in *P.euphraticus*. A- without Immobilization of (*C. vulgaris*), B- with using Immobilized *C. vulgaris*



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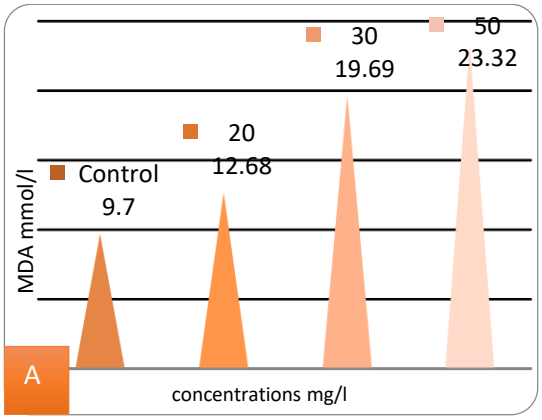

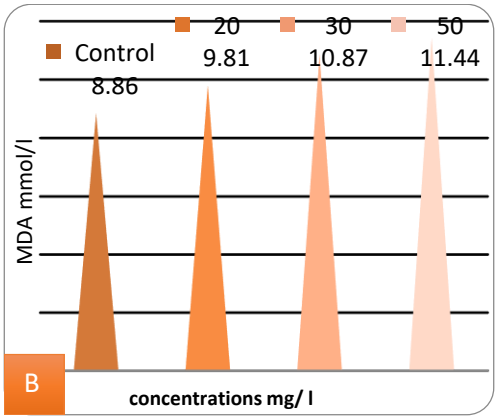

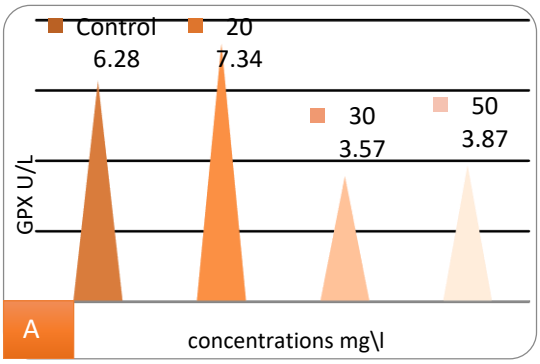

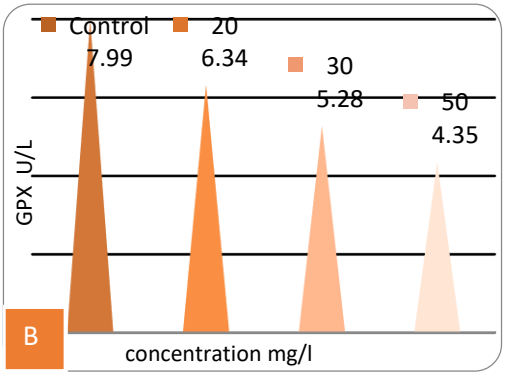

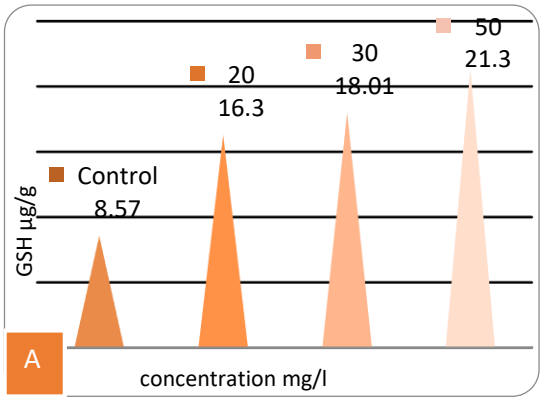

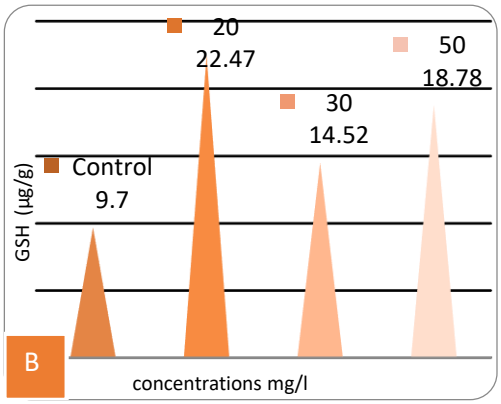

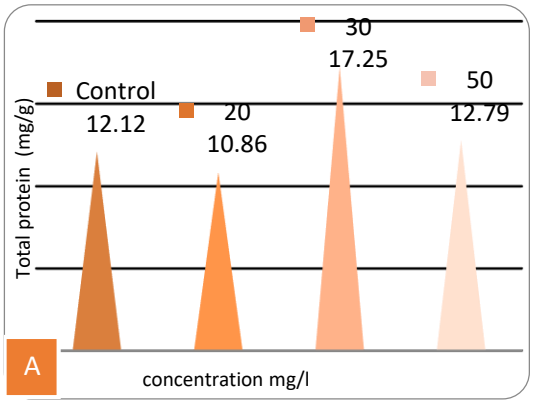

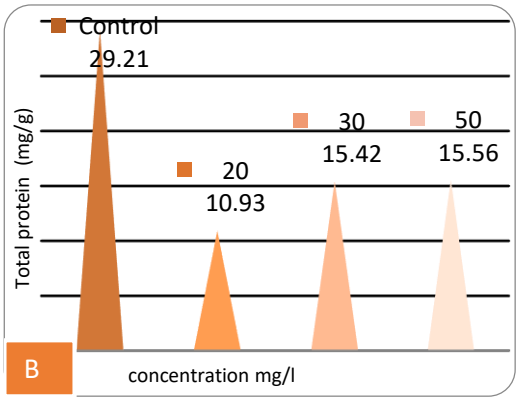

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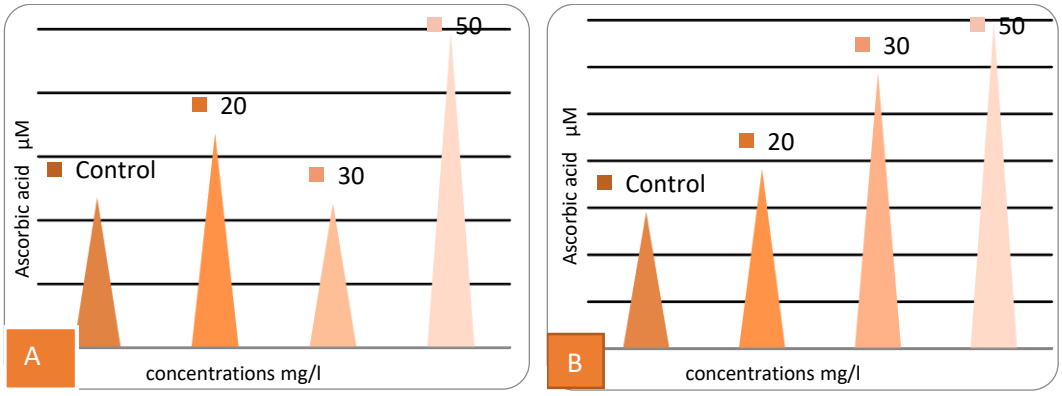

Figure 2. Effect of the Potassium Dichromate ($K_2Cr_2O_7$) on mean of biochemical markers SOD,CAT,ROS,MDA,GPX, GSH, Total Protein and Ascorbic Acid in mussel in *P.euphraticus*. : c- without Immobilization of (*C. vulgaris*), D- with Immobilization of the alga.

Table 1. The mean of biochemical markers *in P. euphraticus* during acute exposure period to antibiotic amoxicillin (Min., Max., Mean±SDof three replicates).

Biochemical markers	Without Immobilization of (*C. vulgaris*)				Immobilization with (*C. vulgaris*)			
	control	100 (mg/l)	200 (mg/l)	300(mg/l)	control	100 (mg/l)	200 (mg/l)	300 (mg/l)
ROS (µg/mg)	9.63-11.92 11.77± 0.94	10.56 – 17.00 12.99 ± 3.49	14.83 - 12.38 13.66 ± 1.22	19.35 – 25.93 22.52 ± 3.29	8.54 – 13.54 10.91 ± 2.37	7.22-11.26 8.89 ± 2.10	11.72 - 8.54 9.80 ± 1.68	14.46 – 18.75 16.73 ± 2.15
SOD(U/m)	16.22-18.92 17.51 ± 1.35	18.92- 22.14 20.79 ±1.67	16.49 - 32.43 25.04 ± 8.03	27.03-40.54 33.33 ± 6.80	8.11– 10.81 9.80 ± 1.47	11.62-13.92 12.72 ± 1. 15	10. 81 20.54-16.75 ± 5.21	16.22 – 27.03 21.62 ± 5.40
CAT (KU/L)	24.14-24.9 24.14 ± 0.39	23.93-26.77 25.44 ±1.42	27.08 - 29.15 28.21 ± 1.04	27.39-35.28 30.53 ± 4.18	11.58 – 17.49 14.26 ± 2.99	15.86-18.42 17.56 ±1.47	22.42 - 24. 56 23.41 ± 1.07	23.59 - 26.24 25.15 ± 1.38
MDA (µm/l)	12.19-14.36 13.28 ± 1.06	12.18-23.21 17.69 ± 5.51	25.90-25.90 25.9± 0.00	40.01-44.87 42.43 ± 2.43	7.69-9.62 8.89 ± 1.04	11.60-11.69 11.60 ± 0.00	12.79-15.13 13.96 ± 1.17	20.51 - 26.47 23.97 ± 3.09
GPX (U/L)	16.82 - 21.82 18.48 ± 2.88	6.94 – 9.78 8.36 ± 1.42	6.50 - 7.38 6.91 ± 0.44	5.26 - 6.02 5.70 ± 0.39	11.90 - 15.90 13.96 ± 2.00	5.62 – 15.14 9.58 ± 4.95	6.14 - 9.43 7.50 ± 1.71	4.98 - 5.06 5.02 ±

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								0.04
GSH(µg/g)	5.82 - 6.00 5.92 ± 0.09	5.64 -8.81 7.55 ±1 .68	5.46 -11.81 8.79 ± 3.18	9.11 - 17.32 10.37 ± 1.25	3.96 - 6.90 5.14 ± 1.55	5.55 - 7.31 6.54± 0.90	4.86 - 9.40 6.95 ± 2.29	7.25-12.13 9.46± 2.47
Total protein (mg/g)	12.74-14.38 13.17 ± 0.58	9.37-12.58 11.14 ± 1.63	9.04-11.39 10.38 ± 1.20	8.67-10.45 9 .65 ± 0.905	11.09-13.95 12.44 ± 1.43	13.59-17.18 15.60 ±1.83	14.43-15.06 14.74 ± 0.31	12.66 - 15.45 14.25 ± 1.43
Ascorbic acid (AA) µM	10.95-12.28 11.59 ± 0.91	10.08-15.65 13.41 ± 2.94	11.66-16.66 13..37± 2.91	12.49-17.14 14.48± 2.39	10.34-12.65 11.47 ± 1.13	10.47-11.99 11.31 ± 0.77	7.11-13.51 10.31± 3.19	11.80 - 14.27 13.40 ± 1.39

Table 2. The mean of biochemical markers *in P. euphraticus* during acute exposure period to Potassium dichromate. (Min., Max., Mean±SD of three replicates)

Biochemical markers	without Immobilization of (*C. vulgaris*)				Immobilization with (*C. vulgaris*)			
	control	20(mg/l)	30 (mg/l)	50(mg/l)	Control	20(mg/l)	30(mg/l)	50(mg/l)
ROS (µg/mg)	10.74–14.77 12.58 ± 1.76	30.70-44.17 37.50 ± 6. 73	36.31-45.94 40.97 ±4.82	45.08 – 57.20 51.05 ± 6.06	9.53 – 13.54 11.80 ± 2.05	31.62 – 36.55 33.88± 2.48	34.03 – 38.69 36.86 ± 2.48	40.06 – 43.64 42.06 ± 1.82
SOD(U/m)	21.62–27.03 24.02 ± 2.75	27.62-29.05 28.36 ±0.71	29.73-32.43 31.10 ± 1.35	33.55 - 36.04 33.40 ± 1.44	19.11–21.62 20.35 ± 1.255	24.32-29.73. 23.36 ±1.18	25.62 - 28.92 27.43 ± 1.67	33.25 – 29.34 31.41 ± 1.96
CAT (KU/L)	22.67 – 25.13 23.60 ± 1.33	26.61 - 27.16 26.88 ±0.38	30.52 - 31. 20 30.86 ± 0.48	30.99-35.41 33.32 ± 2.22	21.59–24.59 22.82 ± 1.57	25.08-26.45 25.76 ± 0.68	24.11 - 29.25 26.30 ± 2.65	25.27-28.92 27.16 ± 1.82
MDA(µmol/l)	8.00 - 11.70 9.70± 1.86	10.77-14.6 12.68 ± 2.70	17.38-12.38 19.69 ± 2.52	22.38-24.13 23.22 ± 0.87	5.77 – 5.86 8.86 ± 0.09	8.40–11.22 9.81 ± 1.41	8.97-12.82 10.87± 1.92	11.5-11.73 11.44 ± 0.41
GPX (U/L)	6.18-9.06 7.99 ± 1.57	4.26 – 8.42 6.34 ± 2.08	2.78 – 7.42 5.28 ± 2.34	3.70 - 5.34 4.35 ± 0.86	6.14 - 6.14 6.28 ± 0.14	6.66–8.02 7. 34 ± 0.68	3.10 – 4.34 3.57± 0.67	3.02 – 5.10 3.87 ± 1.08
GSH(µg/g)	6.42 - 10.79 8.57 ± 2.18	14.38 - 18.46 16.30 ±2.05	18.15-16.06 18.01 ± 1.88	18.99 - 25.56 21.30 ± 3.69	6.42 - 10.79 9.7 ± 2.89	18.46-25.70 22.47 ±3.68	11.03-18.01 14.52 ± 4.39	15.00 - 22.77 18.78 ± 3..88

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Total protein (mg/g)	10.29 – 13.95 12.12 ± 1.83	10.16 - 11.39 10.86 ± 0.62	15.42 - 17.25 17.25 ± 1.83	11.03-14.43 12.79 ± 1.70	28.24 – 30.07 29.21 ± 0.92	9.93 - 12.22 10.93 ± 1.17	12.49 - 18.35 15.42± 2.93	14.40-15.50 15.56 ± 1.06
Ascorbic acid (AA) µM	24.52 - 26.54 25.36 ± 1.05	26.35 - 27.11 26.37 ± 0.38	24.14 - 26.29 25.27± 1.08	27.68-28.32 27.93± 0.33	23.57 - 24.27 23.92 ± 0.34	22.86 - 26.10 24.83 ± 1.73	26.73-26.99 26.88 ± 0.13	27.03 - 28.86 27.94 ± 0.91

Discussion

In this study, it was found that exposure to AMX and $K_2Cr_2O_7$ enhanced the production of ROS, this is consistent with many studies which indicated that these pharmaceutical substances led to an increased production of ROS in aquatic organisms [33-35]

Previous studies showed that AMX and $K_2Cr_2O_7$ caused significant changes in the activity of antioxidant enzymes and induced oxidative stress [36,37].

In this study, increased levels of the antioxidant enzymes CAT, SOD, GSH, and MDA were observed in oysters' *P. euphraticus* after exposure to the pharmaceuticals AMX and $K_2Cr_2O_7$ than the control group, which possibly due to increased oxidative stress on the clams (Tables 1,2 and Figs.1,2. respectively) .

The Super oxide dismutase SOD and CAT are the most important first lines of defence to remove Reactive oxygen radicals in antioxidant enzymes. They are mostly used as an indicator of oxidative stress to determine pollution stress on organisms [38, 39], and could indicate that the cells are attempting to defend itself against the scenario of oxidative stress. It is a protective mechanism for the conversion of excess oxygen and free radicals resulting from exposure to hydrogen peroxide [10,40]. Cellular biomarkers have a prognostic or diagnostic value for long-term toxicological or ecological effects by early identifying the onset of biological changes induced by chemical pollutants [41]. Our results are consistent with Elizaldi-Velasquez who reported that the AMX induced oxidative stress, and it was also responsible for raising the activity of the enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) in the gills, kidneys, and brain of *C. carpio* during acute exposure[42]. Also the study of [43], showed that Cr^{6+} affects antioxidant responses and causes increased SOD-CAT activity, DNA damage and apoptosis in fish Channapunctatus. In this study, GPx levels in *P. euphraticus* decreased considerably following chromium and AMX exposure, this may be due to the fact that Cr(VI) compounds cause a decrease in glutathione concentrations due to an increase in glutathione disulphide (GSSG), which is an important marker of oxidative stress in cells as reported by [44,45].

The enzyme activity of the glutathione system can be induced by pharmaceutical preparations in bivalves, as in *M. galloprovincialis* and *Curbicula fluminea*[46], hexavalent chromium increased GSH in *Venus verrucosa* soft tissues, due to oxidative stress[45].

The enzyme activity of the glutathione system can be induced by pharmaceutical preparations in bivalves, as in *M. galloprovincialis* and *Carbicula fluminea* [46]. According to a study carried out by Shaaban et al. ,Hexavalent chromium increased GSH in *Venus verrucosa* soft tissues, due to oxidative stress[45]. MDA is widely used as a biomarker of oxidative stress, and increased level of oxidative damage in terms of lipid oxidation has been reported in different species of snails exposed *in vitro* to environmental pollutants [47]. Excess ROS produced within the organism's body may react with the lipid of the cell membrane, forming lipid peroxides that are further degraded into malondialdehyde. The MDA formation is an indicator of cell damage, which leads to tissue damage and, in extreme cases, death of the organism [48]. Rusdi et al. suggested that the elevated

2024, 21(2): 0289-0304
https://doi.org/10.21123/bsj.2023.8214
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
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MDA level in green-lipped mussels (*Pernaviridis)* indicated that an organism has experienced oxidative stress [49]. Proteins are the most important organic molecules in a living system. Proteins play an essential role in an organism's physiology and providing an information on an animal's general energy mobilization. Proteins are broken down into amino acids under stress conditions by organisms to meet their metabolic needs [50].In clams, an environment with high levels of pollution leads to a rise in protein breakdown and a decrease in cell protective proteins [49].

The study carried out by Ahmad et al. showed that potassium dichromate caused a considerable reduction in renal tissue proteins, albumin levels and hepatic tissue proteins when compared the control group of mollusks [50]. Ascorbic acid or vitamin C is a primary nutrient that, acts as a reducing factor and a non-enzymatic antioxidant in the cell. Ascorbic acid is used as a reducing agent for potassium dichromate from Cr (VI) to Cr (III). According to Chaâbane et al. chromium (VI) causes a significant increase in the levels of both GSH and vitamin C in soft tissues of *Venus verrucosa*[51]. Increased Catalase, SOD enzyme activity and MDA level as well as reduced GPx activity significantly indicated that *P. euphraticus* clams had experienced oxidative stress.

The present study indicated that *C.vulgaris* has a distinct role in relieving oxidative stress in clams through its pronounced effect on biochemical biomarkers during the study period. A gradual decrease in the values of ROS, SOD, CAT, GSH, TP and MDA was observed compared to untreated samples ($p<0.05$). Because *C. vulgaris* has been used to remove many environmental pollutants, (such as (heavy metals, organic compounds or pharmaceuticals), due to its widespread occurrence in aquatic habitats, rapid growth rates, and tolerance to harsh environmental conditions [52, 53]. Algae can at the same time utilize many methods or mechanisms that supplement each other to remove medicines and other toxic compounds from the environment. These mechanisms included intracellular and extracellular biodegradation, adsorption, bioaccumulation photolysis and hydrolysis [54] Additionally, Xiong et al. used *C. vulgaris* to eliminate some antibiotics such as levofloxacin and fluoroquinolones; with an initial concentration was of 5 mg/L, after 7 days, about 15% of the antibiotic had been eliminated [55]. There are several studies indicating the ability of *C. vulgaris* to clear the amoxicillin antibiotic, such as the study by Rickey et al. who indicated the susceptibility of *C. vulgaris* to removal of AMX (by 37%) from the medium by a biodegradation mechanism[56]. In a similar the study of Xiao et al. [57] who used *Chlorella pyrenoidosa* to remove amoxicillin which achieved about 91% clearance, within 6 hours. As well as study performed by Zhao et al., AMX was removed by *C. vulgaris* with an efficiency of 25% [58]

Many investigators reported that cell immobilization could protect the organism's growth against the toxicity of both heavy metals at LC50 as compared to lethal concentrations and maintain metabolic cell activity for a longer period [58, 59]. This makes it more effective in removing these compounds from the medium and thus reducing toxic effects on the organism. Immobilization of microalgal cells are recently used to remove many pollutants such as heavy metals, nitrogen, phosphorus, and pharmaceutical materials from polluted wastewater, because microalgae have a high ability to adapt to various and harsh environmental conditions [60] and can act as a good biological absorbent, and provide a high absorption capacity for minerals and nutrients. This study agrees with the results of Xie et al. who reported that immobilized *C. vulgaris* disrupted the toxicity of SMX and increased the removal efficiency by 85.1% and 86.2% SMX, respectively from the medium [61].

The possibility of using *C. vulgaris* as a cheap and effective sorbent material to remove chromium ions from wastewater without the need for pretreatment. The maximum chromium ion removal (99.75%) was under the following conditions; pH, 60 min., contact time, 60 mg/50 mL at a concentration of 100 ppm [62]. The alga removed chromate by adsorption, the alga contain functional groups, such as carboxyl (COO^-), amino (NH_2^-), sulfate (SO_4^{2-}), and hydroxyl (OH^-), which acted as binding sites for metals [63]. Also, the possibility of using green algae as a good bio absorbent for

2024, 21(2): 0289-0304
https://doi.org/10.21123/bsj.2023.8214
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
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removing Cr(VI) from aqueous solutions in the environment, and showed that *C. glomerata* dry biomass is a suitable candidate to remove Cr(VI) from aqueous solutions[64].

Conclusion

According to the obtained results of the present study, the pharmaceutical substances; AMX and $K_2Cr_2O_7$ cause oxidative stress in *P. euphraticus* due to increased ROS formation and CAT, SOD, MDA, GSH, and Ascorbic Acid activities in clams. So it might suggest the critical role of these enzymes in cell protection against the deleterious effects of pharmaceutical compound. Also, it showed that $K_2Cr_2O_7$ is the most harmful and effective toxin in clams. A recent study confirmed the high adaptability of immobilized *C. vulgaris* to environments containing pharmaceuticals, as well as its ability to reduce the toxic effects of these substances and thus reduce oxidative stress on non-target aquatic organisms. More researches are needed to determine whether immobilized *C. vulgaris* can reduce the toxic impacts of other pharmaceuticals on aquatic creatures that are not targets.

Acknowledgment

The authors would like to thank the Department of Biology, College of Science, University of Babylon, Iraq for their assistance with this project.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Babylon.

Authors' Contribution Statement

This work was carried out in collaboration between all authors. Z.H.O. diagnosed the cases then collected the samples and doing the tests. J M S, wrote and edited the manuscript with revisions idea. N.F.K ., wrote and analyzed the data with revisions idea . All authors approved the final manuscript.

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دورطحلب *Chlorella vulgaris* المقيد في تقليل سمية بعض المخلفات الصيدلانية في محار *Pseudodontopsis euphraticus*

زهراء حسين عبيد[1]، نهى فالح[2]، جاسم محمد سلمان[2]

[1]مركزالبحوث والدراسات البيئية، جامعة بابل، بابل، العراق.
[2]قسم علوم الحياة ـكلية العلوم ـجامعة بابل، بابل، العراق.

الخلاصة

تم في الآونة الأخيرة زيادة تطبيقات الطحالب الدقيقة المقيدة في الدراسات البيئية. تشمل الاستخدامات الحالية للطحالب الدقيقة المقيدة *Chlorella vulgaris*تقليل المواد الصيدلانية مثل الأموكسيسيلين AMXوثنائي كرومات البوتاسيوم $K_2Cr_2O_7$على محار المياه العذبة*Pseudodontopsis euphraticus* كنموذج حيوي. أشار البحث الحالي إلى حدوث تغيير في المؤشرات الحيوية للإجهاد التأكسدي في تقييم السمية المستحدثة. حيث تم تعريض المحار لتركيزات مختلفة100 ، 200 ، 400 ملغم / لتر لمدة 7 أيام و20 ، 30 ، 50 ملغم / لتر لمدة 5 أيام من أموكسيسيلي نوثنائي كرومات البوتاسيوم على التوالي. وأظهرت النتائج أن التعرض لـ AMX و K2Cr2O7أدى إلى حدوث تغير كبير في نشاط الإنزيمات المضادة للأكسدة ، مع زيادات كبيرة في إنتاجROS أعلى قيمة مسجلة لـ ROS كانت 51.05 ميكروغرام / ملغم بتركيزات 50 ملغم / لتر منK2Cr2O7 ، وأعلى نسبة مسجلة كانت SOD و CAT و MDAو GSH ، على النحو التالي: 33.40وحدة/مل ، 33.32كيلو وحدة/لتر، 23.22 ميكرو مول / لتر و 21.30 ميكروغرام / غرام على التوالي ، بتركيزات 50ملغم / لتر من $K_2Cr_2O_7$ غير المعالجة. لوحظ في هذه الدراسة أن ثنائي كرومات البوتاسيوم كان أكثر فعالية من الأموكسيسيلين في إحداث السمية. وفقًا للدراسة الحالية ، كان لنوعطحالب المقيدة*C. vulgaris* دورًا أساسيًا في تقليل سمية المواد الكيميائية ، من خلال تخفيف الإجهاد التأكسدي على محار*P. euphraticus*، حيث سجل انخفاضًا كبيرًا في قيم ROS والإنزيمات المؤكسدة مثل GSH و SOD و CAT و MDA ، وكذلك حامض الاسكوربيكAA ، البروتين الكلي و GPX في العينات المعالجة.

الكلمات المفتاحية: المؤشرات الكيموحيوية، *Chlorella vulgaris* ,محار المياه العذبة،الطحالب المقيدة، المخلفات الصيدلانية.