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Dunaliella salina **Extract Alleviates The Toxic Impact of Dioxin Induced Endocrine Disruption in Nile Tilapia**

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POLLUTION of lakes and seas in Egypt become a severe problem as it produces a lot of wastes and toxic chemicals. The water pollution induces a lot of endocrine disruptions in aquatic organisms. Several endocrine organs are influencing by pollution such as testes, ovaries, pituitary, hypothalamus, thyroid, and adrenal organs. Propositions of upsetting endocrine chemicals (EDCs) can meddle with fish larval advancement affecting the delicate balance of the biological ecosystem. Endocrine disruption effects on reproductive cycles may affect reproductive behaviour and the development of secondary sexual characteristics. So the main objective of this research is to use marine algae, *Dunaliella salina*, extract in fish immunity enhancement against pollutants. Nile tilapia fish were exposed to Dioxin (as the main pollutant in the Egyptian environment) after feeding on algae extracts for one month. The results revealed that *Dunaliella salina* was able to protect the fish against pollution as it enhances the immune capability of Nile tilapia fish due to increase the levels of the antioxidant enzymes and decrease the protein oxidation.

Additionally, *Dunaliella salina* extract was able to decrease the alteration in the expression of genes encoding endocrine hormones such as LH and FSH in the pituitary of Nile tilapia exposed to Dioxin in comparison to control fish. Moreover, the *Dunaliella salina* extract decreased significantly the DNA damage in brain tissues of Nile tilapia exposed to Dioxin compared with control fish. The results conclude that *Dunaliella salina* could be used as ameliorative compounds against toxicity of Dioxin.

Keywords: Marine algae extract, Water pollution, Endocrine disruption, Gene expression, DNA damage.

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Introduction

A few chemical substances can enter the sea and disrupt the hormonal (endocrine) balance of the organisms, these chemicals named 'endocrine disrupters' (EDs). Currently, the research on endocrinedisrupters focuses on vertebrate sex steroid hormone frameworks, yet the field is widening to incorporate other vertebrate and additionally invertebrate structures [1].

The impacts of EDs might be exceptionally extreme. Also, endocrine disrupters can meddle with sex steroid union and digestion system [2,3] and with the pituitary, thyroid and internal hormone frameworks [4,5].

Countless chemicals have been accounted to have endocrine tweaking action. These include: characteristic items (e.g. coumestrol, genistein), and a few individuals from the accompanying classifications of chemicals: pesticides, fungicides and bug sprays (e.g. dieldrin, toxaphene, endosulfan, phenyl phenol, DDT and its metabolites, Methoxychlor, vinclozolin), medicinal medications (e.g. hydroxyflutamide, nilutamide, tamoxifen, diethylstilbestrol, oral contraceptives, for example, ethynylestradiol), business and/or mechanical chemicals, for example, bisphenol-An, alkylphenols (nonylphenol), polychlorinated biphenyls (PCBs), phthalate plasticisers, PAHs and some metals [6].

Most endocrine disturbance research has focused on the impacts of estrogens, which can have a feminising effect. In human, conceptive clutter and decreased sperm numbers have been ascribed to estrogens [7, 8]. Estrogenic impacts in fish, both in the research centreand in the field, have significantly been reported, including affectation of the female yolk protein in guys (vitellogenesis: e.g. [9, 10] anomalous gonad improvement [11] and adjustments in sex steroid titres [12].

Algae constitute a major group of living organisms that are an essential source of a variety of useful products with widespread applications in the biodiesel, food, and pharmaceutical industries, as well as in biotechnology [13]. To prevent oxidative damage, and disease conditions are arising from such loss, a combination of bioactive compounds such as carotenoids and omega-3 fatty acids possessing different chemical characteristics can be used. The unicellular marine alga *Dunaliella*is a most interesting green cell factory to produce carotenes, vitamins, and fatty acids under extreme environmental conditions. Therefore, the main objective of the present study was designed to evaluate the effect of *Dunaliella salina* as marine algae in enhancing the fish immunity against water pollutants.

Materials and Chemicals

Reagents for water quality analysis, determination of total protein, biochemical and molecularanalyses were purchased from Sigma-Aldrich and Invitrogen (Germany). All chemicals and reagents were from the highest purity available.

Experimental Fish

The fish (Nile tilapia, *Oreochromis niloticus*) used in this study were brought from National Research Centre farm (Nubaria, Egypt). Fish were transferred to the laboratory of biotechnology and biodiversity conservation group in large plastic water containers accompanied by battery aerators as a source of oxygen with de-chlorinated tap water (24.5±2.1°C and pH 7.2-8.2). Fish were fed*on libitum* standard fish food during the experiment. Eighty fish were used, after one week of acclimatization, fish were divided into eight groups (10 fish/ group) and were placed into fish aquariums.

Water quality Parameters:

Water quality parameters were determined by the procedures recommended in the standard methods for the examination of water and wastewater [14].

Cultivation of Dunaliella salina

Cultivation and harvesting of *Dunaliella salina* microalgaeare illustrated in Figure 1. *Dunaliella salina* was used in the following experiments as fish feed. The algae were isolated from salt deposition basins of the Egyptian Salts and Minerals Company, EMISAL) and grown on BG11 media containing NaCl with a concentration of 100 g/L and $NaNO_3$ 0.3 g/L. The cultivation was carried out on small ponds

with a capacity of 50 L containing a volume of 40 L of microalgae culture. The temperature of the culture was adjusted to be 22±3°C. Fluorescent light was used to supply constant light intensity for the culture, which was ≈2500 lux with continuous aeration. Media was added twice a week. After two weeks' culture was let to settle down, the biomass was withdrawn and collected by the electro flocculation [15].

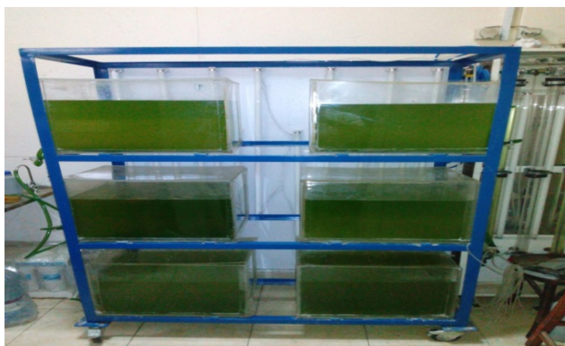

Figure. 1. Cultivation and harvesting of *Dunaliella salina* micro algae. (a) Cultivation of *Dunaliella salina* in open ponds. (b) Harvesting using electrofloculation.

Experimental design

After the adaptation period, Nile tilapia fish were allocated into the following groups: The first group contained fish with only standard fish diet; the second to fourth groups including fish fed on different concentrations of microalgae (*Dunaliella salina*) in addition to standard fish feed as follows; 5%, 10% and 20%. Fish groups from 5-7 contained fish diets with 5%, 10% and 20% of *Dunaliella salina* microalgae meal in addition to Dioxin (1/10 of the LD50~22 mg/kg), respectively; while the eights group contained fish fed on diet with Dioxin only with concentration of1/10 of the LD50~22 mg/kg. The experiment duration was one month,and the fish were usedat the end of the treatment to determine the toxicity of Dioxin and to be compared with other groups after feeding.

Biochemical analysis

Determination of enzyme activity

The activities of glutathione-S-transferase (GST) and glutathione peroxidase (GPx) were measured in brain samples referring to Miranda et al. [16] and Elmegeed et al. [17], respectively. The reaction mixture contained the following: 8 mM H2O2, 40mM guaiacol, 50 mM sodium acetate buffer, pH 5.5 in addition to a suitable amount of enzyme preparation. The absorbance change at 470 nm due to guaiacol oxidation was followed every 30 s intervals. The definition of glutathione peroxidase activity one unit represents the enzyme amount that increases O.D. 1.0/min under standard assay conditions.

Protein analysis

The analysis of protein oxidation through cellular protein carbonyl (PC) content was determined to refer to the method stated by Baltacıog et al. [18], by some modifications using 2,4-dinitrophenylhydrazine (DNPH) reagent. Carbonyl content was calculated from the peak absorbance at 340 nm, with an absorption coefficient of 22,000/M/cm.

Comet assay for DNA strand break determination

Isolated hepatic cells of all groups of Nile tilapia were subjected to the modified single-cell gel electrophoresis or comet assay [19]. To obtain the cells, a small piece of the liver was washed with an excess of ice-cold Hank's balanced salt solution (HBSS) and minced quickly into approximately one mm^3piece while immersed in HBSS, with a pair of stainless steel scissors. After several items of washing with cold phosphate-buffered saline (to remove red blood cells), the minced liver was dispersed into single cells using a pipette [20]. In brief, the protocol for electrophoresis involved embedding of the isolated cells in agarose gel on

microscopic slides and lysing them with detergent at high salt concentrations overnight (in the cold). The cells were treated with alkali for 20 min to denature the DNA and electrophoresis under alkaline conditions (30 min) at 300 mA, 25 V. The slides were stained with ethidium bromide and examined using a Zeiss fluorescence microscopy axiostar plus (USA)with a green filter at × 40 magnification. For each experimental condition, about 100 cells (approximately25 cells per fish) were examined to determine the percentage of cells with DNA damage that appear like comets. The nonoverlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with length between 1× and 2× the nuclear diameter; and class 3 = tail longer than 2× the widthof the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus [21,22]. A total damage score for each slide was derived by multiplying the number of cells assigned to each class of damage by the numeric value of the class and summing up the values. Slides were analyzed by one observer to minimize the scoring variability.

Gene expression analysis

Isolation of total RNA

TRIzol® Reagent (cat#15596-026, Invitrogen, Germany) was used to extract total RNA from pituitary gland tissues of Nile tilapia per the manufacturer's instructions with minor modifications. Briefly, tissue samples (50 mg) were homogenized in 1 ml of TRIzol® Reagent. Afterwards, the homogenized sample was incubated for 15 min at room temperature. A volume of 0.2 ml of chloroform per 1 ml of TRIzol® Reagent was added. Then, the samples were vortexed vigorously for 15 seconds and incubated at room temperature for 3 min. The samples were centrifuged for no more than 12 000 g for 15 min at 4 °C. Following centrifugation, the mixture was separated into a lower red, phenol-chloroform phase, interphase, and a colourless upper aqueous phase. RNA remained exclusively in the aqueous phase. Therefore, the upper aqueous phase was carefully transferred without disturbing the interphase into a fresh tube. The RNA was precipitated from the aqueous phase by mixing with isopropyl alcohol. A volume of 0.5 ml of isopropyl alcohol was added per 1 ml of TRIzol® Reagent used for the initial homogenization. Afterwards, the samples were incubated at 15 to 30 °C for 10 min and centrifuged at not more than 12,000 x g for 10 min at 4 °C. The RNA was precipitated which was often invisible before centrifugation, formed a gel-like pellet on the side and bottom of the tube. The supernatant was obliterated. The RNA pellet was washed once with 1 ml of 75% ethanol. The samples were mixed by vortexing and centrifuged at no more than 7 500 g for 5 min at 4 °C. The supernatant was removed, and the RNA pellet was air-dried for 10 min. RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water by passing solution a few times through a pipette tip.

Total RNA was treated with 1 unit of RQ1 RNase-free DNAse (Invitrogen, Germany) to digest DNA residues, re-suspended in DEPC-treated water and quantified photo-spectrometrically at 260 nm. The purity of total RNA was assessed by the 260/280 nm ratio, which was between 1.8 and 2.1. Additionally, integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis (data not shown). Aliquots were used immediately for reverse transcription (RT). Otherwise, they were stored at -80°C.

Reverse transcription (RT) reaction

The complete Poly(A)$^+$ RNA isolated from Nile tilapia tissues was reverse transcribed into cDNA in a total volume of 20 µl using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Germany). An amount of total RNA (5µg) was used with a master mix (MM). The MM consisted of 50 mM $MgCl_2$,10x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), tenmM of each dNTP, 50 µM oligo-dT primer, 20 IU ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity) and 50 IU MuLV reverse transcriptase. The mixture of each sample was centrifuged for 30 sec at 1000 g and transferred to the thermocycler. The RT reaction was carried out at 25°C for 10 min, followed by oneh at 42°C, and finished with a denaturation step at 99°C for 5 min. Afterwards, the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for cDNA amplification through Real-Time polymerase chain reaction (RT-PCR).

Quantitative Real-Time- PCR (qRT-PCR)

StepOne™ Real-Time PCR System (USA) was used to determine the Nile tilapia cDNA copy number. PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.), 0.5 µL 0.2 µM sense primer, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template. Each experiment included a distilled water control. The sequences of specific primer of the genes used [23] and product sizes are listed in Table 1. At the end of each qRT-PCR, a melting curve analysis was performed at 95.0°C to check the quality of the used primers. The relative quantification of the target to the reference was determined by using the$\Delta\Delta C_T$ method if E for the goaland the reference primers (β-Actin) are the same.

TABLE 1. Primers sequences used for RT-PCR.

Gene	Primer sequence (5′–3′)[a]	Annealing Tm (°C)
FSH	F: TCAGTCACCTGACGATCTGCAA R: TCCTGCAGGTCCAGCAGAAACG	60
LH	F: CTTCTCAACCTCAATGAAATCTTC R: GGATATACTCAGATAACGCAGCTT	60
β-Actin	F: TGG GGC AGT ATG GCT TGT ATG R: CTC TGG CAC CCT AAT CAC CTC T	55

[a] **F: forward primer; R: a reverse primer. Tm: temperature**.

Statistical analysis

The data were examined using the General Linear Models (GLM) procedure of the Statistical Analysis System [24] followed by Scheffé-test to detect the significant differences amongfish groups. The values were expressed as mean±SEM. The statements of significance were based on the probability of $P < 0.05$.

Results and Discussion

Water quality Parameters

Since the quality of water influences the survival and performance of fish. Some of these key parameters are more likely to be involved with fish loss, such as dissolved oxygen and temperature while others such as salinity and total dissolved solid affect fish but usually are not directly toxic, during the experiment period some of the physicochemical parameters were measured using laboratory equipment, and result showed that all the measured parameters were within normal range for optimum fish performance. Table 2 represents water quality parameters measurements. The results revealed that the quality of water parameters was decreased in the water contained Dioxin compared with control water. However, the water quality was improved in the water contained fish fed on *D. salina* microalgae.

In agreement with the current results, several studies established that certain types of algae have the potential for integrated use in water purification from aquaculture and biomass production [25] Some microalgae species such as *C. vulgaris*, *N. oculata*, *T. Chui* exhibited the high potential of accumulation for nitrogen and phosphorus compounds contained in the wastewater from aquaculture and could be used for their treatment [26, 27].

TABLE 2. Water quality parameters

	Disolved oxygen (mgO_2/l)	pH	COD mgO_2/l	BOD mgO_2/l	TDS (mg/l)	Salinity (‰)	Alkalinity mg/l
Diet+ *D. salina* (5%)	7.2	7.9	11	7	469	420	140
Diet+ *D. salina* (20%)	7.5	8.0	11	6	494	440	140
Diet+ *D. salina* (5%)+Dioxin	4.5	7.0	12	8	358	486	132
Diet + *D. salina* (20%)+Dioxin	5.0	7.2	13	8.5	366	490	130
Diet+Dioxin	2.4	6.0	14	10	226	500	136
Control	8.0	7.5	10	6	458	450	130

Biochemical analysis:

Table 3, shows levels of antioxidant enzymes such as GST and GPxin brain tissues of Nile tilapia exposed to Dioxin in water with/ or without micro-algae (*Dunaliella salina*). The results indicated that GST and GPx activity levels were significantly lower in brain tissues collected from fish exposed to Dioxin than those collected from control fish. However, fish fed on diet contains 5, 10 and 20% micro-algae (*Dunaliella salina*) exhibited significantly higher levels of GST and GPx compared to those in fish exposed to Dioxin. Also, micro-algae (*Dunaliella salina*) wasable to increase the levels of GST and GPx in fish exposed to Dioxin when *Dunaliella salina* was supplemented to fish exposed to Dioxin. In the same line with our results, Zainuri et al. [28] reported that *Dunaliella salina* exhibited high antioxidant capacity in different polluted media. *Dunaliella salina* is one of the most potent natural feeds as a used feed additive and feeds supplement in aquaculture [28]. Based on research conducted by Abd EL-Baky [29], *D. salina* contain a high portion of carotenoids (12.6 % of the dry weight) which comprising β-carotene (60.4 %), α-carotene (17.7 %), zeaxanthin (13.4 %), lutein (4.6 %) and cryptoxanthin (3.9 %). Salinity is one of the environmental stressors that can affect the increase of β-carotene content in *D. salina.*

TABLE 3. Activity levels of GST and GPx in the brain tissues of Nile tilapia exposed to Dioxin in water with/ or without micro-algae (*Dunaliella salina*)

Treatments	GST (nmol/mg P/min) (Mean±SD)	GPx (µU min^{-1} mg^{-1} P) (Mean±SD)
Control	267±14^{a}	136±15^{a}
D. salina (5%)	269±22^{a}	138±12^{a}
D. salina (10%)	271±18^{a}	139±11^{a}
D. salina (20%)	272±21^{a}	142±13^{a}
D. salina (5%)+ Dioxin	196±16^{c}	111±09^{bc}
D. salina (10%)+ Dioxin	213±13^{bc}	116±11^{bc}
D. salina (20%)+ Dioxin	231±17^{b}	121±14^{b}
Dioxin	158±19^{d}	84±12^{c}

$^{a,b,c,d:}$ Values within the same column with different superscripts are significantly different (P<0.05). *D. salina*: *Dunaliella salina*

Protein oxidation levels

Table 4, shows levels of protein carbonyl (PC) in gills and liver tissues of Nile tilapia exposed to Dioxin in water with/ or without micro-algae (*Dunaliella salina*). The results revealed that cellular (PC) contents were significantly higher in gills and liver tissues collected from fish exposed to Dioxin than those collected from control fish. However, fish fed on diet contains 5, 10 and 20% micro-algae (*Dunaliella salina*) exhibited significantly lower levels of PC compared to those in fish exposed to Dioxin. Also, micro-algae (*Dunaliella salina*) was able to decrease the levels of PC in fish exposed to Dioxin when *Dunaliella salina* was supplemented to fish exposed to Dioxin. These results are in great agreement with Singh et al. [30], who reported that *Dunaliella salina* decreases the protein oxidation and increase the antioxidant activity.

Moreover, they indicated that different stress conditions which may lead to an increase in carotene production by the *Dunaliella salina*. Further, antioxidant and cytotoxic effects of *Dunaliella salina* in oxidized cells suchas breast cancer cell lines were found to be significantly higher in some stress conditions. This could be due to an increase in carotene production or may be due to the involvement of some other natural bioactive compounds.

TABLE 4. Levels of protein carbonyls (PC, nmol/mg protein) in the liver and gills of Nile tilapia exposed to Dioxin in water with/ or without micro-algae (*Dunaliella salina*)

Treatments	Cellular protein carbonyl Mean±SD Gills	Mean±SD Liver
Control	1.19±0.43[d]	0.82±0.06[d]
D. salina (5%)	1.18±0.02[d]	0.83±0.04[d]
D. salina (10%)	1.19±0.06[d]	0.82±0.05[d]
D. salina (20%)	1.17±0.04[d]	0.81±0.02[d]
D. salina (5%)+ Dioxin	2.42±0.06[b]	2.32±0.06[b]
D. salina (10%)+ Dioxin	1.73±0.08[c]	2.14±0.04[b]
D. salina (20%)+ Dioxin	1.46±0.04[c]	1.75±0.04[c]
Dioxin	3.41±0.09[a]	2.82±0.07[a]

[a,b,c,d:] Values within the same column with different superscripts are significantly different ($P<0.05$).

Expression of genes encoding endocrine hormones (FSH and LH)

Expression levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) related genes in the pituitary gland cells of Nile tilapia exposed to Dioxin in water with/ or without micro-algae (*Dunaliella salina*) in the diet are summarized in Figures 2 and 3. The results revealed that expression levels of FSH and LH genes were significantly ($P<0.01$) lower in the pituitary gland tissues collected from fish exposed to Dioxin than those collected from control fish. In contrary, fish fed on diet contains 5, 10 and 20% micro-algae (*Dunaliella salina*) exhibited significantly higher levels of the expression of FSH and LH genes compared to those in fish exposed to Dioxin alone. Moreover, micro-algae (*Dunaliella salina*) was able to increase the levels of the expression of FSH and LH genes in fish exposed to Dioxin when *Dunaliella salina* was supplemented to fish exposed to Dioxin. In agreement with our findings, several studies indicated that *Dunaliella salina* could modulate the expression profile of several gene pathways. Miyashita [31] has reported that fucoxanthin (as a pigment in the chloroplasts of microalgae algae) can significantly affect human health by altering the gene expression profiles of proteins involved in cell metabolism. Many studies have tested the antioxidant effects of fucoxanthin on different cell lines and animal models [32,33]. Another vitalcarotenoid exhibiting strong antioxidant activity is astaxanthin,which shows higher levels of antioxidant activity than other carotenoids such

as β-carotene, zeaxanthin,and canthaxanthin [34]. Rodrigues et al. [35] have reported that astaxanthin acts as a scavenger of various reactive species. Several studies have reported that dietaryintake of carotenoids can protect humans and animals from oxidative damage to lipophilic parts ofcells; this is because carotenoids can limit lipid peroxidation events by scavenging the ROS formed during photo-oxidative processes [36].

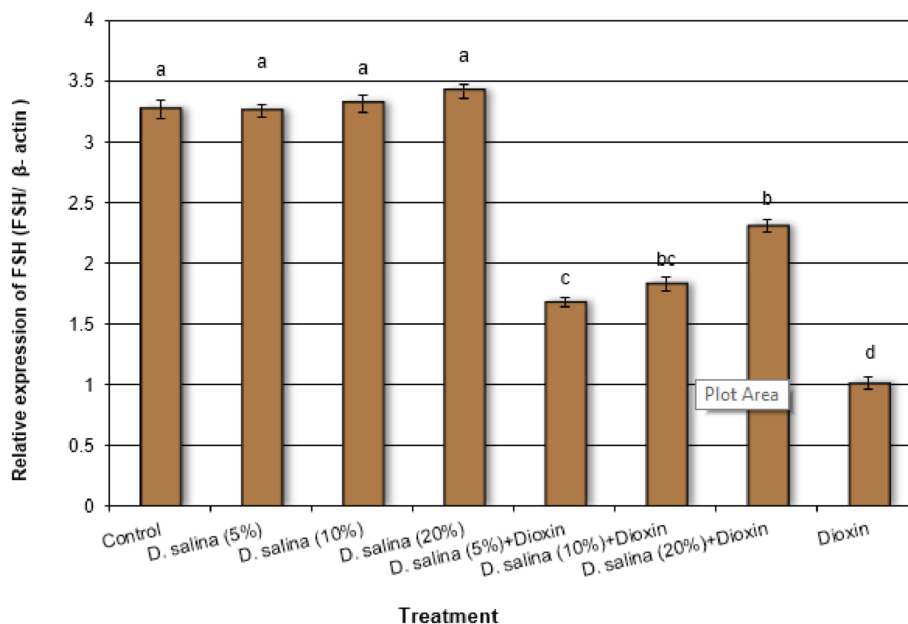

Figure. 2. Expression levels of FSH gene in the pituitary tissues of Nile tilapia treated with dioxin and/or *D. salina* extract determined by qRT-PCR. [a, b]: Within each column, means superscripts with different letters are significantly different (P < 0.05).

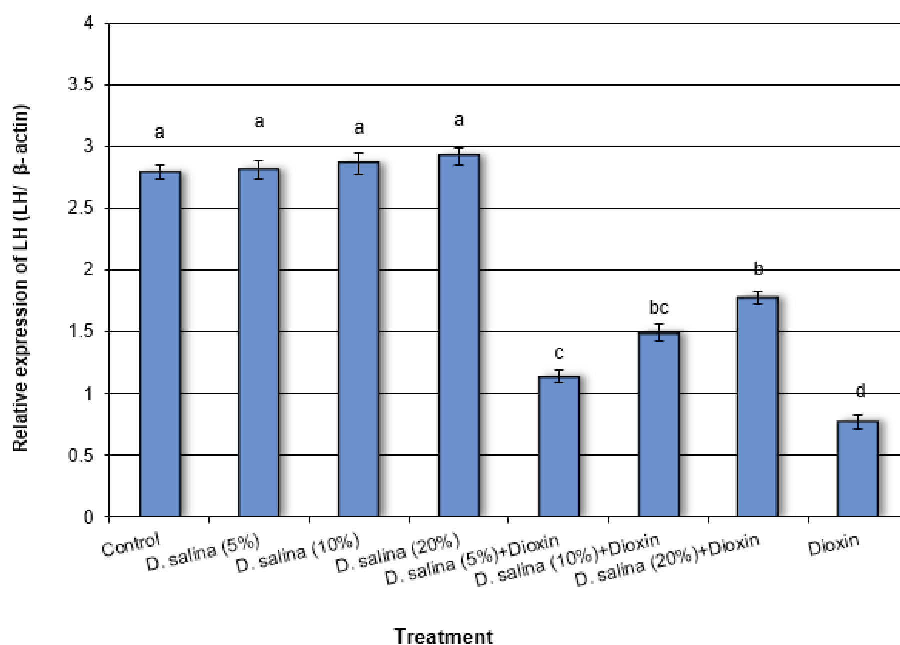

Figure. 3. Expression levels of LH gene in the pituitary tissues of Nile tilapia treated with dioxin and/or *D. salina* extract determined by qRT-PCR. [a, b]: Within each column, means superscripts with different letters are significantly different (P < 0.05).

DNA damage assessed by comet assay

Assessment of the DNA damage in liver tissues ofNile tilapia exposed to Dioxin in water with/ or without micro-algae (*Dunaliella salina*) in the dietwas summarized in Table 5.

The results revealed that rates of DNA damage in the liver tissuescollected from fish exposed to Dioxin were significantly (P<0.01) higher than those collected from control fish. On the contrary, DNA damage in the liver tissues of fish fed on diet contains 5, 10 and 20% micro-algae (*Dunaliella salina*) was significantly lower compared to those in fish exposed to Dioxin alone. Moreover, micro-algae (*Dunaliella salina*) wasable to decrease the rates of DNA damage in fish exposed to Dioxin when *Dunaliella salina* was supplemented to fish exposed to Dioxin. Additionally, the high dose of *Dunaliella salina* extract exhibited the lowest DNA damage rates compared with low and medium doses of *Dunaliella salina* extract.

The following findings come in agreement with our results as Park et al. [37] reported that *Dunaliella salina* was able to decrease the DNA damage in different human cell types, which was attributed to Astaxanthin. They indicated that astaxanthin content is a powerful, naturally occurring carotenoid pigment in *Dunaliella salina.* In this study, the women who ingested 2 mg of astaxanthin for eightweeks had lower blood levels of C-reactive protein, indicating that this compoundhas anti-inflammatory activity. Also, the study found that astaxanthin could reduce ROS production by down-regulating *NF*-kappa *B* and *AP-1* transcription factors, as well as inflammatory cytokineproduction. From these results, it is clear that astaxanthin ingestion can decrease DNA damage, reduceacute phase protein levels, and enhance immune responses in healthy young women [37].

TABLE. 5. Rate of DNA damage in liver tissues of Nile tilapia treated with dioxin and *D. salina* extract using comet assay.

Treatment	No. of cells		Class[¥] of comet				DNA damaged cells (mean ± SEM)
	Analyzed	Total comets	0	1	2	3	
Control	500	38	462	27	11	0	7.6±0.17[d]
D. salina (5%)	500	37	463	25	12	0	7.4±0.33[d]
D. salina (10%)	500	36	464	26	8	2	7.2±0.42[d]
D. salina (20%)	500	32	468	24	5	3	6.4±0.34[d]
D. salina (5%)+Dioxin	500	103	397	37	44	22	20.6±0.56[b]
D. salina (10%)+Dioxin	500	87	413	38	29	20	17.4±0.58[bc]
D. salina (20%)+Dioxin	500	68	432	33	21	14	13.6±0.46[c]
Dioxin	500	141	359	41	52	48	28.2±0.82[a]

[¥]: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.(*): No of cells analyzed were 100 per animal.

Conclusions

The results of the current findings may suggest that *Dunaliella salina* was able to enhance the immune competence of Nile tilapia due to increase the levels of the antioxidant enzymes and decrease the protein oxidation. Additionally, *Dunaliella salina* extract was able to reduce the alteration in the expression of genes encoding endocrine hormones such as LH and FSH in the pituitary of Nile tilapia exposed to Dioxin in comparison to the control fish. Moreover, the *Dunaliella salina* extract decreased significantly the DNA damage in brain tissues of Nile tilapia exposed to Dioxin compared with control fish. The results conclude that *Dunaliella salina* could be used as ameliorative compounds against toxicity of Dioxin and to enhance the immune system and reduce the hormonal disruption. These valuable results may be attributed to the rich content of

antioxidant within the algal tissues, among other natural products that will be revealed in other research.

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مستخلص *Dunaliella salina* يخفف من التأثير السام لاضطراب الغدد الصماء الناتج عن الديوكسين في أسماك البلطي النيلي

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أصبح تلوث البحيرات والبحار في مصر مشكلة حادة لأنها تنتج الكثير من النفايات والمواد الكيميائية السامة. و يتسبب تلوث المياه في حدوث الكثير من اضطرابات للغدد الصماء في الكائنات المائية. ويؤثر التلوث على العديد من أعضاء الغدد الصماء مثل الخصيتين والمبيض والغدة النخامية وما تحت المهاد والغدة الدرقية والأعضاء الكظرية. ويمكن لهذا الخلل بإعداد المواد الكيميائية للغدد الصماء (EDCs) أن يؤثر على تقدم يرقات الأسماك التي تاثير على التوازن الدقيق للنظام الإيكولوجي. ويؤثر إضطرب الغدد الصماء على الدورات التناسلية وتطور الخصائص الجنسية الثانوية. لذلك فإن الهدف الرئيسي للبحث هو استخدام مستخلص الطحالب البحرية ، *Dunaliella salina* لتعزيز مناعة الأسماك ضد الملوثات.

وفى هذة الدراسة تعرض سمك البلطي النيلي للديوكسين (باعتباره الملوث الرئيسي في البيئة المصرية) بعد ان تمت تغذية أسماك البلطى على مستخلصات الطحالب لمدة شهر. ولقد أظهرت النتائج أن *Dunaliella salina* كانت قادرة على حماية الأسماك من التلوث لأنها تعزز القدرة المناعية لأسماك البلطي النيلي بسبب زيادة مستويات إنزيمات مضادات الأكسدة وتقليل أكسدة البروتين.

بالإضافة إلى ذلك ، تمكن مستخلص *Dunaliella salina* من تقليل التغير في التعبير عن الجينات التي ترمز لهرمونات الغدد الصماء مثل LH و FSH في الغدة النخامية لبلطي البلطي المعرض للديوكسين مقارنةً بالسيطرة على الأسماك. علاوة على ذلك ، خفض مستخلص *Dunaliella salina*بشكل كبير التلف الخاص بالحمض النووي في أنسجة المخ سمك البلطي النيلي المعرض للديوكسين مقارنة مع الأسماك الضابطة. ويمكن الاستنتاج من هذة النتائج أن *Dunaliella salina* يمكن استخدامها كمركبات تحسينية ضد الديوكسين السام.