ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology, Volume 2 Number 3: 222-240, 2003
ISSN 1682-2978

Spirulina Species as a Source of Carotenoids and α-Tocopherol and its Anticarcinoma Factors

Hanaa H. Abd El-Baky, Farouk K. El Baz and [1]Gamal S. El-Baroty
Agriculture deviation, National Research Center, Dokki, Cairo, Egypt
[1]Department of Biochemistry, Faculty of Agriculture, Cairo University, Egypt

Abstract: In the present study blue-green alga ***Spirulina platensis*** and ***Spirulina maxima*** were grown in batch culture at different nitrogen and NaCl concentrations, respectively. Both species were found to respond to nitrogen deficiency and high NaCl level by accumulation of large amounts of commercially important chemicals such as carotenoids and tocopherols. The higher carotenoids and α-tocopherol contents was obtained when ***Spirulina sp*** grown at low nitrogen level (51 ppm N), with values ranged from 19.82 to 24.1 mg g^{-1} and from 533.2 to 978.5 μg Kg^{-1} (dry weight, d.w.), respectively. Whilst, these values in cells grown in free nitrogen medium were ranged from 30.15 to 31.13 mg g^{-1} and from 960.4 to 1325.7 μg Kg^{-1} (d. w), respectively. The high performance liquid chromatography (HPLC) elution patter of total caroteniods extracted from ***S. platensis*** and ***S maxima*** culture contained β-carotene (49.6 to 319.5 μg g^{-1}), lutein (0.06 to17.21 μg g^{-1}), astaxanthin (6.61 to 160.27 μg g^{-1}), zeaxanthin (1.25 to 18.55 μg g^{-1}) and cryptoxanthin (1.41 to 20.13 μg g^{-1}). ***S. maxima*** accumulated a large amount of cryptoxanthin and zeaxanthin than that found in ***S. platensis***. The effect of ***Spirulina*** species extracts containing various carotenoid compounds and tocopherols on the viability of Ehrlich ascites carcinoma cells (EACC) were evaluated. All algae extracts at different concentration of 200 and 400 ppm significant reduced the cell viability ranged from 89.11 to 5.25%. These extracts did not induce any significant changes in DNA fragmentation of treated EACC compared with untreated cells. But lactate dehydrogenase (LDH) and glutathione-S- transferase (GST) enzyme activities and glutathione level in treated EACC were significantly higher than that in untreated cells. These finding suggest that algae extracts may be reduce cell viability by other mechanism such as membrane lyases instead of apoptosis. Thus, ***Spirulina*** extract rich in carotenoids and tocopherols could be used as chemopreventive agents since they are relative non-toxic.

Key words: ***Spirulina***, nitrogen and salt stress, carotenoids, tocopherols, antitumor, viability and blue green algae

Introduction

In last decade algae biotechnology has made major advance and many algae metabolites are being production commercially (Borowitzka, 1992 and Morimoto ***et al.***, 1995). A number of microalgae such as ***Duneliella***, ***Chlorella*** and ***Spirulina*** are grown for food, feed or for the

extraction of valuable chemical such as carotenoids, antibiotics, fatty acids and antioxidant vitamins (Borowitzka, 1986; El Baz ***et al.***, 2002a and b; Abouel Enein ***et al.***, 2003; Abd El-Baky ***et al.***, 2002).

Carotenoids are naturally occurring pigment in algae that are involved in light-harvesting reaction and protection of algal organelle cells against singlet oxygen- induced damage. The alga species ***Duneliella*** and ***Chlorella*** show considerable differences in metabolisms of carotenoids. The differences are attributed to unfavorable conditions of growth, especially deficiency of nitrogen and or iron and high light intensity and high salinity (Abd El-Baky ***et al.***, 2002; El-Baz ***et al.***, 2002a and Borowitzka, 1986) leads to accumulation of secondary carotenoids, mainly ketocarotenoids. However, the increase of ketocarotenoids level in cells of these algae accompanied by the disappearaence of chlorophyll and a decrease in primary carotenoids such as, α and β carotene and lutein (Burczyk, 1987). ***Spirulina*** has a higher content of β-carotene, cryptoxanthin and zeaxanthin compared to most other natural source (Mathew ***et al.***, 1995). Also, ***Spirulina*** offer a wide scope for production of large quantities of natural carotenoids directly in an edible state. It has also been demonstrated to be safe food item (Seshadri ***et al.***, 1992 and Mathew ***et al.***, 1995).

Epidemiological and animal studies indicate the inhibitory effect of micronutrients, such as vitamin E, C and carotenoids on carcinogensis. Studies have also shown that ***Spirulina*** extracts exert chemoprephylactic actions on certain cancers (Schwartz and Shklar, 1987; Schwartz ***et al.***, 1988; Govannucci, 1999; Abd El-Baky ***et al.***, 2002). For example, the extracts of ***Spirulina*** and ***Dunaliella*** algae have been demonstrated to be capable of regressing carcinomas of the hamster buccal pouch. The preventive potential of micronutrients should preferably be realized by modulation of diet rather than in the form of pharmacological supplements (Mathew ***et al.***, 1995).

In the present work the effect of nitrogen and NaCl on accumulation of caroteniods and tocopherols in ***Spirulina maxima*** and ***Spirulina plantensis*** were studied. Also, the antitumor activity of these compounds was evaluated.

Materials and Method

Algae source: ***Spirulina maxima*** and ***Spirulina plantensis*** were obtained from the Culture Collection of Texas University, Austin, USA.

Growth conditions

Algae was cultured in 2 L Erlenmeyer flasks containing Zarrouk's medium as described by Zarrouk, (1966). $NaNO_3$ was used as a nitrogen (N) source at four different concentrations; 410 ppm N (control), 205 ppm N, 102.5 ppm N and 51 ppm N and zero nitrogen. Also NaCl was used at different concentrations 0.02 M (control), 0.1 M and 0.2 M. The cultures were gassed with 0.03% CO_2 in air and the algae were cultivated at 25°C±3. The pH of the medium was adjusted to 10.5 with 1 M NaOH prior to autoclaving. The cultivated flasks were illuminated by continuous cool white fluorescent lamps at 400 W.

Growth measurements

The growth of ***Spirulina maxima*** and ***Spirulina plantensis*** was measured by dry weight methods and optical density at 450 nm (Vonshak, 1997).

Harvesting

After 2 weeks (end of logarithmic-phase), the algae cells were harvested at 4°C by centrifugation at 10000 × g for 10 min.

Extraction of carotenoids and tocopherols

Carotenoids and tocopherols were extracted from algal cells with tetrahydrofuran (THF) 1: 10 (w/v) in present 30 mg L^{-1} of BHT (2,6 ditertra-butyl-p-cresol) and magnesium carbonate (0.1 g^{-1} sample). After 24 h, the aliquot of the clear extracted pigments was filtered and evaporated to 5 ml under a stream of nitrogen. The extracted pigments were saponified with 25 ml of 10% (v/w) methanolic potassium hydroxide for 2 h at room temperature, then the carotenoids and tocopherols were extracted with dichloromethane. The solvent layer was then separated by separatory funnel, washed several times with distilled water and dried on $NaSO_4$. The organic solvent was evaporated to dryness under nitrogen (Farag ***et al.***, 1998).

Determination of algal carotenoids

The total carotenoids were determined by spectrophotometric method at 450 nm, β-carotene served as a standard compound and was used for preparing the calibration curve (Semenko and Abdullaev, 1980).

Identification of carotenoids

The thermoseparation Liquid Chromatography system consisted of a spectra system UV 2000 detector and Spectra System P2000 pump was used. The carotenoids pigments were separated on a 250×4.6 mm (i.d) column packed with Chromosil C_{18} material, 5 µm particle size and eluted with 80:10 (v/v) acetonitrile : methanol. Flow rate was 1 ml min^{-1} and detection was carried out at 438 nm. Some available standard carotenoids: β-carotene, zeaxanthin, lutein, astazenthin and cryptoxanthin (Sigma), were also run by the same HPLC method (Honya ***et al.***, 1994).

Determination of algal tocopherol

Tocopherols were determined by high performance liquid chromatography (HPLC) equipped with Spectra System UV2000 detector at 290 nm. Tocopherol were separated on a 250×4.6 mm (i.d) column packed with Vydac and eluted with 90:10 acetonitrile: methanol (v/v) at flow rate was 1 ml min^{-1} and α- tocopherol standard (Sigma) was ran under the same conditions.

Viability of Ehrlich ascites carcinoma cells (EACC)

The tumor cell line

The original tumor cells was obtained from Cell Biology Department, National Cancer Institute, Cairo University, Egypt. The tumor cells were maintained in female mice as cell line in Biochemistry Department, Faculty of Agriculture, Cairo University. The mice were injected with a 0.2 ml aliquot (for each mice) of a 10% suspension of minced tumor cell line in saline.

Viability of tumor cells

The viability percentages of tumor cells were measured by the modified cytotoxic trypan blue exclusion technique of Bennett ***et al.*** (1976).

Determination of glutathione (GSH)

The GSH content was determined in tumor cells solution (2 ml containing 4×10^6 cells). The cells incubated with and without the test extracts as well as control based on the reaction with 5,5 ` dithiobis -2- nitrobenzoic (DTNB) reagent to give a compound that absorbed at 412 nm (Silber ***et al.***, 1992). GSH was expressed as $\mu g10^{-6}$ cells.

Determination of glutathione-S- transferase activity (GST)

The activity of GST in treated and untreated tumor cells were determined according to method of Habig ***et al.***, (1974).

Determination of lactate dehydrogenase (LDH)

LDH activity was determined in tumor cells after incubation with algal extract as described by Bergmeyer (1974).

DNA fragmentation assay

After EACC treated with algal extracts for 2 h a portion of treated cells were washed three times with cold phosphate buffer (pH 8.0). Then the cells were lysed with a lysis buffer (50 mM Tris-HCl, pH 8.0, 0.2 mM EDTA, 10 mM NaCl, 2% SDS, 50 mg L^{-1} proteinase K) at 50 °C for more than 4 h and chilled in ice. Proteins were precipitated with saturated NaCl and removed by centrifugation at 15000 × g for 10 min, leaving the supernatant containing DNA fragments (Liu ***et al.***, 2000). The DNA fragment was evaluated by spectrophotometric method at 600 nm. As described by Perandones ***et al.*** (1993).

Determination of protein

Protein content of treated tumor cells was extracted by phosphate buffer (pH 8.0) and determined spectrophotometrically at 595 nm, using comassein blue g 250 as a protein binding dye (Bradford, 1976). Bovine serum albumin (BSA) was used as a protein standard.

Statistical analysis

The data are presented as mean with standard deviation (SD). All results were analyzed by one- way ANOVA and Scheffe' F-test to identify significant differences between groups. $P < 0.01$ were considered significant. All analyses were performed using Co Stat software version 4 (Abacus Concepts, Inc., Berkeley, CA, USA).

Results and Discussion

The concentration of nitrogen used in the culture medium affected in the carotenoids content of blue green alga ***Spirulina plantensis*** and ***Spirulina maxima*** at the end of logarithmic phase is shown in Table 1, 2 and Fig 1. The higher levels of carotenoids produced by both species were obtained when grown at low nitrogen level (51 ppm N), with values of 19.82 and 24.21 mg g^{-1} (d w), respectively. Whereas, the highest levels of carotenoids were occurred in cells grown in free nitrogen medium, with value of 31.13 and 30.15 mg g^{-1} (d.w), respectively. In contrary, *S. **plantensis*** and *S. **maxima*** grown in medium contained high nitrogen level (410 ppm N) produced

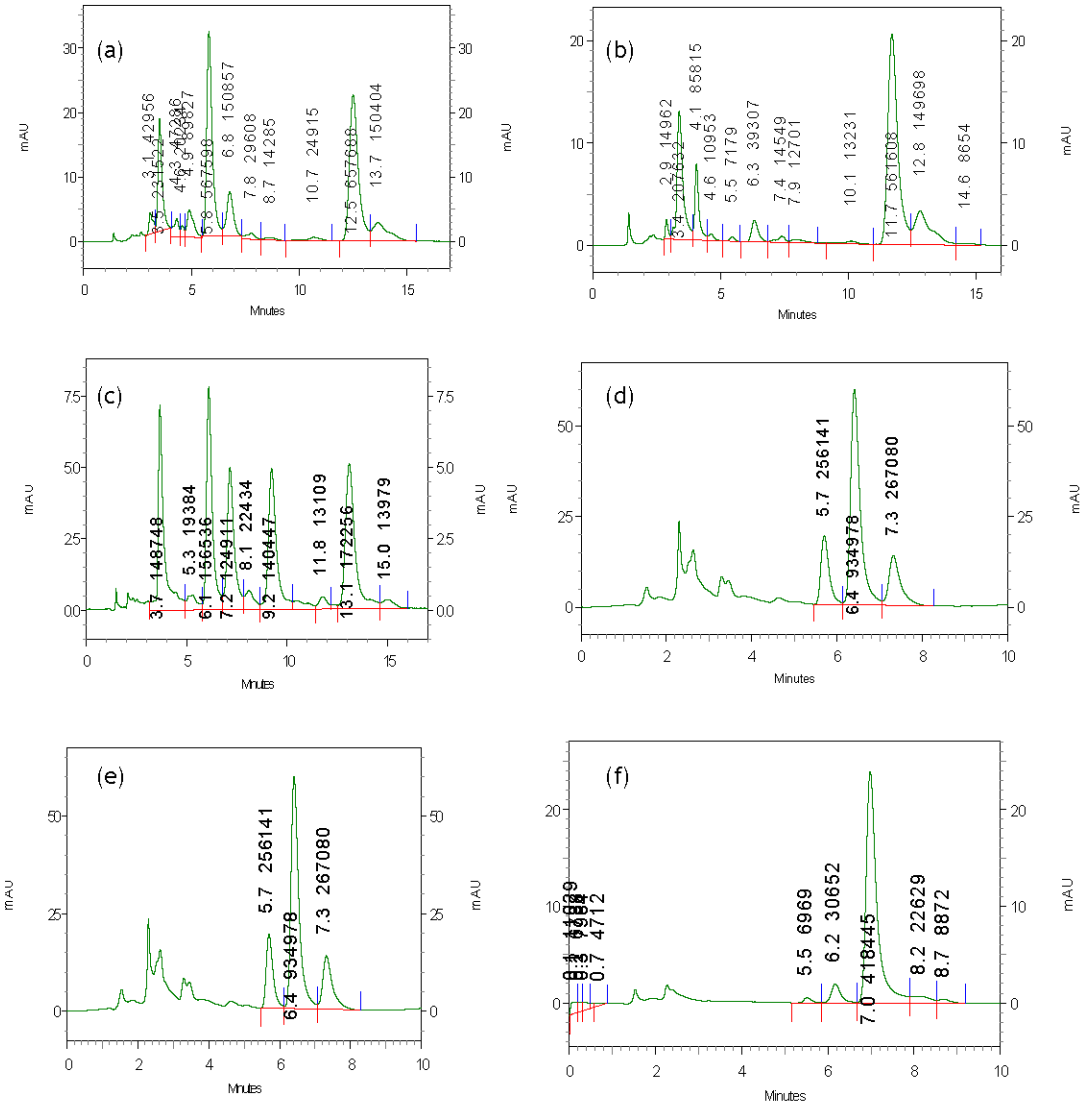


Fig. 1: HPLC profile of carotenoids and tocopherol of ***Spirulina plantensis*** and ***Spirulina maxima***
a: ***Spirulina plantensis*** (carotenoids), b: ***Spirulina maxima*** (carotenoids)
c: Standard of Carotenoids, d: ***Spirulina plantensis*** (Tocopherol)
e: ***Spirulina maxima*** (Tocopherol), f: Standard Aplha tocopherol

Compound	RT
Astazanthin	3.6-4.2
Lutein	5.5-6.2
Cryptoxanthine	8.9-9.5
β-carotene	12.0-13.5
Zeaxanthin	7.2-8.2

Table 1: Influence of nitrogen and salt stress on caroteniods in *Spirulina plantensis*

	B-carotene		Astaxanthin		Lutein	
Treatment	µg g^{-1}	Ratio of control	µg g^{-1}	Ratio of control	µg g^{-1}	Ratio of control
Extract of Sp.grown under control conditions (410 ppm N+ 0.02M NaCl)	49.61	1	6.61	1	0.06	1
Extrac of Sp.grown in medium containe 205 ppm N	70.11	1.41	8.32	1.26	0.41	6.83
Extrac of Sp.grown in medium containe 102.5 ppm N	91.77	1.85	33.57	5.1	1.07	17.83
Extrac of Sp.grown in medium containe 51 ppm N	144.18	2.91	58.23	8.81	2.1	35.0
Extrac of Sp.grown in zero N	171.63	3.46	153.75	23.26	3.58	59.67
Extrac of Sp.grown in medium containe 0.1M NaCl	301.11	6.1	33.27	5.03	1.33	22.2
Extrac of Sp.grown in medium containe 0.2 M NaCl	261.00	5.26	76.21	11.53	1.42	23.7
Extracof Sp.grown in medium containe 102.5 ppm N+0.1 M NaCl	182.05	3.67	64.1	9.7	1.71	28.5

	Zeaxanthin		Cryptoxanthin		Total carotenoids		
Treatment	µg g^{-1}	Ratio of control	µg g^{-1}	Ratio of control	mg g^{-1}	%	Ratio of control
Extract of Sp.grown under control conditions (410 ppm N+ 0.02M NaCl)	1.25	1	1.41	1	4.75	0.475	1
Extrac of Sp.grown in medium containe 205 ppm N	2.41	1.93	1.95	1.38	8.32	0.832	1.75
Extrac of Sp.grown in medium containe 102.5 ppm N	3.51	2.81	2.45	1.74	14.21	1.421	2.99
Extrac of Sp.grown in medium containe 51 ppm N	5.77	4.6	3.42	2.42	19.82	1.982	4.2
Extrac of Sp.grown in zero N	11.51	9.21	7.79	5.52	31.13	3.31	6.55
Extrac of Sp.grown in medium containe 0.1M NaCl	2.71	2.17	1.99	1.41	7.82	0.782	1.65
Extrac of Sp.grown in medium containe 0.2 M NaCl	2.98	2.38	2.53	1.79	13.2	1.32	2.78
Extracof Sp.grown in medium containe	4.57	3.66	2.71	1.92	34.84	2.98	7.33

lower amount of carotenoids, with value 4.75 and 5.31 mg g^{-1} (d.w), respectively. Therefore, the variation in carotenoids content in blue green algae occurred as a function of nitrogen levels. However, at all nitrogen levels the *S. **maxima*** contained relatively large amount of total caroteniods than in *S. **plantensis***.

Table 2: Influence of nitrogen and salt stress on caroteniods in *Spirulina maxima*

	B-carotene		Astaxanthin		Lutein	
Treatment	μg g^{-1}	Ratio of control	μg g^{-1}	Ratio of control	μg g^{-1}	Ratio of control
Extract of Sp.grown under control conditions (410 ppm N+ 0.02M NaCl)	54.72	1	13.21	1	1.23	1
Extrac of Sp.grown in medium containe 205 ppm N	80.12	1.46	15.32	1.16	2.78	2.26
Extrac of Sp.grown in medium containe 102.5 ppm N	101.2	1.85	40.21	3.04	6.32	5.14
Extrac of Sp.grown in medium containe 51 ppm N	162.15	2.96	67.81	5.13	8.31	6.76
Extrac of Sp.grown in zero N	192.5	3.52	160.27	12.13	17.21	13.99
Extrac of Sp.grown in medium containe 0.1M NaCl	250.1	4.57	40.57	3.1	2.95	2.4
Extrac of Sp.grown in medium containe 0.2 M NaCl	319.50	5.84	75.73	5.73	4.87	4.0
Extracof Sp.grown in medium containe 102.5 ppm N+0.1 M NaCl	202.7	3.7	70.85	5.36	5.35	4.35

	Zeaxanthin		Cryptoxanthin		Total carotenoids		
Treatment	μg g^{-1}	Ratio of control	μg g^{-1}	Ratio of control	mg g^{-1}	%	Ratio of control
Extract of Sp.grown under control conditions (410 ppm N+ 0.02M NaCl)	2.54	1	3.35	1	5.31	0.531	1
Extrac of Sp.grown in medium containe 205 ppm N	3.92	1.54	4.52	1.27	12.51	1.251	2.36
Extrac of Sp.grown in medium containe 102.5 ppm N	8.21	3.2	9.21	2.7	19.52	1.952	3.68
Extrac of Sp.grown in medium containe 51 ppm N	13.21	5.2	15.21	4.5	24.21	2.421	4.56
Extrac of Sp.grown in zero N	18.55	7.3	20.13	6.01	30.15	3.015	5.68
Extrac of Sp.grown in medium containe 0.1M NaCl	4.85	1.9	3.98	1.19	9.72	0.972	1.83
Extrac of Sp.grown in medium containe 0.2 M NaCl	5.48	2.16	4.52	1.35	19.52	1.952	3.68
Extracof Sp.grown in medium containe	6.21	2.44	5.33	1.6	28.98	2.898	5.46

Identification of carotenoids pigment

Figure 1 shows the HPLC elution pattern of carotenoids pigment extracted from the *S. plantensis* and *S. maxima* that had been grown in culture medium containing different concentration of nitrogen. The results revealed that at all nitrogen levels, both of algae species grown contained β-carotene (49.6 to 319.5 μg g^{-1}), lutein (0.06-17.21 μg g^{-1}), astaxanthin (6.6 to

Table 3: Influence of nitrogen and salt stress on α - Tocopherol in *Spirulina plantensis* and *Spirulina maxima*

	Spirulina plantensis α-Tocopherol		*Spirulina maxima* α-Tocopherol	
Treatment	μg Kg^{-1}	%	μg Kg^{-1}	%
Extract of Sp.grown under control conditions (410 ppm N+0.02M NaCl)	144.8	0.014	266.3	0.026
Extrac of Sp.grown in medium containe 205 ppm N	292.8	0.029	445.5	0.044
Extrac of Sp.grown in medium containe 102.5 ppm N	363.9	0.036	501.7	0.05
Extrac of Sp.grown in medium containe 51 ppm N	533.2	0.053	978.5	0.098
Extrac of Sp.grown in zero N	960.4	0.096	1325.7	0.13
Extrac of Sp.grown in medium containe 0.1M NaCl	427.3	0.043	533.5	0.053
Extrac of Sp.grown in medium containe 0.2 M NaCl	641.5	0.064	878.9	0.087
Extracof Sp.grown in medium containe 102.5 ppm N+0.1 M NaCl	1375.9	0.13	1670.7	0.167

160.3 μg g^{-1}), caryptoxanthin (1.4 to 20.1 μg g^{-1}) and zeaxanthin (1.2 to 18.5 μg g^{-1}) (Table 1 and 2). The amount of these carotenoids varies with nitrogen concentration in medium. However, both *S. plantensis* and *S. maxima* had the capacity to accumulate large amount of β-carotene by decrease nitrogen levels. Among all carotenoids, lutein was accumulated most in both species in free N medium, followed by astaxanthin.. However, the *S. maxima* was accumulated large amount of caryptoxanthin and zeaxanthin than in *S. plantensis*.

Influence of salinity stress on carotenoids

The effect of NaCl stress on quantity and quality of *S. plantensis* and *S. maxima* carotenoids are shows in Table 1 and 2. The values vary with different NaCl and nitrogen levels. At higher NaCl, the grown cells contained higher significant amount of carotenoids reaching to 3% of the cells d.w. For instance, the carotenoid content of *S. plantensis* and *S. maxima* grown at 0.1 and 0.2 (in parentheses) M NaCl were 7.82 (13.2) and 9.72 mg g^{-1} (19.52 mg g^{-1}) of the cells d.w, respectively. Whereas, these values were 34.84 and 38.98 mg g^{-1} (d.w) in cells grown under combination condition of low N level (102.5 ppm nitrogen) and 0.1M NaCl.

Table 1, 2 and Fig. 1 shown the carotenoids profile of *S. maxima* and *S. plantensis* grown under NaCl stress. The carotenoids profile was varied in both species as results to increase NaCl level in nutrient medium. At higher NaCl level, the grown cells contained higher significant amount of β-carotene than other carotenoids. The accumulated values of β-carotene in *S. plantensis* and *S. maxima* (in parentheses) grown at 0.1 and 0.2 M NaCl concentration being 6.1 (4.57) and 5.26, (5.84), respectively times as high as that at 0.02 M NaCl. In addition of β-carotene, these algae cells accumulated significant amount of astaxanthin 5.03 (3.1) and 11.53 (5.73), lutein

22.2 (2.4) and 23.7 (4.0), zeaxanthin 2.17 (1.9) and 2.38 (2.16) and carpytoxanthin 1.41 (1.19) and 1.79 (1.35), respectively times as great as that found in cells grown at 0.02 M NaCl.

Influence of nitrogen on the production of α- tocopherol

Influence of nitrogen on the production of α-tocopherol from ***S. plantensis*** and ***S. maxima*** is shown in Table 3 and Fig 1 (HPLC profile). The proportion of α-tocopherol in ***S. plantensis*** and ***S. maxima*** increased with decreasing nitrogen level in nutrient medium. The maximum values of α-tecopherol in ***S. plantensis*** and ***S. maxima*** were obtained when grown in free N medium, with values 960 and 1325 μg Kg^{-1} (d.w), respectively. Whereas, minimum values were found in cells grown in adequate nitrogen medium (410 ppm N), with values 144.8 and 266.5 μg Kg^{-1} (d.w).

Influence of salt stress on the production of α- tocopherol

Table 3 shown the influence of NaCl stress on tocopherol content. In both algae ***S. plantensis*** and ***S. maxima***, the α-tecopherol content increased with increasing NaCl concentrations. The α-tecopherol values were 641.5 and 878.9 μg Kg^{-1} and 427.3 and 532.5 μg Kg^{-1} in ***S. plantensis*** and ***S. maxima*** grown in 0.2 M and 0.1 M NaCl culture, respectively. However, these amounts decreased significantly with decreasing nitrogen concentration to 102.5 ppm N in 0.1 M NaCl medium (Table 3 and Fig. 1).

It seems that the mechanism suggested by Arad ***et al.*** (1993); Rice ***et al.*** (1994) and El-Baz ***et al.*** (2002) for the acclimation of carotenoids in ***Chlorella*** and ***Dunaliella*** to growth under stress condition also applies to ***Sprinulina***. It seems that the division of algae cells grown under N starvation are blocked, while photosynthesis continues, leading to storage specific compounds such as carotenoids, carbohydrates and triglycerides. The accumulation of these compounds was attributed to the fact that carbohydrates and carotenoids do not require nitrogen for their synthesis. Also, it has previously been suggested that the carbohydrates and carotenoids synthesizing enzymes may be less susceptible to disorganization than in the system responsible for other compounds synthesis (Fogg, 1975).

Early studies on vitamin E production by microorganism showed that most of the prokaryotes and yeast showed little or no tocopherol (Hughest and Tove, 1982). But, some microalgae such as ***Euglena gracilis*** and ***Dunaliella*** are considered a potential source for tocopherols (with vitamin E, values between 1.12 - 1.35 mg g^{-1} d.w) under condition of oxygen or nitrogen starvation and light intensity (El-Baz ***et al.***, 2002 [a]; Venkataraman ***et al.***, 1994 and Ogbonna ***et al.***, 1998). In the present study, α- tocopherol (vitamin E) content of ***Spirulina*** grown under condition of nitrogen stress, varied between 1.72- 2.0% of the dry weight and this content probably can be increased under other conditions. However, this content is higher than in conventional food traditionally considered rich in vitamin E (Fabregas and Herrero, 1990 ; El-Baz ***et al.***, 2002[a]). Finally, the metabolic pathway for accumulation of fatty material leading to catabolism by β-oxidation to produce the excess of acetyl CoA. Thus, the biosynthesis of carotenoids and tocopherol in ***Spirulina*** cells cultivated under nitrogen starvation increase, which acetyl CoA serves as a precursor for synthesis of tocopherol and carotenoids. In other words, the accumulation of

Table 4: Inhibitory effect of carotenoids and tocopherol extracts from *Spirulina plantensis* on the viability of Ehrlich Ascites carcinoma cells (EACC)

Treatments	concentration of algal extract ppm	% of viable cells	% of dead cells
Tumor cells (negative control)	0	95.5	4.5
Tumor cells + β-carotene	200	34.89	65.11
	400	10.35	89.65
Tumor cells +astazanthin	200	39.22	60.78
	400	24.72	75.28
Tumor cells + β-carotene +astazanthin	200	20.00	80.00
1 : 1	400	0.00	100
Tumor cells + α-tocopherol	200	37.22	62.78
	400	15.36	84.64
Tumor cells + β-carotene +astazanthin + a-tocopherol	200	10.31	89.69
1 : 1 : 1	400	0.00	100.0
Tumor cells + extract of Sp.grown	200	90.5	9.5
under control conditions (410 ppm N)	400	80.4	19.6
Tumor cells + extract of Sp.grown	200	74.9	25.1
in medium containe 205 ppm N	400	65.1	34.9
Tumor cells + extract of Sp.grown	200	51.6	48.4
in medium containe 102.5 ppm N	400	33.9	66.1
Tumor cells + extract of Sp.grown	200	35.7	64.3
in medium containe 51 ppm N	400	14.2	85.8
Tumor cells + extract of Sp.grown	200	22.2	77.8
in zero N	400	10.7	89.3
Tumor cells + extract of Sp.grown	200	90.9	9.1
in medium containe 0.1M NaCl	400	88.8	11.2
Tumor cells + extract of Sp.grown	200	91.6	8.4
in medium containe 0.2 M NaCl	400	87.5	12.5
Tumor cells + extract of Sp.grown	200	77.6	22.4
in medium containe	400	62.6	37.4
102.5 ppm N +0.1 M NaCl			

2 ml of cell solution containing $4x10^6$ cells

β-carotenoids and α-tocopherol in algae cells grown under nitrogen starvation may be due to stimulation of lipolysis process which produced a large amount of acetyl CoA (Abd El-Baky, 1999). However, acetyl CoA could be incorporated to phytyl pyrophosphate. It is known that tocopherol is synthesized by condensing the phytyl pyrophosphate with homogentistic acid to methyphyty-l quinones and subsequently methylation (Jsniszowska, 1987; Ogbonna ***et al.***, 1998).

The effect of algal extracts on viability of Ehrlich ascites carcinoma cells

The effect of algal extracts, β-carotene, astaxanthin, α-tocopherol and their mixture on viability of Ehrlich ascites carcinoma cells (EAAC) were examined by the trypan blue exclusion method. As shown in Table 4 and 5, treatment of cells with 200 and 400 ppm of β-carotene and astaxanthin (in parentheses) significant reduced cell viability to 34.9 (39.2) and 10.4% (24.7%), respectively. While, their mixture of β-carotene and astaxanthin (1: 1, w/w) reduced cell viability

Table 5: Inhibitory effect of carotenoids and tocopherol extracts from *Spirulina maxima* on the viability of Ehrlich Ascites carcinoma cells (EACC)

Treatments	concentration of algal extract ppm	% of viable cells	% of dead cells
Tumor cells (negative control)	0	95.5	4.5
Tumor cells + β-carotene	200	34.89	65.11
	400	10.35	89.65
Tumor cells +astazanthin	200	39.22	60.78
	400	24.72	75.28
Tumor cells + β-carotene +astazanthin	200	20.00	80.00
1 : 1	400	0.00	100
Tumor cells + α-tocopherol	200	37.22	62.78
	400	15.36	84.64
Tumor cells + β-carotene +astazanthin + α-tocopherol	200	10.31	89.69
1 : 1 : 1	400	0.00	100
Tumor cells + extract of Sp.grown	200	88.00	22.00
under control conditions (410 ppm N)	400	70.00	30.00
Tumor cells + extract of Sp.grown	200	70.21	29.79
in medium containe 205 ppm N	400	51.25	48.75
Tumor cells + extract of Sp.grown	200	40.92	59.08
in medium containe 102.5 ppm N	400	22.11	77.89
Tumor cells + extract of Sp.grown	200	30.21	69.79
in medium containe 51 ppm N	400	11.53	88.47
Tumor cells + extract of Sp.grown	200	18.33	81.67
in zero N	400	5.25	94.75
Tumor cells + extract of Sp.grown	200	89.11	10.89
in medium containe 0.1M NaCl	400	75.52	24.48
Tumor cells + extract of Sp.grown	200	85.34	14.66
in medium containe 0.2 M NaCl	400	72.13	27.87
Tumor cells + extract of Sp.grown	200	73.52	26.48
in medium containe	400	59.21	40.79
102.5 ppm N +0.1 M NaCl			

2 ml of cell solution containing $4x10^6$ cells

greater than those did by each one alone. The α-tocopherol at 200 and 400 ppm significantly reduced the cell viability, with 37.2 and 15.4 %, respectively. The mixture of β-carotene, astaxanthin and α tocopherol (1:1:1, w/w/w) at 200 and 400 ppm was significant reduced the cell viability over than those obtained by each one alone, with 10.3 and 0.0%. Thus, cell viability was dependent of carotenoids and α-tocopherol concentration as well as the carotenoid structure.

Algal extracts of S. ***plantensis*** and ***S. maxima*** significantly reduced the viability of EACC is compared with untreated cells. The algae extracts of ***S. plantensis*** and ***S. maxima*** grown in free nitrogen medium which contained relatively large amounts of carotenoids and α-tocopherol were significantly reduced the cell viability greater than those did by other algae extracts. At a concentration of 200 and 400 ppm (in parentheses), these extracts significantly reduced cell viability to 22.20 % (10.70%) and 18.33 (5.25%), respectively. The extracts obtained from ***Spirullina sp***. grown under NaCl stress condition that contained lower amount of carotenoids and

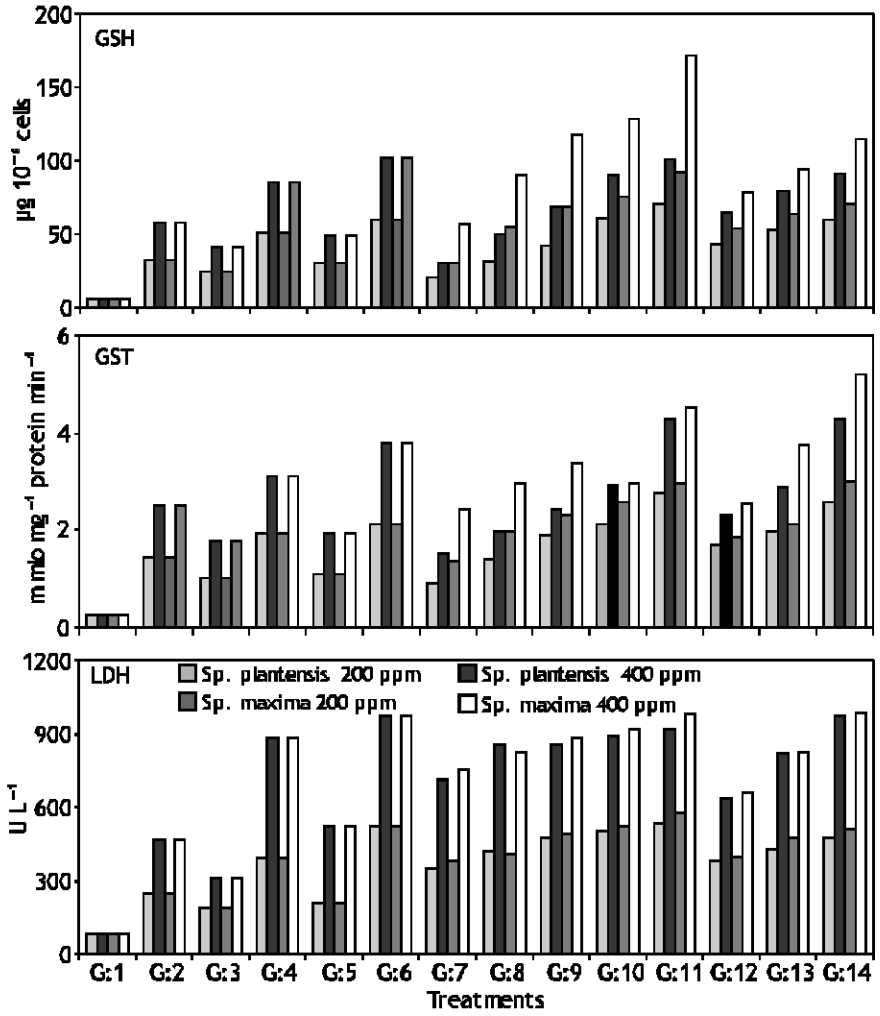
Table 6: Effect of carotenoids and trocopherol extracts from *Spirulina plantensis* and *Spirulina maxima* on DNA fragmentation

Treatments	concentration of algal extract ppm	*Spirulina plantensis* DNA fragmentation %	Spirulina maxima DNA fragmentation %
Tumor cells (negative control)	0.00	0.09	0.09
Tumor cells + cis-platinum (50 mM)	0.00	7.25	7.25
Tumor cells +β-carotene	200	0.04	0.04
	400	0.03	0.03
Tumor cells +astazanthin	200	0.04	0.04
	400	0.03	0.03
Tumor cells +β-carotene +astazanthin	200	0.02	0.02
1 : 1	400	0.00	0.00
Tumor cells + α-tocopherol	200	0.03	0.03
	400	0.02	0.02
Tumor cells+β-carotene +astazanthin + α-tocopherol	200	0.01	0.01
1 : 1 : 1	400	0.00	0.00
Tumor cells + extract of Sp.grown	200	0.45	0.31
under control conditions (410 ppm N)	400	0.28	0.17
Tumor cells + extract of Sp.grown	200	0.33	0.27
in medium containe 205 ppm N	400	0.21	0.15
Tumor cells + extract of Sp.grown	200	0.25	0.24
in medium containe 102.5 ppm N	400	0.18	0.11
Tumor cells + extract of Sp.grown	200	0.18	0.21
in medium containe 51 ppm N	400	0.15	0.09
Tumor cells + extract of Sp.grown	200	0.13	0.18
in zero N	400	0.04	0.07
Tumor cells + extract of Sp.grown	200	0.35	0.15
in medium containe 0.1M NaCl	400	0.20	0.05
Tumor cells + extract of Sp.grown	200	0.28	0.13
in medium containe 0.2 M NaCl	400	0.11	0.03
Tumor cells + extract of Sp.grown	200	0.04	0.09
in medium containe	400	0.03	0.01
102.5 ppm N +0.1 M NaCl			

α-tocopherol reduced the cell viability, but less than those alga extracts contained large amount carotenoids and α-tocopherol contents. Thus, cell viability was depended on the of carotenoids and tocopherol content in these alga extracts.

DNA fragment assay

Exposure of ***Spirullina*** extracts, β-carotene, astaxanthin and α tocopherol and those mixtures at 200 and 400 ppm to the EACC clearly did not induce any significant change in DNA fragmentation compared with untreated cell (Table 6). This indicates that carotenoids (β-carotene and astaxanthin), α-tocopherol and algae extracts may not be able to induce apoptosis in the EACC. However, treated of EACC with cis-platinum (apotosis agent) at 50 mM clearly induced the morphological changes characterized by reduction of cell volume (data not shown) and increased the DNA fragmentation to 7.25% of the control (Duthie ***et al.***,1997).



G:1 Tumor cells (negative control), G:2 Tumor cells+β-carotene, G:3 Tumor cells+astazanthin
G:4 Tumor cells+β-carotene+astazanthin(1:1), G:5 Tumor cells+α-tocopherol
G:6 Tumor cells+β-carotene+astazanthin+α-tocopherol (1:1:1)
G:7 Tumor cells+extract of Sp. grown under control conditions (410 ppm N)
G:8 Tumor cells+extract of Sp. grown in medium containe 205 ppm N
G:9 Tumor cells+extract of Sp. grown in medium containe 102.5 ppm N
G:10 Tumor cells+extract of Sp. grown in medium containe 51 ppm N
G:11 Tumor cells+extract of Sp. grown in zero N
G:12 Tumor cells+extract of Sp. grown in medium containe 0.1 M NaCl
G:13 Tumor cells+extract of Sp. grown in medium containe 0.2 M NaCl
G:14 Tumor cells+extract of Sp. grown in medium containe 102.5 ppm N+0.1 M NaCl

Fig. 2: Carotenids and tocopherol extracts from Sp. ***plantensis*** and ***Sp. maxima*** enhanced glutathine level, glutathhine S-transferase activity and lactate dehydrogenase activity of Ehrlich Ascites carcinoma cells (EACC)

Table 7: Carotenids and tocopherol extracts from *Sp. plantensis* and *Sp. maxima* enhanced glutathione level, glutathhine S- transferase activity and lactate dehydrogenase activity of Ehrlich Ascites carcinoma cells (EACC)

				Glutathhine S-transferase		lactate dehydrogenase	
Treatment	Concentration of algal extract ppm	Glutathione μg 10^{-8} cells	Ratio treatment/ control	Specific activity μ mol mg^{-1} protein min^{-1}	Ratio treatment/ control	U L^{-1}	Ratio treatment/ control
Tumor cells (negative control)	0.00	6.1±0.29	1.0	0.25±0.02	1.0	84±1.02	1.0
Tumor cells +β-carotene	200	32.21 ±0.98	5.28	1.45±0.04	5.8	250.7± 2.02	3.0
	400	58.2±0.99	9.54	2.51± 0.32	10.0	471.8 ± 2.62	5.6
Tumor cells +astazanthin	200	24.51±0.54	4.02	1.01±0.05	4.0	191.3±1.02	2.3
	400	40.91±0.89	6.71	1.79±0.08	7.16	311.2±5.02	3.7
Tumor cells + β-carotene	200	50.92±1.21	8.35	1.92±0.08	7.68	390.8±6.2	4.7
+astazanthin 1 : 1	400	85.31±1.25	13.98	3.11±0.31	12.4	882.7±4.5	10.5
Tumor cells+α-tocopherol	200	30.34±1.0	4.97	1.11±0.05	4.4	210.1±3.3	2.5
	400	49.21±1.1	8.1	1.93±0.03	7.8	521.3±4.0	6.2
Tumor cells+β-carotene	200	60.21±1.51	9.87	2.13±0.08	8.5	522.4 ± 6.0	6.2
+astazanthin+α-tocopherol	400	102.34±1.63	16.78	3.78±0.21	15.1	975.7 ± 7.0	11.6
1 : 1 : 1							
Tumor cells+extract of	200	30.33±0.5	4.97	1.35±0.05	5.4	382.7±3.3	4.6
Sp.grown under control	400	56.54±0.94	9.27	2.43 ± 0.24	9.7	751.7±5.02	9.0
conditions (410 ppm N)							
Tumor cells+extract of	200	55.22±1.0	9.1	1.98 ± 0.07	7.9	410.8± 3.4	4.9
Sp.grown in medium	400	90.24±1.21	14.79	2.95 ± 0.05	11.8	827.3±4.3	9.9
containe205 ppm N							
Tumor cells + extract of	200	68.95±1.71	11.3	2.32±0.08	9.3	491.3±3.5	5.8
Sp.grown in medium	400	117.6±2.0	19.27	3.39±0.23	13.6	882.7±4.3	10.5
containe 102.5 ppm N							
Tumor cells + extract of	200	75.98±1.91	12.45	2.57±0.11	10.3	522.8±3.7	6.2
Sp.grown in medium	400	128.69±2.41	21.1	2.98±0.21	11.9	921.5±5.02	10.9
containe 51 ppm N							
Tumor cells + extract of	200	92.33±1.56	15.14	2.97±0.08	11.9	578.9±4.9	6.9
Sp.grown in zero N	400	171.34±2.97	28.1	4.5±0.21	18.0	982.5±4.3	11.7
Tumor cells + extract of	200	54.33±1.1	8.91	1.85±0.05	7.4	395.1±4.7	4.7
Sp.grown in medium	400	78.77±1.54	12.9	2.53±0.31	10.1	659.9±3.3	7.9
containe 0.1M NaCl							
Tumor cells+extract of	200	63.94±1.97	10.5	2.11±0.21	8.4	477.5±6.3	5.7
Sp.grown in medium	400	93.64±2.0	15.35	3.77±0.42	15.1	823.7±5.02	9.8
containe 0.2 M NaCl							
Tumor cells + extract of	200	70.34±1.8	11.53	3.01±0.08	12.0	510.5±7.3	6.1
Sp.grown in medium	400	114.84±2.98	18.83	5.21±0.19	20.8	985.3±5.02	11.7
containe 102.5 ppm N +0.1 M NaCl							

± S.D, 2 ml of cell solution containing $4x10^6$ cells, All values are significant at (P< 0.5)

Enzymes induction

To further examine the role of carotenoids, α-tocopherol and algae extracts on the reduction of EACC viability, the intercellular enzyme activity of lactate dehydrogenase (LDH) and glutathine S-transferase (GST) was assayed. Cells treated with algae extracts significantly

enhanced enzyme activities when compared with untreated cells. In general, EAC treated cells with *S. plantensis* and *S. maxima* extracts at 200 and 400 ppm had increased LDH, GST activities (Table 7, 8 and Fig. 2).

The LDH and GST activities in cells incubated with algal extracts obtained from *S. plantensis* and *S. maxima* (in parentheses) grown in free N medium (the most algae extract rich in carotenoids) were markedly increased and dose dependently, in rang 200 and 400 ppm, with values 535.8 (578.9) and 918.4 U L^{-1} (982.5 U L^{-1}), respectively. Compared with that of β-carotene, α-tocopherol, astaxanthin and their mixtures, all algae extracts increased the level of LDH and GST of treated EACC. Treated of EAC cells with β-carotene, astaxanthin, α-tocopherol and their mixture increased at 200 and 400 ppm (in parentheses) significantly ($P<0.05$) increased LDH level, with 3.0 (5.6), 2.3 (3.7), 2.5(6.2) and 6.2 (11.6), respectively time over than that in untreated cells. Also, the GST level was increased, with 5.8 (10.0), 4.0 (7.16), 7.68 (12.4) and 8.5 (15.1) time as high as that in increased.

In general, EAC treated cells with *S. plantensis* and *S. maxima* extracts at 200 and 400 ppm had increased LDH and GST activities. Earlier studies have demonstrated that carotenoid treatment of human oral carcinoma cells increased some antioxidant enzyme activity (Palozza ***et al.***, 2000). Also, Abd El-Baky, (2003) reported that algal extracts rich in phycocayanin, β-carotene and α-tocopherol may modulates the activities of GST and GSH in EACC line. In animal studies that examined the chemopreventive effect of algae ***Dunalliell*** and ***Chlorella*** on chemically induced carcinogensis such as Benzo-***a***-pyrene (B-***a***-P) and Dimethyl antherathine (DAMA), the antioxidant enzyme levels correlated with reduced incidences of tumor formation (Shklar and Schwartz, 1988). Furthermore, we have previously suggested that the increased in GST, SOD, LDH and GSH levels contribute to the anticarcinogesis effect of algal extracts in Benzo-***a***-pyrene-induced carcinogesis in rats (Abd El-Baky ***et al.***, 2002).

GSH level

The influence of algal extracts and β-carotene, α-tocopherol, astazanthin at 200 and 400 ppm on GSH level in EACC as shown in Table 7, 8 and Fig. 2. Algal extracts significant increased GSH level compared with untreated cells. The GSH level of untreated cells was 6.1μg10^{-6} cells. Whilst, treated of EACC cells with S. *plantensis* and *S. maxima* (in parentheses) extracts (obtained from free N culture) at 200 and 400 ppm increased the GSH level to 70.32 (92.33) and 100.73 (171.34) μg 10^{-6} and β-caratene and α-tocopherol, respectively. However, all algae extracts, β-carotene and α-tocopherol increased GSH concentration in treated cells compared with untreated cells.

Recently, Liu ***et al.***, (2000) and Abd El-Baky, (2003) reported that algae extract of S. *plantensis* and *S. maxima* may be able to inhibit the growth of Leukemia-blast crisk 562 and EACC by pathway other than apoptosis. Moreover, Gabai, ***et al.***, (1995), observed that EACC has low susceptibility to apoptosis due to the propensity of these cells to accumulate those heat shock proteins on exposure to apoptosis inducing agents. It is also possible that, the majority of apoplotic cell are not phagocytzed but enter secondary necrosis (Vahrmeijer ***et al.***, 1999). In the present study, carotenoids and α- tocopherol act only as antioxidant but not as prooxidant. The prooxidant action of carotenoids has been suggested to induce apoptosis in tumor cells

Table 8: Carotenids and tocopherol extracts from *Sp. plantensis* enhanced glutathine level, glutathhine S-transferase activity and lactate dehydrogenase activity of Ehrlich Ascites carcinoma cells (EACC)

				GlutathhineS-transferase		lactatedehydrogenase	
Treatment	Concent-ration of algal extract ppm	Glutathione μg 10^{-8} cells	Ratio treatment/ control	Specific activity μ mol mg^{-1} protein min^{-1}	Ratio treatment/ control	U L^{-1}	Ratio treat-ment/ control
Tumor cells (negative control)	0.00	6.1±0.29	1.0	0.25±0.02	1.0	84±1.02	1.0
Tumor cells +β-carotene	200	32.21±0.98	5.28	1.45±0.04	5.8	250.7±2.02	3.0
	400	58.2±0.99	9.54	2.51±0.32	10.0	471.8±2.62	5.6
Tumor cells +astazanthin	200	24.51±0.54	4.02	1.01±0.05	4.0	191.3±1.02	2.3
	400	40.91 ±0.89	6.71	1.79 ± 0.08	7.16	311.2 ± 5.02	3.7
Tumor cells + β-carotene	200	50.92±1.21	8.35	1.92±0.08	7.68	390.8±6.2	4.7
+astazanthin 1 : 1	400	85.31±1.25	13.98	3.11±0.31	12.4	882.7±4.5	10.5
Tumor cells+α-tocopherol	200	30.34±1.0	4.97	1.11±0.08	4.4	210.1±3.3	2.5
	400	49.21±1.1	8.1	1.93 ± 0.03	7.8	521.3 ± 4.0	6.2
Tumor cells+β-carotene	200	60.21±1.51	9.87	2.13±0.08	8.5	522.4 ± 6.0	6.2
+astazanthin+α-tocopherol	400	102.34±1.63	16.78	3.78±0.21	15.1	975.7 ± 7.0	11.6
1 : 1 : 1							
Tumor cells+extract of	200	20.11±1.61	6.3	0.921±0.01	3.7	349.5±3.1	4.2
Sp.grown under control	400	30.33±2.51	13.2	1.53 ± 0.05	6.1	712.7±5.1	8.5
conditions (410 ppm N)							
Tumor cells+extract of	200	31.21±1.51	11.1	1.42 ± 0.08	5.7	421.3±2.5	5.0
Sp.grown in medium	400	50.31±3.51	18.1	1.98±0.05	8.0	852.7± 4.0	10.2
contain 205 ppm N							
Tumor cells + extract of	200	42.42±2.81	19.7	1.89±0.23	7.6	473.8±6.1	5.6
Sp.grown in medium	400	69.11±2.51	31.2	2.44±0.08	9.8	855.5±7.1	10.2
contain 102.5 ppm N							
Tumor cells + extract of	200	60.71±2.41	23.0	2.13±0.21	8.52	505.3±8.1	6.0
Sp.grown in medium	400	90.11±4.51	34.4	2.92±0.31	11.7	891.7±3.6	10.6
contain 51 ppm N							
Tumor cells + extract of	200	70.32±3.71	23.1	2.78±0.05	11.1	535.8±4.1	6.4
Sp.grown in zero N	400	100.73±2.51	39.5	4.31±0.21	17.2	918.4±8.4	10.9
Tumor cells + extract of	200	42.7±1.71	15.2	1.71 ± 0.05	6.8	379.5 ± 5.6	4.5
Sp.grown in medium	400	65.1 ±3.21	23.8	2.33 ± 0.08	9.3	638.4 ± 9.02	7.6
contain 0.1M NaCl							
Tumor cells+extract of	200	53.17±2.31	21.3	1.97±0.11	7.9	427.3± 5.2	5.1
Sp.grown in medium	400	79.75±1.51	31.3	2.89±0.31	11.7	817.2±5.02	9.7
containe 0.2 M NaCl							
Tumor cells + extract of	200	60.11±1.51	34.5	2.58±0.13	10.3	473.5±4.3	5.6
Sp.grown in medium	400	90.7±1.51	47.7	4.28±0.31	17.1	971.7±3.6	11.6
containe 102.5 ppm N +0.1 M NaCl							

± S.D, 2 ml of cell solution containing $4x10^6$ cells, All values are significant at (P< 0.5)
Values represents are mean of three replicates

(Palozza ***et al.***, 2001). People in East Asian countries, in which the mortality rate from cancer is low (Hsing ***et al.***, 2000) ingest astazanthin, fucoxanthin and nexzanthin through edible algae such as ***Undaria pinnatilida.*** Thus, consumption of algae might reduce the risk of cancer (Kotake-Nara ***et al.***, 2001). Schwartz and Shklar, (1987) has reported that extracts of ***Sp. plantensis*** (rich in β-

carotene and other caroteniods) induced tumor regression and stimulated a large number of tumor necrosis factor alpha-positive (TNF-α) macrophages at the tumor site which may be a mechanism for the tumor degeneration and regression.

Finally, this study revealed that the blue-green algae ***Spirulina sp.*** offer a wide scope for accumulation of large quantities and qualitative natural carotenoids and tocopherols. ***Spirulina*** extracts was found to reduce EAC cell viability through other mechanism rather than apoptosis induction. Thus, these results suggest those ingestion ***Spirulina*** rich in β-carotene, astaxanthin and other carotenoids and α-tocopherol might have the chemopreventive potential. Therefore, ***Spirulina*** could be easily incorporated into daily dietary practices since they are relatively non-toxic.

References

Abd El-Baky, H.H., 1995. Chemical and biochemical studies on carotenoids of some green algae. Thesis, M.Sc., Faculty of Agriculture, Biochemistry Dept., Cairo University, Egypt.

Abd El-Baky, H.H., A. Moawd, A.N. El-Behairy. and G.S. El-Baroty, 2002. Chemoprevention of benzo[a]pyrene-induced carcinogen and lipid peroxidation in mice by lipophilic algae extracts (phycotene). J. Med. Sci., 2: 185-193.

Abd El-Baky, H.H., 2003. Over production of phycocayanin pigment in blue green alga ***Spirulina Sp.*** and it's inhibitory effect on growth of Ehrlich ascites carcinoma cells. J. Med. Sci., 3: 314-324.

Aboul-Enein, M.A., F.K. El-Baz, G.S. El-Baroty, A.M. Yousse and H.H. Abd El-Baky, 2003. Antioxidant activity of algal extracts on lipid peroxidation. J. Med. Sci., 3: 87-98.

Arad S., E.Cohen and A. Ben Amotz, 1993. Accumulation of canthaxanthin in ***Chorella emersonii.***Physiol. Plantarum, 87: 232- 236.

Bar E., M. Rise, M. Vishkautsan and A. Shoshana, 1995. Pigment and structural changes in ***Chlorella zofingiensis*** upon light and nitrogen stress. J. Plant physiol., 146: 527 -534.

Bennett, J.M., D. Catovsky, M.T. Danniel, D.A. Galton and C. Sultan, 1976. Proposal for the classification of the acute leukaemias. Br. Haem., 33: 451-458.

Bergmeyer, H.U., 1974. Methods of enzymatic analysis. 2th Ed. Academic Press Inc. pp: 1081-1084.

Borowitzka, M.A., 1992. Algal biotechnology products and processes matching science and economics. J. Appl. Phycol., 4: 267-279.

Borowitzka A.M. and J.L. Borowitzka, 1986. ***Dunaliella*** In Micro-algal biotech. (Ed). Borowitzka A.M. and Borowitzka J.L., Cambridge, New York, pp: 28-58.

Bradford M.M., 1976. A rapid and sensitive method for the quantitation of microgram of protein utilizing of protein -dye binding. Anal. Biochem., 72: 248-258.

Burczyk, J., 1987. Biogenetic relationships between ketocarotenoids and sporopollenins in green alga. Phytochem. 26: 113-119.

Duthie, S.J., W. Johnson and V.L. Dobson, 1997. The effect of dietray flavonoids on DNA damage: (strand breaks and oxidized pyrimdeins and growth in human cells. Mut. Res., 390: 141-151.

El- Baz, F.K., M.A.Aboul -Enein, G.S. El-Baroty, A.M. Youssef and Abd El- H.H, Baky, 2002. Accumulation of antioxidant vitamins in ***Dunaliella salina***. Online J. Biolog. Sci., 2: 220-223.

El-Baz, F.K., M.A. Aboul-Enein, G.S. El-Baroty and H.H. Abd El-Baky, 2002. Anticarcinogenic activity of algal extracts. J. Med. Sci., 2: 243-251.

Fabregas J. and C. Herrero, 1990. Vitamin content of four marine microalgae: Potential use as source of vitamin in nutrition. J.Indust.Microbiol., 5: 259-264.

Farag, R.S., K.H. Elkhwas and M. Mohamed, 1998. Distribution of carotenoids in some fresh and boiled foods. Ads. Food Sci., 20: 1-6.

Fogg, G.E., 1975. Metabolic pattern and growth in: alga cultures and phytoplankton ecology. 2nd Ed., Univ. Wisconsin Press, pp: 52 - 61.

Gabai, L., I.V. Zamulaeva, A.F. Mosin, Y.M. Makarova, K.R. Budagova and A.E. Kabakov, 1995. Resistance of Ehrlich tumor cells to apoptosis can be due to accumulation of heat shock proteins. FEBS Lett., 575: 21-26.

Giovannucci, E., 1999. Tomatoes, tomato-based products lycopene and cancer: Review of the epidemiological literature. J. Nat. Cancer Inst. 91: 317-310.

Habig W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathion S- Transferase.J Biol. Chem., 249: 7130-7139.

Honya, M.K., T. Kinoshita, M. Ishikawa, H. Mori and K. Nisizw, 1994. Seasonal variation in lpipd content of cultured Laminaria japonica fatty acids,srerols,β-carotene and tocopherol.J. Appl. Phycol., 6: 25-29.

Hsing, A.W., L. Tsao and S.S. Devesa, 2000. International trends and patterns of prostate cancer incidence and mortality. Int. J. Cancer Predict. Oncol., 85: 60-67.

Hughest,P.E. and S.B. Tove, 1982.Occurrence of α-tocopherol quinona and α-tocopherol quinol in microogranisms.J.Bacteriol., 151: 1397-1402.

Jsniszowska, W., 1987. Intracellular location of tocopherol biosynthesis in ***Calendula officinalis***. Phytochem., 26: 1403-1407.

Liu, Y., X. Lizhi, N. Cheng, L. Lin and C. Zhang, 2000. Inhibitory effect of phycocyanin from ***Spirulina platensis*** on the growth of human leukemia K562 cells. Appl. Phycol., 12: 125-130.

Kotake-Nara, E., K. Masayo, H.T. Zhang, Sugawara and A. Nagao, 2001.Carotenoids affect proliferation of human prostate cancer cells. J. Nutr., 131: 3303-3306.

Mathew, B., R. Sankaranaryanan and P. Padmanabhan, 1995. Evaluation of chemoperevention of oral cancer with ***Spirulina fusiformis***. Nutr. Cancer, 24: 197-202.

Morimoto, T., A. Nagatu and N. Murakami, 1995. Anti -tumor -promoting glyceroglycolipids from the green alga ***Chlorella vulgaris***. Phytochem., 5: 1433-1437.

Ogbonna, J., T. Shota and H. Tanaka, 1998. Heterotrophic cultivation of ***Euglena gracilis Z*** for efficient production of α-tocopherol. J. Appl. Phycol., 10: 67-74.

Palozza, P., G. Calviello, M. Emilia, S. Serini and G.M. Bartoli, 2000.Canthaxathin supplementation alters antioxidant enzymes and iron concentration in liver of Balb/c mice. J. Nutr., 130: 1303-1308.

Palozza, P., G. Calviello, S. Serini, N. Maggiano, P. Lanza, F. Ranelletti and G.M. Bartoli, 2001. β-carotene at high concentrations induces apoptosis by enhancing oxy-radical production in human adenocarcinoma cells. Free Radic. Biol. Med., 30: 1000-1007.

Perandones, C.E., V.A. Liiera, D. Peckham, L.L. Stunz and R.F. Ashman, 1993. Regulation of apoptosis in vitro in mature spleen T-cell. Immunol., 151: 3521-3528.

Semenenko, E.V. and A.A. Abdullaev, 1980. Parametric control of β-carotene biosynthesis in ***Dunaliella salina*** cells under conditions of intensive cultivation. Fizioloiya Rastenii., 27: 31-41.

Seshadri, C.V. and N. Jeeji-Bai, 1992. ***Spirulina*** Madras, India:Murngappa chettiar Research center.(Ecology,Taxonomy, Technology and applications Natl. symp).

Schwartz, J. and G. Shklar, 1987. Regression of experimental hamster cancer by β-carotene and algae extracts. J. Oral maxilloofac surg., 45: 510-515.

Schwartz, J., G. Shklar, Reid and D. Trickler, 1988. Prevention of experimental oral cancer by extracts of ***Spirulina-Dunaliella*** algae. Nutr. Cancer, 11: 127-134.

Rice, M., E, Cohen, M Vishkautsan, H. E Cojocau, E. Gotrlieb and S. Arad, 1994.Accumulation of secondary carotenoids in ***Chlorella zofingiensis***. J Plant physiol., 144 : 287- 292.

Silber, R., M. Farber, E. Papopoulos, D. Nervla, L. Liebes, M. Bruch and R. Bron, 1992. Glutathione depletion in chronic lymphocytic leukemia B-lymphocytes. Blood, 80: 2038-2040.

Vahrmeijer, A.L., J.H. Van-Dierendonck, J. Schrutrups, J.H. Cornelis and G.J. Mulder, 1999. Effect of glutathione depletion on inhibition of cell cycle progression and induction of apoptosis by Melphalan (L-phenylalanine mustard) in human colorectal cancer cells. Biochem. Pharm., 58: 655-664.

Venkataraman, L.V., T. Somasekaran and E.W. Becker, 1994. Replacement value of blue -green algae (***Spirulina plateensis***) for fish meal and a vitamin,mineral premix for broiler chicks. Br. poultry Sci., 35: 373-381.

Vonshak, A., (Ed) 1997. ***Spirulina platensis*** (Arthrospira). Taylor and Francis U.K.

Zarrouk, C., 1966. Contribution a l'etude d; une cyanophycee. Influene de divers facteurs physiques et chimiques sur la croissance et la photosynthese de ***Spirulina maxima*** (Setch. et Gardner) Geitler. Ph.D. thesis, University of Paris, France.