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Over Production of Phycocyanin Pigment in Blue Green Alga *Spirulina* sp. and It's Inhibitory Effect on Growth of Ehrlich Ascites Carcinoma Cells

Hanaa H. Abd El-Baky

Two species of blue green algae *Spirulina platensis* and *Spirulina maxima* were grown in nutrient medium containing different nitrogen and salt levels. In both species increasing nitrogen levels led to increase in phycocyanin pigments from 12.08 to 22.3% and soluble protein content from 29.7 to 86.1 mg g^{-1}. Also, *Spirulina* has great variety in composition of phycocyanin pigments ranging from C-phycocyanin (C-PC) from 1.65 to 4.02%, allophycocyanin (A-PC) from 2.53 to 6.11% and R-phycocyanin (R-PC) from 5.75 to 12.35% as a results, of changing nitrogen contents and salt stress. *Spirulina platensis* at high nitrogen level gave highest percentage of total phycocyanin 9.94% and R-CP 5.75% was the predominate among phycocyanin pigments. The increasing in NaCl levels in nutrient medium led to production significant in phycocyanin contents and soluble protein in *Spirulina platensis* and *Spirulina maxima* cells. The composition of phycocyanin pigment was changed markedly as results of increasing in NaCl level. Both algal species grown under combined stress (nitrogen deficient and high NaCl level) produced higher amount of phycocyanin than control. The anti-carcinoma activity of *Spirulina* towered Ehrlich Ascites Carcinoma Cells (EACC) was evaluated by cell viability, DNA fragmentation and enzymes assay. Phycocyanin significantly inhibited the growth of EACC in a dose-dependent manner. Phycocyanin did not induce DNA fragmentation in EACC, (no ladder of DNA fragments). However, glutathione (GST), the activity of glutathione S- transferase (GST) and lactate dehydrogenase (LDH) were significantly increased over the control level. These findings indicate that phycocyanin may be able to inhibit the growth of EACC by membrane destructor, which led to increase the leakage of cell constituent and increase LDH and GST enzyme activities. Therefore, algal phycocyanin may have antitumor activity and could be used as a chemoprventive agent.

Key words: *Spirulina*, phycocyanin, antitumor, viability and blue green algae

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Introduction

Spirulina is one of the most promising microalgae, which be utilized for the production of cyanocobalamine (B12), antioxidant pigment like β-carotene, tocopherols and γ-linolenic acid, and can be used as raw material for single cell protein (SCP) (Belay ***et al.***, 1996 and Ortega-Calvo ***et al.***, 1993). Several fine compounds such as essential fatty acids like γ-linolenic acid (GLA), essential amino acids, antioxidant vitamins like tocopherols , minerals and proteins (Richmond, 1980 and Roughan, 1989), are found in ***Spirulina*** species at relatively high concentration and command a high market value (Santillan, 1982 and Cohen, 1995). The deep blue color of phycocyanin and other extractable pigment including myxoxanthophyl and zeaxanthin extracted from microalga ***Spirulina*** has been widely used as a naturally occurring colorant for food additive purposes (Hirata ***et al.***, 2000 and Kato, 1994). Phycocyanin had anticancer, antioxidant, antiviral and anti-inflammatory activities (Romay ***et al.***, 1998; Gonzalez ***et al.***, 1999; Hirata ***et al.***, 2000 and Mathew ***et al.***,1995). Also, phycocyanin is a powerful tonic agent for the immune system in animals and human, which providing protection from variety of diseases (Liu ***et al.***, 2000).

Great variability in the chemical gross composition of ***Spirulina*** species have been shown as a result of several factors such as genotype, the stage in growth cycle, the source and concentration of nitrogen be used in the culture medium (Ruengjitchatchawalya ***et al.***, 2002). These environmental variability can used for producing cells with biochemical contents that can be previously determined as a function of nitrogen source and concentration.

The phycocyanin content in ***Spirulina*** can be also effected by the source and concentration of nitrogen in the culture medium. This pigment may serve as a nitrogen storage material since the phycocyanin content is highest when ***Spirulina*** is cultivated under favorable nitrogen level (Richmond, 1980). In the present work the effect of nitrogen and NaCl on accumulation of Phycocyanin in ***Spirulina maxima*** and ***Spirulina plantensis*** were studied. Also, the antitumor activity of these compounds was evaluated.

Materials and Methods

Algae source

In the present work the effect of nitrogen and NaCl on accumulation of Phycocyanin in ***Spirulina maxima*** and ***Spirulina plantensis*** were obtained from the culture collection of Texas University, Austin, USA.

Growth conditions

Algae was cultivated (in National Research Center during 2003) in Zarrouk's medium (Zarrouk, 1966). $NaNO_3$ was used as a nitrogen source with 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 and medium contain 102.5 ppm N and 0.1 M NaCl. Algae was cultivated in 2L flasks. The cultures were gassed with 0.03% CO_2 in air and algae were cultivated at 25ºC±3, pH 10.5. 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

Stationary-phase cells were harvested at 4°C by centrifugation at 6000 rpm for 10 min.

Extraction and determination of phycocyanin

The concentration of blue phycocyanin pigment including: allophycocyanin (APC), phycocyanin (PC) and R-phycocyanin (R-PC) were determined spectrophotometrically at 650 and 618 nm; 618 and 650 nm and 498, 615 and 650 nm respectively as reported by Kursar and Alberte (1983).

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 aliquot 0.2 ml (for each mice) of a 10% suspension of minced tumor cell line 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) incubated with and without the test extracts as well as control. The reaction is based on the reaction with 5, 5` dithiobis -2- nitrobenzoic (DTNB) reagent to give a compound that absorbed a light at 412 nm (Silber ***et al.***, 1992). GSH was expressed as μg 10^{-6} cells.

Determination of glutathione-S- transferase activity (GST)

The activity of GST in 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) using biosystems kit.

DNA fragmentation assay

After EACC treatment with algal extracts for 2 h, a portion of treated cells were washed three times with cold phosphate buffer-saline (pH 7.8) and then, they 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) at 50°C for more than 4 h and then chilled in ice. Proteins were precipitated by saturated NaCl and removed by centrifugation at 1500 g, for 10 min, the supernatant contained DNA fragments (Liu ***et al.***, 2000). Then the DNA fragment was evaluated spectrophotometrically 200 μl of supernatant were transferred to test tube containing a 200 µl diphenyl amine (0.088 M DPA, 98 v/v glacial acetic acid, 1.5% v/v sulfuric acid and 0.5% acetyladehyde) then kept at 4°C for 48 h. The developed bluish color was recorded at 600 nm (Perandones ***et al.***, 1993).

Data represent the means±SD. Results were analyzed by one-way ANOVA and Scheffe' F-test to identify significant differences between groups. P-values < 0.01 were considered significant. All analyses performed using Co Stat software version 4 (Abacus Concepts, Berkeley, CA).

Determination of protein

Protein content of treated tumor cells was extracted by phosphate buffer 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. Data represent the means±SD. Results were analyzed by one-way ANOVA and Scheffe' F-test to identify significant differences between groups. P-values <0.01 were considered significant. All analyses performed using Co Stat software version 4 (Abacus Concepts, Berkeley, CA).

Results and Discussion

In Table 1 and 2 the two of blue green alga ***Spirulina*** species are compared in phycocyanin production at different growth conditions. Decreasing the nitrogen concentration in the nutrient medium led to a decrease in the phycocyanin content (total phycocyanin). The most significant decrease was observed when ***Spirulina*** algae was grown in free nitrogen medium (0.0%N). Under this conditions total phycocyanin content in ***S. plantensis*** and ***S. maxima*** was 3.31 and 1.7% (D.W), respectively and with increasing nitrogen concentration these quantities were increased slowly and reached to high values 12.08 and 9.94%, respectively and with increasing nitrogen concentration 410 ppm N. as $NaNO_3$, However, at comparable nitrogen levels, the ***S. plantensis*** algae generally produced higher amount of phycocyanin than in the ***S. maxima*** (12.89-9.94%). Phycocyanin: composed of C-PC, APC and RPC were determined by spectrophotometric method. Both ***Spirulina*** species have a great variety of phycocyanin pigments ranging from C-PC, A-PC and R-PC. The percentages of these pigments changed markedly by nitrogen concentrations variation. By decreasing of nitrogen concentration, ***Spirulina*** species mainly produced R-PC and lower amount of C-PC. At nitrogen levels were (51 and 410 ppm), the % of C-PC, A-PC and R-PC in ***S. plantensis*** were 0.71 (2.7), 1.78 (3.57) and 2.22% (6.80%), respectively. While, in ***S. maxima*** were 0.53(1.65), 0.83 (2.53) and 1.53% (5.75), respectively at the same nitrogen level.

Effect of NaCl stress

In Table 1 and 2 the ***S. plantensis*** and ***S. maxima*** are compared in production of phycocyanin became pigments when grown in medium containing 5 at (0.1 M NaCl) and 10 at 0.2 M NaCl fold level became of NaCl over than the optimum NaCl level (0.02 M NaCl). Increasing NaCl in nutrient medium led to produced significant amount of phycocyanin content when compared to the control. The amount of total phycocyanin in ***S. plantensis*** grown under NaCl stress (0.1 and 0.2 ppm) were 1.38(22.3) and 1.85(16.63%), respectively times over the control (12.08%). Whereas, these levels were 1.46 (14.47) and 1.89(18.87), respectively of the control (9.94%), in ***S. maxima*** algae. However, composed phycocyanin pigment have great variety in both species and the amount of each pigment was changed markedly as a results of increased NaCl concentration.

S. plantensis and ***S. maxima*** grown under combined stress of nitrogen deficient (102 ppm N) and high NaCl concentration (0.1 MnaCl), produced lower amount of phycocyanin content than that grown in medium containing enough nitrogen and high NaCl level concentration.

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

Treatment Nitrogen starvation	Kind of phycocyanin pigment % CPC%	APC%	R-PC%	Total Phycocyanin %	Ratio treatment/ control	soluble protein mg g^{-1}	Ratio treatment/ control
Extract of Sp.grown under control conditions (410 ppm N + 0.02M NaCl)	2.705	3.577	6.8	12.08	1.0	29.7	1.0
Extract of Sp.grown in medium contain 205 ppm N	1.83	2.53	4.84	9.2	0.761	27.7	0.933
Extract of Sp.grown in medium contain 102.5 ppm N	1.04	1.909	2.59	5.54	0.459	20.00	0.673
Extract of Sp.grown in medium contain 51 ppm N	0.705	1.788	2.22	4.71	0.389	15.3	0.515
Extract of Sp.grown in zero N Salt stress	0.351	1.23	1.73	3.31	0.274	10.1	0.340
Extract of Sp.grown in medium contain 0.1M NaCl	2.81	4.61	9.21	16.63	1.376	45.7	1.54
Extract of Sp.grown in medium contain 0.2 M NaCl	4.02	5.93	12.35	22.3	1.84	86.1	2.89
Extract of Sp.grown in medium contain 102.5 ppm N +0.1 M NaCl	2.85	2.63	8.52	14.0	1.2	35.1	1.18

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

Treatment Nitrogen starvation	Kind of phycocyanin pigment % CPC%	APC%	R-PC%	Total Phycocyanin %	Ratio treatment/ control	soluble protein mg g^{-1}	Ratio treatment/ control
Extract of Sp.grown under control conditions (410 ppm N + 0.02M NaCl)	1.659	2.53	5.75	9.94	1.0	22.1	1.0
Extract of Sp.grown in medium contain 205 ppm N	1.321	1.95	4.21	7.48	0.752	18.5	0.837
Extract of Sp.grown in medium contain 102.5 ppm N	0.907	1.07	2.91	4.89	0.492	14.3	0.647
Extract of Sp.grown in medium contain 51 ppm N	0.531	0.833	1.53	2.89	0.291	9.21	0.417
Extract of Sp.grown in zero N Salt stress	0.225	0.561	0.921	1.707	1.18	4.51	0.204
Extract of Sp.grown in medium contain 0.1M NaCl	2.41	4.55	6.51	14.47	1.46	38.9	1.76
Extract of Sp.grown in medium contain 0.2 M NaCl	3.05	6.11	9.72	18.88	1.89	57.8	2.62
Extract of Sp.grown in medium contain 102.5 ppm N +0.1 M NaCl	2.87	3.75	5.31	11.9	1.20	24.1	1.09

Values represents are mean of three replicates and based on dry weight, All values are significant at ($P<0.5$)

Table 3: Inhibitory effect of phycocyanin extract from *Spirulina plantensis* on the viability of Ehrlich Ascites Carcinoma Cells (EACC)

Treatment	concentration of algal extract ppm	% of viable cells	% of dead cells
Tumor cells (negative control)	0	95.5	4.5
Tumor cells + extract of Sp.grown	200	64.2	35.8
under control conditions (410 ppm N)	400	35.8	64.2
Tumor cells + extract of Sp.grown	200	71.2	28.8
in medium contain 205 ppm N	400	43.5	56.5
Tumor cells + extract of Sp.grown	200	86.3	13.7
in medium contain 102.5 ppm N	400	55.5	44.5
Tumor cells + extract of Sp.grown	200	90.3	9.7
in medium contain 51 ppm N	400	65.4	34.6
Tumor cells + extract of Sp.grown	200	94.2	5.8
in zero N	400	74.9	25.1
Tumor cells + extract of Sp.grown	200	54.8	45.2
in medium contain 0.1M NaCl	400	29.6	70.4
Tumor cells + extract of Sp.grown	200	41.7	58.3
in medium contain 0.2 M NaCl	400	23.6	76.4
Tumor cells + extract of Sp.grown	200	66.4	33.6
in medium contain	400	35.8	64.2
102.5 ppm N +0.1 M NaCl			

Table 4: Inhibitory effect of phycocyanin extract from *Spirulina maxima* on the viability of Ehrlich Ascites Carcinoma Cells (EACC)

Treatment	concentration of algal extract ppm	% of viable cells	% of dead cells
Tumor cells (negative control)	0	95.50	4.50
Tumor cells + extract of Sp.grown	200	70.36	29.64
under control conditions (410 ppm N)	400	39.11	60.89
Tumor cells + extract of Sp.grown	200	79.55	20.45
in medium contain 205 ppm N	400	49.12	50.88
Tumor cells + extract of Sp.grown	200	89.12	11.88
in medium contain 102.5 ppm N	400	60.09	39.91
Tumor cells + extract of Sp.grown	200	91.64	91.36
in medium contain 51 ppm N	400	63.95	36.05
Tumor cells + extract of Sp.grown	200	93.02	6.98
in zero N	400	70.12	29.88
Tumor cells + extract of Sp.grown	200	59.25	40.75
in medium contain 0.1M NaCl	400	30.44	69.56
Tumor cells + extract of Sp.grown	200	45.92	54.08
in medium contain 0.2 M NaCl	400	26.20	73.80
Tumor cells + extract of Sp.grown	200	69.34	30.66
in medium contain	400	36.33	63.67
102.5 ppm N +0.1 M NaCl			

2ml of cell solution containing 4×10^6 cells

Soluble protein

In both algae ***Spirulina*** species, the total soluble protein was increased with the increased of nitrogen and high NaCl level (Table 1 and 2). At comparable nitrogen levels 0, 51, 102.5, 205 and

410 ppm N in the medium, the soluble protein content in *S. plantensis* and *S. maxima* (in parenthesis) were 10.1 (4.51), 15.3 (9.21), 20.6 (14.3), 27.7 (18.5) and 29.7 (22.1 mg g^{-1}), respectively. Also, the soluble protein content of *Spirulina* species were increased as results of NaCl increase in present of sufficient nitrogen levels in nutrient medium (Table 1 and 2).

By varying the concentration of the nitrogen in nutrient medium, *S. plantensis* and *S. maxima* can be manipulated with respect to their total phycocyanin and soluble protein content. The *Spirulina* grown in medium with high nitrogen levels yielded a maximum phycocyanin pigment (up to 12.2%), whereas with decreasing nitrogen level the phycocyanin content of *Spirulina* cells was dropped (Becker, 1994). Piorreck *et al.* (1984) grew *Spirulina platensis* and other three unicellular algae at different nitrogen levels and they observed significant changes in pigment and total protein content. Which decreasing nitrogen concentration led to decrease in chlorophyll and protein content due to breakdown of the whole chloroplast apparatus. However, *Spirulina* protein can accumulate in considerable amount (up to 70%) in stationary-phase cells when grown in nutrient medium rich in nitrogen. However, the protein fraction of *Spirulina* species was containing up to 20% of cyanophycin granules, a water-soluble blue pigment (Becker, 1994 and Ciferri, 1983).

Effect of phycocyanin extract of *Spirulina* species on viability of EACC

Phycocyanin extracts of two species of blue green microalga *Spirulina maxima* and *Spirulina plantensis* on the viability of EACC were examined by means of the trypan blue exclusion method. After 2 h incubation of tumor cells in fresh medium with or without algal phycocyanin, the cell viability was measured. As shown in Table 3 and 4, treatment of cells with *Spirulina* - phycocyanin caused significant reduction in cell viability. Generally as the concentration of phycocyanin algal extract increased, the viability of EACC were reduced, which suggested that the effect of PC-*S. maxima* and *S. plantensis* on the growth of EACC was dose dependent. Further, the increase of phycocyanin content (% of dry weight) in the phycocyanin algal extracts led to a great decrease in cell viability. The most significant decreases in cell viability were observed in phycocyanin algal extract of *S. plantensis* and *S. maxima* containing total phycocyanin 22.3 and 18.88%, respectively, which reduced the cell viability to 23.6 and 26.2%, respectively. In contrast, the extract of *S. plantensis* and *S. maxima* contain less level of phycocyanin, 3.3 and 2.89% did not, produce any significant change in cell viability at concentration level of 200 ppm, whereas at 400 ppm these extracts gave significant effect on reduction of cell viability. Thus, the cell viability was depended on phycocyanin content and phycocyanin type.

Cells constituents and enzyme levels

The levels of glutathione (GSH) and activities of glutathione S-transferase (GST) and lactic dehydrogenase (LDH) were determined in treated EACC, in relation to reduction of tumor cells viability with algal phycocyanin. As shown in Table (5 and 6) all algal extracts were markedly increased the level of cellular GSH and GST and LDH activities in the tumor cells when compared with the control, especially with *S. Plantensis* extracts rich in phycocyanin (22% of DW). Thus, as the concentration of algal extracts increased, the level of GSH and enzyme levels were increased, which suggest that the effect of algal extracts on the cellular constituents of EACC

Table 5: Phycocyanin extract from *Spirulina plantensis* enhanced glutathione level , glutathione S- transferase

Treatment	Concentration of algal extract ppm	glutathione µg 10^{-6} cells	Ratio treatment/ control	Glutathione S-transferase: specific activity µ mole mg^{-1} protein min^{-1}	Glutathione S-transferase: Ratio treatment/ control	Lactate dehydrogenase: U/L	Lactate dehydrogenase: Ratio treatment/ control
Tumor cells (negative control)	0.00	6.1± 0.21		0.25±0.02	1.0	84 ± 1.02	
Tumor cells + extract of Sp.grown	200	60.51±0.95	9.92	1.49±0.09	5.96	457.1 ± 2.8	5.44
under control conditions (410 ppm N)	400	91.24±1.21	14.96	2.42±0.08	9.68	764.5 ± 5.2	9.1
Tumor cells + extract of Sp.grown	200	50.41±1.11	8.26	1.24±0.11	4.96	312.1± 2.12	3.72
in medium contain 205 ppm N	400	73.57±1.54	12.06	1.84±0.02	7.36	463.5 ± 3.52	5.52
Tumor cells + extract of Sp.grown	200	42.84±1.09	7.02	1.02±0.09	4.08	289.9 ± 2.02	3.45
in medium contain 102.5 ppm N	400	64.11±1.15	10.51	1.57±0.02	6.28	387.3 ± 3.12	4.61
Tumor cells + extract of Sp.grown	200	37.45±1.54	6.14	0.84±0.07	3.36	211.1 ± 4.11	2.51
in medium contain 51 ppm N	400	54.19±1.12	8.88	1.13±0.02	4.52	301.9 ± 2.2	3.59
Tumor cells + extract of Sp.grown	200	29.54±1.14	4.84	0.61±0.06	2.44	189.8 ±1.2	2.26
in zero N	400	48.94±1.25	8.02	1.05±0.08	4.2	250.7 ± 2.02	3.0
Tumor cells + extract of Sp.grown	200	72.25±1.44	11.84	1.89±0.02	7.56	596.3 ±4.22	7.1
in medium contain 0.1M NaCl	400	137.64±1.54	22.56	2.36±0.31	9.44	854.4 ±6.02	10.2
Tumor cells + extract of Sp.grown	200	89.47 ± 1.24	14.67	2.33±0.13	9.32	674.1 ± 5.12	8.02
in medium contain 0.2 M NaCl	400	167.41± 1.59	27.44	3.24±0.14	12.96	994.5 ± 7.4	11.84
Tumor cells + extract of Sp.grown	200	50.21 ± 1.61	8.23	1.22±0.09	4.88	351.6 ± 5.3	4.19
in medium contain	400	78.65 ± 1.54	12.89	2.41±0.21	9.64	544.6 ± 2.45	6.48
102.5 ppm N +0.1 M NaCl							

Table 6: Phycocyanin extract from *Spirulina maxima* enhanced glutathione level , glutathione S- transferase activity and lactate dehydrogenase activity of Ehrlich Ascites Carcinoma Cells (EACC)

Treatment	Concentration of algal extract ppm	glutathione µg 10^{-6} cells	Ratio treatment/ control	Glutathione S-transferase: specific activity µ mole mg^{-1} protein min^{-1}	Glutathione S-transferase: Ratio treatment/ control	Lactate dehydrogenase: U/L	Lactate dehydrogenase: Ratio treatment/ control
Tumor cells (negative control)	0.00	6.1±0.21		0.25±0.02		84±1.02	
Tumor cells+extract of Sp.grown	200	54.32±1.21	8.9	1.23±0.04	4.92	301.11 ±1.41	3.58
under control conditions (410 ppm N)	400	79.55±1.11	13.0	1.98±0.32	7.92	489.21±1.57	5.82
Tumor cells+extract of Sp.grown	200	41.25±2.21	6.76	0.89±0.05	3.56	234.15±2.02	2.79
in medium contain 205 ppm N	400	60.21±1.21	9.87	1.31±0.08	5.24	354.09±1.49	4.21
Tumor cells+extract of Sp.grown	200	33.54±2.31	5.49	0.64±0.11	2.56	201.61±1.41	2.4
in medium contain 102.5 ppm N	400	48.7±2.33	7.98	1.01±0.04	4.04	314.24±2.2	3.74
Tumor cells+extract of Sp.grown	200	24.83±0.91	4.1	0.59±0.02	2.36	175.36±2.6	2.1
in medium contain 51 ppm N	400	40.89±1.23	6.7	0.98±0.05	3.92	245.37±2.9	2.92
Tumor cells+extract of Sp.grown	200	18.99 ± 1.01	3.11	0.41 ± 0.01	1.64	139.87 ± 2.8	1.67
in zero N	400	36.84 ± 2.34	6.04	0.63 ± 0.02	2.52	200.47 ± 2.4	2.39
Tumor cells+extract of Sp.grown	200	64.31± 3.11	10.54	1.51± 0.21	6.04	374.61 ± 3.02	4.46
in medium contain 0.1M NaCl	400	96.91 ± 5.51	15.88	2.06± 0.23	8.24	459.11 ± 1.41	5.46
Tumor cells+extract of Sp.grown	200	76.94 ± 2.31	12.61	1.71 ± 0.25	6.84	499.44 ± 3.3	5.95
in medium contain 0.2 M NaCl	400	110.6 ± 6.21	18.13	2.54 ± 0.14	10.16	798.14 ± 3.5	9.5
Tumor cells+extract of Sp.grown	200	45.98 ± 1.51	7.53	0.94 ± 0.06	3.76	278.16 ± 3.7	3.31
in medium contain	400	70.34 ± 2.41	11.53	1.95 ± 0.02	7.8	436.94 ± 4.12	5.2
102.5 ppm N+0.1 M NaCl							

± S.D, 2ml of cell solution containing 4×10^6 cells, All values are significant at (P< 0.5), Values represents are mean of three replicates

were dose dependent. Phycocyanin of ***S. plantensis*** and S. ***maxima*** grow in medium contain 0.2 M NaCl at 400 ppm increase in GSH, GST and LDH levels about 27.4 (18.13), 12.9 (10.16) and 11.8 (9.5), respectively, times as that found in untreated cells. Whereas, the algal extracts of ***S. plantensis*** and S. ***maxima*** grown in free nitrogen medium at 400 ppm significant increase GSH, GST and LDH to, 8.02 (6.04), 4.2 (2.52) and 3.0 (2.39) time over the in untreated cells.

Table 7: Effect of phycocyanin extract from *Spirulina plantensis* on DNA fragmentation

Treatment	concentration of algal extract ppm	DNA fragmentation % of viable cells
Tumor cells (negative control)	0.00	0.0
Tumor cells + cis-platinum (50 mM)	0.00	7.25
Tumor cells + extract of Sp.grown	200	0.89
under control conditions (410 ppm N)	400	0.44
Tumor cells + extract of Sp.grown	200	1.05
in medium contain 205 ppm N	400	0.67
Tumor cells + extract of Sp.grown	200	1.54
in medium contain 102.5 ppm N	400	0.98
Tumor cells + extract of Sp.grown	200	2.11
in medium contain 51 ppm N	400	1.84
Tumor cells + extract of Sp.grown	200	2.99
in zero N	400	2.01
Tumor cells + extract of Sp.grown	200	0.77
in medium contain 0.1M NaCl	400	0.32
Tumor cells + extract of Sp.grown	200	0.41
in medium contain 0.2 M NaCl	400	0.18
Tumor cells + extract of Sp.grown	200	1.00
in medium contain	400	0.42
102.5 ppm N +0.1 M NaCl		

Table 8: Effect of phycocyanin extract from *Spirulina maxima* on DNA fragmentation

Treatment	concentration of algal extract ppm	DNA fragmentation %
Tumor cells (negative control)	0.00	0.0
Tumor cells + cis-platinum (50 mM)	0.00	7.25
Tumor cells + extract of Sp.grown	200	1.12
under control conditions (410 ppm N)	400	0.84
Tumor cells + extract of Sp.grown	200	1.54
in medium contain 205 ppm N	400	1.07
Tumor cells + extract of Sp.grown	200	1.78
in medium contain 102.5 ppm N	400	2.64
Tumor cells + extract of Sp.grown	200	3.45
in medium contain 51 ppm N	400	2.08
Tumor cells + extract of Sp.grown	200	4.13
in zero N	400	2.51
Tumor cells + extract of Sp.grown	200	0.97
in medium contain 0.1M NaCl	400	0.42
Tumor cells + extract of Sp.grown	200	0.65
in medium contain 0.2 M NaCl	400	0.23
Tumor cells + extract of Sp.grown	200	1.13
in medium contain	400	0.65
102.5 ppm N +0.1 M NaCl		

Alga ***Spirulina*** have a higher content of phycocyanin other than plant source (Vadiraja ***et al.***, 1998). Phycocyanin has various medical properties, which may inhibit the growth of much type of tumor cells by pathways other than apopotosis (Liu ***et al.***, 2000). Because phycocyanin has characteristic stability and solubility in aqueous solution and non-toxicity, it has been used in many research applications. Cyanobacteria-phycocyanin (C-PC) could reduce the viability of mouse myeloma cells, when cultured with 250 mg C-PC for 3 days (Morcos ***et al.***, 1988). Also, Liu ***et al.*** (2000) reported that phycocyanin of *S.* ***plantensis*** inhibited the growth of human Leukemia K 562 cells in a dose and time dependent manner by a potential pathway other than apoptosis.

In this study the phycocyanin extracts of two algal species inhibited the growth of EACC in a dose-dependent, the algal extracts contain a large amount of phycocyanin had a higher destructive effect on EACC. From this observation, it is clear that the anticarcinoma or antitumor activity of algae extracts were mostly due to phycocyanin compound present in these extracts. The EACC were killed in treated solution, 2 h after incubation, these indicated that phycocyanin extracts did not induce apoptosis. Similar finding were obtained by Liu ***et al.*** (2000) who found that phycocyanin of alga *S.* ***plantensis*** and S. ***maxima*** kiled the human leukemia K 562 cells by a potential pathway other than apoptosis. This study revealed that the *S.* ***plantensis*** algae extracts may induce cell death of EACC by membrane destruction, which lead to increase the leakage of cell constituent (GSH and LDH and GST enzymes).

DNA fragmentation

The whether of phycocyanin algal extracts could induce apoptosis in EACC was performed using calorimetric method. After DNA was extracted from the treated EACC, the percentage of DNA fragment was calculated as shown in Table 7 and 8. Apoptosis-induce cis- platinum (50 nM) produced 7.25%. DNA fragmentation. Compared with apoptosis induce treat, the phycocyanin algal extract of *S.* ***plantensis*** and *S.* ***maxima*** content high level of phycocyanin 22.3 and 18.88% were most significant decreased the DNA fragment to 0.18 and 0.23%, respectively. The algal extracts *S.* ***plantensis*** and *S.* ***maxima*** contents less phycocyanin% were produced DNA-fragment with 2.99 and 4.13%, respectively. This suggests that phycocyanin algal extracts may not be able to induce the apoptosis in the EACC. Consecontly, the phycocyanin algal extracts apparently reduce cell viability by anther mechanisms such as cell membrane lyases. However, the results revealed that after 2 h of incubation of algae extract with tumor cells clear showed lower DNA fragment, than control. These mean that no intranucleosm degradation of DNA (Ladder DNA) was occurred in treated EACC Reddy ***et al.*** (1997) reported that DNA fragmentation (Ladder DNA) was not essentially produced as a results of apoptosis pathways. In addition the algae extracts may induce chromosomal abnormalities in EACC (Duthie ***et al.***, 1997). Finally, the phycocyanin had an antitumor activity, and could be used chemo-preventive agent.

In conclusion, *S.* ***plantensis*** and *S.* ***maxima*** can be manipulated a big yield of phycocyanin when grown in medium containing 0.2 M NaCl. These, phycocyanin pigments inhabited the growth of EACC in a dose depended manner by a potential pathway other than apoptosis.

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