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ANTIOXIDANT ACTIVITY OF SPIRULINA PLATENSIS UNDER DIFFERENT GROWTH CONDITIONS

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ABSTRACT

Spirulina platensis was allowed to grow under different levels of, Se, NaCl and H_2O_2 or in its combinations for 9 days to study its efficiency to uptake Se during the incubation period. Antioxidant activities and inhibition of lipid peroxidation by phycocyanin extracted from S. platensis were studied. The obtained results showed that S.platensis has a good efficiency to accumulate Se during the incubation period, where it was 26.77 ppm in Se treated cells, 14.45ppm in 0.25M NaCl and 17.39 in 0.2 mM H_2O_2.The only substance stimulate the soluble protein, was Se with a percentage 8% more than control .Also, Se enhanced the biosynthesis of phycobiliprotein, while NaCl alone made a great reduction. Meanwhile their combinations elevate this fraction especially phycocyanin (1.2 mg/g dry wt.). Addition of Se to the media containing NaCl or H_2O_2 improved the adverse effect of stress factors on yield of total nitrogen. Phycocyanin has a good efficiency to inhibit lipid peroxidation by (72%) and the rate of inhibition was significantly increased when alga treated with Se, it gave (88%) in case of phycocyanin and 83% in bilipigments. An obvious reduction was attained especially when the alga treated with (Se+NaCl),where the percentage of inhibition was 27%; meanwhile, it has a moderate level (61%) as the alga treated with Se +H_2O_2.Moreover,the antioxidant efficiency of treated phycocyanin gave a valuable response toward (OH)scavenging through determination of IC_{50} of different stress factors including control (4.13μg/ml),selenium (2.7 μg/ml),hydrogen peroxide (12.66μg/ml) and sodium chloride (9.98 μg/ml) ,while Se+NaCl (5.59/ml),Se +H_2O_2 (11.7 μg/ml). The cytotoxicity of phycocyanin was tested against HeLa cells line (ovarian cell line), the results indicated that phycocyanin obtained from cells treated with selenium was superior inhibitor, since IC50=11.25μg/ml, and the percentage of its inhibition reached 92.7% as compared with control phycocyanin. IC_{50} of other treated phycocyanin with NaCl was 47.75 μg/ml and 23.13 μg/ml in case of phycocyanin treated with H_2O_2. A good confirmatory indication for the cytotoxicity of phycocyanin against HeLa cell line was its DNA fragmentation.

Keywords: *Spirulina platensis, Antioxidant activities, free radical's scavenger, Selenium, salinity, hydrogen peroxide.*

INTRODUCTION

Spirulina platensis is well known to have antioxidant properties, due to the presence of molecules as phycocyanin, β-carotene, tochopherol, γ-linoleic acid and phenolic compounds,(Chopra, *et al.,* 2008). These natural

antioxidants act by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Hanan and Mohamed, 2015). Moreover, antioxidant substances which scavenger free radicals play an important role in prevention of free radical-induced disease ,by donating hydrogen radicals, the primary radicals are reduced to non-radical chemical compounds, then converted to oxidized antioxidant radicals. Selenium-containing phycocyanin of Spirulina platensis has a strong superoxide and hydrogen peroxide scavenging activities (Hung, *et al.,* 2007).

Selenium is an essential micro-element to living organisms. It is an integral part of some enzymes called selanoenzymes.However; excess amount of selenium in living cells induced a level of toxicity for non accumulator organisms. On the other hand, The long-term exposure to selenium led to resistant strain (Burton *et al.,* 1987).Selenium compounds (selenite, SeO_3) have different abilities to generate superoxide ($O^{-2)}$ in vitro by reacting with thiol glutathione (Garberg *et al.,* 1988) forming selano-trisulphide,then it reacts with other compounds containing thiol by redox catalysis generating free radical as super oxide (Imura and Sek, 1977). Selenium supplementation was found to be an effective in reducing the incidence of cancer including prostate, liver, lung and cancer (Yu *et al.,* 2009).The dose, chemical form and metabolic state are the determinants of its anticancer activity (Rikiishi, 2007). Many evidences have supported that apoptosis is a critical cellular event in cancer chemoprevention and chemotherapy by selenium compounds, (Sinha and El-Bayoumy 2004). Recently selenocystine (SeC) was identified as a novel agent with higher antitumor activity than selenomethionine, Se-methyl-seleno-cysteine, selenite and selenate (Chen *et al.,* 2006).

Many studies have shown that Spirulina platensis is a good carrier for Se accumulation (Chen and Wong, 2004). The accumulated Se was incorporated into proteins of *Spirulina platensis* cells. Phycocyanin is the major protein component of *Spirulina* (Benedetti *et. al.,* 2006). Many characterized selenoproteins is enzymes that catalyze oxidation-reduction reactions and contain SE-c in their active site. Structurally, SE-c is identical to cysteine (Cys) except it contains selenium instead of sulfur. Se-c has a distinct functional advantage because the senol group is more fully ionized than thiol group of cysteine at physiological pH (Stadtman, 1996). When Se-cysteine is replaced with cysteine, the catalytic activity of a selenoenzyme is drastically reduced (Axley *et al.,* 1991). Two of glutathione peroxidases (GPx) protect cells against peroxidative damage by reducing hydrogen peroxide and free fatty acid hydroperoxide. Another (GPx) family member, phospholipids hydroperoxide glutathione peroxidase (PHGpx) reduces phospholipids, cholesterol and cholesteryl ester hydroperoxide ,so protecting cells against lipid peroxidation (Flohe *et al.,* 2001).

Lipid peroxidation defined as the oxidative deterioration of lipids containing a number of carbon-carbon double bonds (Rice and Diplock 1993) which affect membranes bounds cellular organs (Raha and Robinson 2000). Also, attack PUFAs in phospholipids of bilayer of biological membranes affecting biophysical properties, decrease membrane fluidity, change its phase characters and decrease

electrical resistance. So, the study of lipid peroxidation (LP) processes has become a rapidly growing field in medicine and biology. Since (LP) gives complex products e.g. hydroperoxides,cleavage products as aldehyde and polymers which exert cytotoxicity and genotoxic effects (Esterbauer, 1995). The more recently role of (LP) products is acting signaling messengers (Poli *et al.,* 2004).

Regarding to free radicals they defined as atoms or molecules bearing unpaired electron in the outer orbit (Gilbert ,2000). Human being is generally exposed to continuous risks from the different free radical generated as biological molecules from different metabolic activity e.g. lipid peroxidation (Devasagayam *et al.,* 2003), protein oxidation by (ROS/RNS), breaking single or double strand of DNA (Halliwell and Chirico, 1993). Free radicals are capable of altering all major classes of biomolecules as lipid, nucleic acids and proteins with changing in their structure and function (Guyton and Kensler, 1993). The prime targets of free radicals are the polyunsaturated fatty acids in cell membranes and their interaction results in lipid peroxidation (Khanzode *et al.,* 2004).

Phycocyanin is considered to be a physiologically important antioxidant against reactive species (Stocker *et al.,* 1987). It inhibits oxidative modification of plasma proteins and aromatic amino acids residue. Scavenging of oxygen radical by phycocyanin protect serum albumin as well as other biological targets (Neuzil and Riggs, 1994).

C- Phycocyanin (C-PC) is a major type of (C-PC) in *Spirulina*, it has been suggested to exhibit radicals scavenging property (Zhou *et al.,* 2005) to reduce anti -inflammatory response (Manconia *et al.,* 2009) and oxidative stress (Zhou *et al.,* 2005) .This phycobiliprotein also induces HeLa cell apoptosis (Li, *et al.,* 2009). Moreover, C-PC has been proven to have therapeutic including antioxidant, neuroprotective, hepatoprotective and anti-cancer activities (Ou *et al.,* 2010).

The objective of this study was to examine the combined effect of selenium plus phycocyanin on the antioxidant defense mechanism and to study the free radicals.

Scavenger ability, study the effect of combination of selenium, hydrogen peroxide and sodium chloride on antioxidant activity of Spirulina phycocyanin. Also to test phycocyanin cytotoxicity against Hella cell line. This was confirmed by DNA fragmentation.

MATERIALS AND METHODS

Isolation of Spirulina platensis :

Water samples were collected from River Nile during October 2011. Few ml were spread on sterile agar Zarrouk medium Zarrouk (1966).The petri dishes were incubated at (8:16) light: dark photoperiods under light intensity (5000 lux) at 27±2°_ for 30 days. The different petri-dishes were examined every three days by naked eyes and by light microscope. Samples from each blue green patch were taken by sterilized needle and examined by light microscope up to find the respective *Spirulina platensis*.

Preparation of algal inoculums :

After isolation of Spirulina platensis a piece

of pure Spirulina platensis trichomes were dipped in sterile 250 ml Erlenmeyer flasks, each containing 100 ml Zarrouk medium, and allowed to grow under optimum conditions of growth. An equivalent inoculums 1 ml of algal cells (1ml =10^4 cells) was inoculated in the following preparations Se (10ppm), NaCl (0.25 M), H_2O_2 (0.2 mM) and the salt combinations Se+ NaCl and Se+H_2O_2 against the control Zarrouk media, (five replicates for each concentration were prepared). Cultures were incubated under suitable conditions for 9 days. At the end of incubation period the algal cells were harvested by centrifugation at 10,000 rpm for 5 minutes. The algal mass was collected and air dried.

Isolation and purification of phycocyanin:

C-phycocyanin was extracted from blue green alga *Spirulina* platensis according to Boussiba and Richmond (1979) and the concentration of C-phycocyanin (CPA), APC (Allophycocyanin) and phycoerythrin (PE) were calculated according to Bennett and Bogorad (1973).

Biochemical Analysis :

Protein extraction was carried according to Lobban *et al.,* (1988). Both soluble and insoluble protein were quantified according to method of Lowery *et al.,* (1951). Total nitrogen was estimated by micro-kjeldahl method according to Allen (1953). The total inorganic and organic selenium concentrations was estimated by using atomic absorption method (Linore *et al.,* 1998). Lipid peroxidation was estimated at different pH according to Hoyland *et al.,* (1991).

Efficacy of phycocyanin as antioxidants:

Hydroxyl radical scavenging was assayed as described by Elizabeth and Rao, (1990). Hypochlorous acid scavenging was assayed by method described by Aruoma *et al.,* (1990). Reducing power was determined by method Oyaiza, (1986), with slight modification. Iron chelating was evaluated by Standard methods, (2012) and IC_{50} was determined.

Cell line culture and phycocyanin treatment:

HeLa cells were routinely grown at 37°C in Dulbecco's modified Eagle_s medium (DMED) containing 10 %FBS in humidified atmosphere containing 5% CO_2 and used for experiment when confluent. The cultured cells were sub-cultured twice each week. HeLa were plated at 5x10^5 Cells/ cm and placed in a humidified incubator containing 5 %CO_2 and 95% air, over night at 37°C. For application of phycocyanin, the medium was replaced with DMEM enriched with 10 % FBS and phycocyanin was added.

Analysis of DNA fragmentation :

Extraction methods which allow the isolation of only fragmented DNA without contaminating genomic DNA according to Hermann *et al.* (1994), and the colorimetric estimation of DNA content was carried out according to Perandones *et. al.* (1993).

Biological analysis

Measurement of cytotoxicity to cells :

This test based on ability of mitochondrial succinate trazolium reductase system to convert the yellow tetrazolium MTT to purple formazon dye and the amount of the produced dye measured according to Mossman, (1983).

RESULTS AND DISCUSSION

Regarding to Se accumulation by the tested alga Table (1) revealed Spirulina platensis has a good efficiency to accumulate Se during incubation period where 26.77ppm of the original concentration was taken up by alga. Meanwhile, the rate of uptake deceased to 14.45 ppm when NaCl was added, and to 17.39 ppm in case of H_2O_2 addition. Also, the rate of bioconversion of inorganic Se to organic one reached 1.23% in Se and to 0.97, 0.69 in case of NaCl and H_2O_2 respectively. These results are in concomitant with Zhi-Yong e*t al.,* (2003) ,who reported that, sodium selenite addition into the culture medium of *Spirulina Platensis* can enhance organic selenium. On the other hand, reduction of the uptake of Se in case of NaCl addition is in agreement with Lauchli (1993). Plant uptake of Se depend on numerous factors including the salinity and the ion composition of saline medium ,also there is a reduction in Se uptake and its accumulation by crops in response to chloride salinity. Moreover, the reduction induced by H_2O_2 may be due to H_2O_2 breaks down into water and single atomic oxygen bearing unpaired of electron i-e free radical (Helwig, 2000).

Table 2 showed that the amount of phycocyanin was increased when the alga treated with either H_2O_2 or NaCl with special reference to the former one. Regarding to soluble protein, the only substance stimulate this fraction is Se with percent 8% more than control. This percent was decreased to a minimum value when alga grown in medium containing Se+NaCl (4.98 mg/g dry wt.). The other treatment gave a moderate amount of soluble protein. The previous stimulatory effect explained the counter effect of both NaCl and H_2O_2 to Se. These results are in agreement with Abdel-Rahman (2005), explained the toxicity of hyper concentrations of salts to the increment concentration of other cations (K,Ca). Also, the increase in salinity caused the increase in protein content of *Spirulina platensis*. Moreover, Abdel-Rahman (2005) stated that, the higher salt concentrations, reduced growth, carbohydrates and protein content of both *Chlorella vulgaris* and *Chrococcus humicola*. In addition to phycocyanin decreased significantly in salt stressed cells (Lu *et al.,* 2002). Zeng and Vonshak (1998) said that salt stress result in a lower protein biosynthesis capacity .This fact can probably explain lower protein content in our results.

Data in Table 3 showed that Se enhanced the biosynthesis of biliprotein when compared with control. While the effect of NaCl or H_2O_2 was small compared to control except in (PE) treated with NaCl. On the other hand, combination between Se and H_2O_2 or NaCl led to decrease the adverse effects of these two factors. The previous values were elevated when Se added to medium containing H_2O_2.

Regarding the effect of selenium on total nitrogen content of *Spirulina* phycocyanin and bilipigment (Table 4), revealed that there is an increase in total nitrogen of S. platensis cells, this was accompanied with an increase in total nitrogen of phycocyanin and biliprotein pigment when alga grown in presence of Se, Se and NaCl or Se and H_2O_2. The only inhibitory effect was found in total nitrogen of H_2O_2-treated *Spirulina platensis* cells. This indicates that the presence of Se diminished the toxic effect of H_2O_2 in total nitrogen of

Spirulina platensis. The total nitrogen in phycocyanin represents 94 % of the total nitrogen in bilipigment in control. This percent was vibrated up and down according to the condition of algal growth.

Table (1) : Efficiency of *Spirulina platensis* for Selenium uptake grown in combination of selenium (10 ppm) and either H_2O_2 (0.2mM) or NaCl (0.25M).

Different treatments	Initial concentration of Se (ppm)	Residual of Se (ppm)	uptake of Se (ppm)	Inorganic Se (ppm)	Organic Se (ppm)	% of Conjugation of Se with phycocyanin
Control	0.00	0.00	0.00	0.00	0.00	0.00
Se	100	73.23	26.77	0.027	26.50	1.23
H_2O_2(0.2mM)	100	75.027	17.39	0.029	17.36	0.69
NaCl (0.25M)	100	77.43	14.45	0.031	14.43	0.97

Table (2) : Effect of combination between selenium (10 ppm) with either H_2O_2 (0.2mM) or NaCl (0.25M) on content of soluble protein and phycocyanin (mg/g dry wt.)in *Spirulina platensis.*

Parameters in combination	Control		NaCl (0.25M)		H_2O_2(0.2mM)	
	Protein	Phycocyanin	Protein	phycocyanin	Protein	Phycocyanin
0.0	5.46 ± 0.81	1.03 ± 0.092	5.16 ± 0.82	1.02 ± 0.07	5.59 ±0.50	1.65 ±0.16
Se	5.90 ± 0.94	1.47 ± 0.230	4.98 ± 0.60	1.2 ±0.10	5.26 ±0.30	1. 35 ±0.14

Table (3) : Combination effect of Selenium (10 ppm) and either H_2O_2 (0.2mM) or NaCl (0.25M) on biliprotein pigment (mg\g dry wt.) extracted from *Spirulina platensis*.

Concentration	Control			NaCl (0.25M)			H_2O_2(0.2mM)		
	Crude extract			Crude extract			Crude extract		
	C-PC	APC	PE	C-PC	APC	PE	C-PC	APC	PE
0.0	2.30 ± 0.13	2.33 ± 0.14	0.78 ± 0.03	1.36± 0.38	1.45 ± 0.26	0.7o± 0.11	2.35 ± 0.09	1.56 ± 0.16	0.66 ± 0.03
Se	2.45 ± 0.36	2.48 ± 0.49	0.83 ± 0.04	1.90± 0.12	2.06 ± 0.22	0.74± 0.02	1.46 ± 0.06	2.21 ± 0.44	0.71 ± 0.02

Table (4) : Effect of Se combination to either H_2O_2 (0.2mM) or NaCl (0.25M) on total nitrogen contents (mg/ g dry wt.) Of Spirulina platensis tissue, phycocyanin and biliprotein extract.

Sample	Control			NaCl(0.25M)			H_2O_2(0.2mM)		
	TN in Tissue	TN in phycocyanin	TN in bilipigment	TN in Tissue	TN in phycocyanin	TN in biliprotein	TN in Tissue	TN in phycocyanin	TN in biliproteinin
0.0	35.0±0.15	3.08±0.70	3.28±0.90	32.5±1.9	3.1±0.5	3.65±0.6	28.0±1.2	3.13±0.6	3.55±0.3
Se	38.5±0.20	4.36± 0.10	4.5±0.20	35.0±1.7	3.4±0.3	3.54±0.4	35.8±1.9	3.48±0.2	3.74±1.4

In this context, addition of Se to H_2O_2 elevate the percentage of nitrogen in phycocyanin extracted from *S. platensis* treated with H_2O_2 from 88% to 90.2% .In a similar results, addition of Se led to an obvious incorporation of nitrogen from 84% to 98%. These results are in agreement with Wang *et. al.* (2012) who stated that the water soluble phycobiliprotein was reduced under elevated salt concentration. This may be due to the great reduction of total nitrogen and protein biosynthesis under the stress condition through the proteomic hydrolysis. Also, under environmental stress, chloroplasts are damaged leading to disturbed photosynthesis. However, addition of appropriate level of Se reduces the damage of chloroplasts and increase chlorophyll contents (Filek *et al.,* 2009).

Results in Table 5 clarified that; phycocyanin has a good efficiency to inhibit lipid peroxidation rather than bilipigment (72 and 67 % respectively). The rate of inhibition was significantly increased when the alga treated Se, it gave 88 %and 83 % for phycocyanin and biliprotein pigment respectively .It is obvious that the nature of phycocyanin and its efficiency were changeable and variables according to the conditions and factors affecting growth .In this regard the inhibitory percentages of phycocyanin extracted from *Spirulina platensis* treated with NaCl and H_2O_2 were obviously reduced with remarkable reduction especially when the alga treated with (NaCl + Se) or (H_2O_2 + Se). The percentages of inhibition were 27 and 61 %. These data indicated that, NaCl and H_2O_2 alone accelerate the lipid peroxidation but addition of Se to NaCl or H_2O_2 showed a clear antagonistic effect. These results may be explained according to Noctor and Foyer (1998) who mentioned that H_2O_2 concentration in the culture medium enhance the production of hydroxyl radical (OH-) which led to lipid peroxidation process. Salinity also increases the content of H_2O_2 and induces oxidative stress in plant tissues. On the same hand, Duran *et. al.* (1997) stated that the antioxidant activity of Se enriched garlic, was increased and oxidative stress lowered with increased Se concentration. Parallels, Xu, *et al.* (2003) reported that Se could enhance the antioxidant activity of green tea extract and the effect was dose dependent. Moreover, Iivan *et al.* (2011) claimed that the lipid peroxidation system may be based on Fenton reaction which caused by metal ion (ferrous) with traces of hydroperoxides forming an alkoxyl radical, which in turn propa-

gates the free radical chain reaction. Considering these facts, our data suggest that C-phycocyanin may inhibit this process at its earliest stage by removing the alkoxyl radical or by binding the metal ions.

Results in Table 6 cleared that treatment of *Spirulina platensis* with Se enhance the efficiency of bilipigment to scavenger the different free radicals (hydroxyl ion, iron chelating, reducing power and hypochlorous acid) where IC50 was indicated. While, *Spirulina platensis* treated with NaCl and H_2O_2 reduced the efficiency of bilipigment to scavenger hydroxyl radical, iron chelating, reducing power and hypochlorous acid. In this regard both of NaCl, H_2O_2 elevated IC_{50} of the extract as compared with control and /or Se. Addition of Se to NaCl and H_2O_2 led to moderate efficiency for phycocyanin as antioxidant component. The most potent treatment to scavenger the different radicals are bilipigment extracted from Se treated cells, followed by the extracts obtained from cells treated with Se+NaCl and finally Se+H_2O_2.

Table 7 showed that pure phycocyanin extracted from *Spirulina platensis* treated with Se have a potent scavenger to the different free radicals when compared with phycocyanin extracted from *Spirulina platensis* treated with other substances. The order of scavenger efficiency was arranged as follow OH>HOCl >Fe chelating and reducing power. On the other hand, the scavenger properties of phycocyanin treated with either NaCl or H_2O_2 led to reduce the scavenger activities and IC_{50} reached in most cases to 2 or 3 times increase in their values. The previous results may be explained on basis of that either NaCl or H_2O_2 antagonized the activity of Se. On the other hand, the order of scavenger when phycocyanin extracted from *Spirulina platensis* treated with the combination effects between Se+NaCl or H_2O_2+Se summarized as HOCl>Fe chelating >OH >reducing power and OH >HOCl>Fe chelating >reducing power respectively .From the previous results it is obvious that the order of efficiency of phycocyanin and Se was parallel to those obtained by phycocyanin obtained from following H_2O_2+NaCl but with more IC_{50}. These results are in accordance with Stocker *et al.,* (1987), who stated that phycocyanin is physiologically important antioxidant against reactive species. Also; biliprotein extracted from Cyanobacteria has a strong antioxidant property. Moreover, Zhou *et al.* (2005) suggested that C-phycocyanin exhibits radical scavenging properties and reduce oxidative stress.Moreover, the minor addition of Se to the growth substances can reduce the excess of ROS generation, especially O_2 and/or H_2O_2 in plants subjected to environmental stress, (Cartes *et. al .,* 2010).

Survival cells assay for HeLa cells using MTT reduction method (Table 8) revealed that the proliferation of untreated HeLa cells was successively survived with maximum increment in its number after 24 h. The applicable untreated C -Phycocyanin resulted in the least toxicity (IC_{50}=21.20 ug/ml).While the survival cells of treated HeLa showed variable inhibition as it grown with different concentration of C-phycocyanin extracted from S. platensis treated cells with different substrates inducing stress. The maximum inhibitory effect was recorded in C-phycocyanin taken from Se-treated *Spirulina platensis* where IC_{50}=11.23 ug/ml C-Phycocyanin, with

Percent of inhibition reached 92.7% compared with C-phycocyanin control. In a similar manner the other treatments of C –Phycocyanin gave a more similar effect with ascending response to that of C-phycocyanin obtained from NaCl treated cells gave more apoptotic than C-Phycocyanin taken from H_2O_2 treated cells. The previous results evaluated that the percentage of cytotoxicity of C-PHYCOCYANIN was dose dependent.

Regarding the effect of C-PC extracted from *Spirulina platensis* and treated with Se, H_2O_2 and NaCl on DNA fragmentation of HeLa cells Figure 1 cleared that the different applicable forms of phycocyanin have a more or less similar effects on the DNA fragmentation of the carcinogenic HeLa cell. The most effective phycocyanin for the degradation of DNA was manifested in Figure (1). In lane 4 the untreated phycocyanin led to an obvious apoptosis to HeLa cell. This was appeared from gradual DNA smear. Lane (2, 3). There were also an obvious apoptotic DNA induced by C-PC –Se and C-PC -NaCl treated phycocyanin respectively with more intensive bands rather than the corresponding smear in C-lane as well as lane 1 through which the cancer cells treated with H_2O_2 (appear in lane 2). The results explain the differences between the untreated cells of cancer in lane (1) which appear as undegradable DNA induced a significant effect on the nucleoplasm of uterus tissues. Our results are in accordance to Morcos *et al.* (1988) who stated that C-phycocyanin specifically binds to cancer cells and can be used for anatomical imaging of tumors in vivo .Moreover,(Pardhasardhi *et al.,* 2003) explained C-phycocyanin induce apoptosis by down regulating of Bc-2 (a known inhibitor of apoptosis) and by generation of ROS. Also, (Chen *et al.,* 2006) reported that phycocyanin extracted from *Spirulina platensis* inhibited the growth of human hepatocellular carcinoma cell line SMM (7721). On the same hand, (Li *et al.,* 2009) stated that phycocyanin induces apoptosis in the existing and proliferating cancer cells and being antioxidant, it also helps getting rid of any cancer promoting oxidative stress.

Table (5) : % inhibition of lipid Phycoerythrienroxidation by phycocyanin and total bilipigment extracted from Spirulina platensis treated with Se, NaCl (0.25M) and H_2O_2 (0.2mM) or in their combination .

Treatmnets	Control		NaCl (.25M)		H_2O_2 (.2mM)	
	Phycocyanin	Bilipigment	Phycocyanin	Bilipigments	Phycocyanin	Bilipigments
0.00	72± 1.7	67±1.0	45±2.3	42 ± 2.1	70± 3.5	65± 3.3
Se	88 ±1.3	83 ± 1.4	27 ± 1.1	15 ± 1.6	61 ± 1.7	38 ± 1.8

Table (6) : IC_{50} (µg/ ml) for Fe chelation ,scavenging of hydroxyl radical, reducing power and hypochlorous acid by bilipigments extracted from *Spirulina platensis* treated with Se in combination with NaCl or H_2O_2.

sample	Control				NaCl				H_2O_2			
	Fe chelation	$^{\cdot}HO$	reducing power	HCLO	Fe chelation	$^{\cdot}HO$	reducing power	HCLO	Fe chelation	$^{\cdot}HO$	Reducing Power	HCLO
0.0	5.26 ± 0.3	4.13 ± 0.3	10 ± 1.1	7.4 ± 0.7	11.87 ± 1.0	9.98 ± 0.6	17.5 ± 2.3	10.30 ±1.2	12.55±1.8	12.66 ±1.5	22.5 ± 3.8	14.45 ±1.5
Se	3.75 ± 0.3	2.7 ± 0.1	8.5 ± 1.3	4.33 ± 0.5	9.45 ± 0.7	5.59 ± 0.4	10.25 ±1.5	8.77 ±0.80	8.05 ± 1.0	11.7 ± 0.8	13.7 ± 1.7	7.75 ± 0.6

Table (7) : IC_{50} (µg/ ml) for Fe chelation, scavenging of hydroxyl radical, reducing power and hypochlorous acid by phycocyanin extracted from *Spirulina platensis* treated with Se in combination with NaCl or H_2O_2

sample	Control				NaCl				H_2O_2			
	Fe chelation	$^{\cdot}HO$	reducing power	HCLO	Fe chelation	$^{\cdot}HO$	reducing power	HCLO	Fe chelation	$^{\cdot}HO$	reducing power	HCLO
0.0	4.7 ± 0.2	3.37 ± 0.1	7.2 ± 0.8	3.91 ± 0.4	7.90 ± 0.9	7.5 ± 0.4	7.54 ±1.1	6.88 ± 0.1	16.6± 0.9	8.54 ± 0.5	13.45± 1.8	4.22 ± 0.6
Se	3.38 ± 0.5	2.43 ± 0.3	5.0 ± 0.5	2.76 ± 0.3	5.02 ± 1.1	5.29 ± 0.2	7.33 ± 0. 6	4.36 ± 0.2	7.8 ±1.2	5.19 ± 0.3	12 ± 0. 9	6.84 ± 0.3

Table (8) : IC_{50} of phycocyanin extracted from Spirulina platensis treated with different substances including stress against HeLa cell.

Treatmnets	Phycocyanin			
Dose	Control	Se(10ppm)	NaCl(0.25M)	H_2O_2(0.2mM)
IC_{50} Ug/ml	21.20	11.25	47.75	23.13

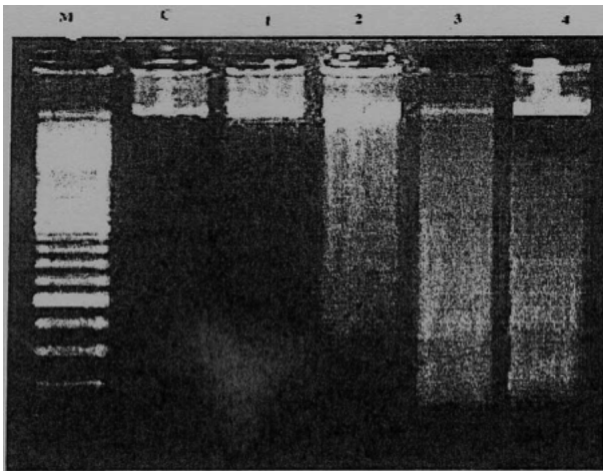

Fig (1) : Agarose gel electrophoresis of genomic DNA extracted from HeLa cells , M:Marker , C: positeeive control 1 : phycocyanin treated with NaCl, 2 :phycocyanin treated with H_2O_2, 3: phycocyanin treated with selenium, 4: phycocyanin untreated.

CONCLUSION

The antioxidants activities and inhibition of lipid peroxidation by phycocyanin extracted from *Spirulina platensis* previously grown under stress assistance clarified the phycocyanin has a good efficiency to inhibit lipid peroxidation and exhibits free radicals scavenging property.

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الملخص العربى

نشاطات مضادات الأكسدة لطحلب سبيرولينا بلاتينسيس تحت ظروف مختلفة من النمو

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تهدف هذة الدراسة إلى زيادة قدرة phycocyanin الفيكوسيانين المستخلص من طحلب سبيرولينا بلاتنسيس لاستخدامه فى تثبيط الشوارد الأيضية. ولتحقيق هذا الهدف سمح للطحلب بالنمو تحت مستويات مختلفة من عنصر السيلينيوم عند تركيز (10جزء فى المليون) ,كلوريد الصوديوم عند تركيز (0.25, مول) و فوق أكسيد الهيدروجين عند تركيز (0.2 مللى مول) وكذلك تنميته فى مخاليط لهذه المواد لمدة تسعة أيام. تم فصل الطحلب وتجفيفه وقياس تراكم السيلينيوم فى خلاياه وكذلك قدرته على تحويل عنصر السلينيوم من الصورة غير العضوية إلى الصورة العضوية . ولقد بينت النتائج ما يلى:-

زادت قدرة الفيكوسيانين المستخلص من طحلب سبيرولينا والمعالج بالسيلينيوم على تراكم عنصر السلينيوم بنسبة (26.77%) , وبنسبة (17.39%) فى حالة النمو تحت تأثير كلوريد الصوديوم , وبنسبة (14.45%) فى حالة النمو تحت تأ ثير فوق أكسيد الهيدروجين. وفى الوقت نفسة أدت مخاليط هذه المواد إلى رفع قدرة الفيكوسيانين على تراكم السيلينيوم خصوصا فى حالة السيلينيوم + كلوريد الصوديوم حيث بلغت 1.2ملج/جم/وزن جاف . ومن الجدير بالذكر أن إضافة السيلينيوم إلى الوسط الغذائى الذى يحتوى على كلوريد الصوديوم أو فوق أكسيد الهيدروجين حسن ذلك من التأثير السلبى لعوامل الإجهاد المختلفة على إنتاجية النيتروجين الكلى للطحلب.

لوحظ أن الفيكوسيانين يمتلك كفاءه جيدة فى منع أكسدة الدهون (27%) وحدثت زيادة فى هذه القدرة عند معالجة الطحلب بالسيلينيوم حيث بلغت النسبة % 83, 88% فى حالة bilipigments. ومن ناحية أخرى لوحظ انخفاضا واضحا عند معاملة الطحلب ب سيلينيوم + كلوريد الصوديوم حيث بلغت النسبة 27% بينما سيلينيوم + فوق أكسيد الهيدروجين كانت النسبة .61%

لوحظ أن قدرة الفيكوسيانين على إزالة الشوارد الأيضية قد أعطت نتائج قيمة وذلك من خلال تعيين IC_{50} وكان أفضلها عند إنماء الطحلب على السلينيوم حيث IC_{50}=2.7µg/ml وفى العينة الضابطه , 4.13 g/mlµ وفى عينة كلوريد الصوديوم , 5.59 سيلينيوم + فوق أكسيد الهيدروجين 11,7ميكروجرام. مللى .

دللت النتائج على أن القدرة التدميرية للخلايا السرطانية بصبغ الفيكوسيانين المعامل بعنصر السلينيوم فائقة حيث ازدادت إلى أضعاف العينة الضابطه وذلك من خلال الجرعة المميتة . ومن المؤشرات التأكيدية لقدرة الفيكوسيانين على تثبيط سمية الخلايا ضد Hella cell line هو تفتيت الحمض النووى DNA.

حيث أثبتت النتائج أن الفيكوسيانين المعالج بالسيلينيوم هو أكثر المواد تحليلا ل DNA يليه الغير معالج ثم المعالج بفوق أكسيد الهيدروجين ثم أخيرا الفيكوسيانين المعالج بكلوريد الصوديوم.

JOESE 5

ANTIOXIDANT ACTIVITY OF SPIRULINA PLATENSIS UNDER DIFFERENT GROWTH CONDITIONS

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