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Chemoprevention of Penzo[a]pyrene-induced Carcinogen and Lipid Peroxidation in Mice by Lipophilic Algae Extracts (Phycotene)

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Four algae strains: *Dunaliella salina*, *Chlorella ellipsoidea*, *Scenedesmus acutus* and *Scenedesmus dimorphus* were cultivated under stress condition, where carotenogenesis (reneged from 4.69 to 13.14 g 100 g^{-1} d.w) and tocopherols (1.23 to 3.75 g 100 g^{-1} d.w) accumulation are induced. The carotenoids have been analyzed by high performance liquid chromatography (HPLC) and were found to contain at least 14 different carotenoids. Of which, β-carotene (23.15 to 80.6%), canthaxathin (5.4-41.2%) and astataxanthin (10.2 -31.2%) were identified as major carotenoids. The lipophilic algae extracts (20 mg $dose^{-1}$ every two days /mice) were administrated in continued dosages for 2 weeks to mice pretreated with 0.25% solution of benzo(a)pyrene kg^{-1} [B(a)P] in corn oil as a single dose. The lipophilic algae extracts (phycotene) were shown to significantly increased the activity of the cellular detoxifying enzyme glutathione S-transferase (GST) being about 2.88 - 6.29 times higher than that the untreated mice. The administration of algal extracts induced variable increase in antioxidant defense enzymes activities (catalase, superoxide dismutase (SOD) and peroxidase) in liver and kidney tissues of B(a)P-treated mice. However, catalase, SOD and peroxidase activities in the liver were significant increased with 1.7 - 2.45, 1.81 - 4.23 and 1.3 - 3.2, respectively times of the control group (B(a)P-mice). Also, their algae extracts were increased the cellular glutathione (GSH) level ranged from 7.3 to 9.4 μmol g^{-1} when compared with B(a)P group (2.1 μmol g^{-1}). The algae extracts were inhibited the induction of lipid peroxidation product, when determined in liver (61.47 -79.40%) and kidney (65.67- 81.74%) homogenate of B(a)P-mice as thiobarbituric acid reactive substance (TBARS). The dietary administration of β-carotene and Vit. E mixture was induce increased activities of GST, catalase, SOD and peroxidase and increased the cellular GSH level and inhibited the induction of lipid peroxidation product of B(a)P-mice. These findings support the hypothesis that lipophilic algae extracts (rich in antioxidant compounds) alters the protective ability of tissues against carcinogenesis and oxidative stress caused by B(a)P *in vivo*.

Key words: Benzo(a)pyrene, antioxidant, tocopherols, peroxidation, carotenoids, microalgae, chemoperventation, vitamin E

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are products of combustion, some of which have been shown to be mutagenic and carcinogenic (Edes *et al.*, 1991 and Burczynski and Penning, 2000). PAHs are formed by pyrolysis of petrol and light diesel. Also, they are found in cigarette smoke, industrial combustion fumes, heated fats, charcoal-broiled meat and smoked foods. PAHs possess serious effects on health of living organisms. Toxicologically, PAHs are known to be potent carcinogens and have shown to causes cancer for some organs and tissues such as skin, lung, liver, bladder, stomach and colon in some animals (Wang *et al.*, 1989 and Frank *et al.*, 1998).

PAHs (Benzo(a)pyrene, for example) are ubiquitous environmental procarcinogens that require metabolic activation to exert their carcinogenic effect (Kada *et al.*, 1981 and Burczynski and Penning, 2000). A major pathway of PAHs metabolic activation procarcinogen through the generation of PAH t-dihydrodiols proximate carcinogens. Then, it is oxidized to reactive electrophlies by oxygen, hydrogen peroxide, super oxide radical and other type of free radicals (Ceutti, 1985; Ruch *et al.*, 1989; Zheng *et al.*, 1993; Burczynski *et al.*, 1999). Since most reactive ultimate carcinogenic forms of chemical carcinogens are electrophiles, glutathione (common non-protein thiol compound used for conjugation) and glutathione-S-transferase (phase II enzyme) in liver and other target tissues may play an important role in carcinogen detoxification (Vecchia, 1992 and Room *et al.*, 1998). The conjugation of glutathione to the reactive center of carcinogens such as the epoxide of benzo[a]pyrene and 7, 8, 9, 10-tetrahydrobenzo[a]pyrene. The resulting water-soluble conjugated may be excreted readily. However, the reactive intermediate compounds if not conjugated, may covalently bind with nucleic acids, proteins or membrane components leading to mutation, cytotoxicity and carcinogenicity (Van Ness, 1998).

Algae is untraditional source of foods, so selected 4 algal strain growing under stress conditions, that may be used in human foods and are richer in antioxidant substances (tocopherols and carotenoids). Examined the algae for their ability to increase the induction of the detoxifying enzyme (phase II enzymes) glutathione S-transferase (GST), catalase, superoxide dismutase (SOD) and peroxidase activities (antioxidant enzymes), and also to elevate the cellular glutathione content as well as inhibited the induction of lipid peroxidation product in the liver and kidney of mice exposed to benzo[a]pyrene (B[a]P), which is a ubiquitous carcinogen commonly found in foods, air and soil.

Materials and Methods

Algae source: *Dunaliella salina* was obtained from the culture collection of Dr.W.H. Thomas, La Jolla, CA, USA; *Chlorella ellipsoidea* was obtained from the Algae Culture Collection, University of Gottingen, Germany. *Scenedesmus acutus* 276–3a and *Scenedesmus dimorphus* were obtained from the Culture Center of Algae and Protozoa, Cambridge, UK. Mutant strains of *Chlorella ellipsoidea, Scenedesmus acutus* 276–3a and *Scenedesmus dimorphus* were generated from the parent strains as described by Abdel-Baky (1999).

Growth conditions: Algal cells were cultivated in specific growth medium (National Research Center, during the spring of 2001) and under stress environmental conditions as described by Abdel-Baky (1999) and ElBaz *et al.* (2002).

Extraction of algae lipophilic substances: The total lipophilic substance was extracted from algae cells with acetone: ethanol (7:3, v/v) according to AOAC (1995) methods.

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

Determination of algal tocopherol: Tocopherols were determined by spectrophotometric method at 534 nm using bathophenthrolin as coloring reagent (AOAC, 1995). Alpha-tocopherol served as a standard compound and used for preparing the calibration curve.

Identification of carotenoids: The portion of lipophilic algae extract was purified by washing with distilled water, and the solvent was removed under a stream of nitrogen gas. The residue was shaken with n-hexane and applied onto an alumina column. The carotenoid fractions were collected and analyzed by high performance liquid chromatography (HPLC) using a Finsil C18 column (250 x 4 mm^2 i.d.). Then, eluted with a mixture of a 0.5% chloroform-acetonitrile (Honya *et al.*, 1994).

Animals and treatments: Forty female Swiss mice (weighing 23 – 27 gm), six weeks age, were obtained from Ophthalmology Research Institute (Giza, Egypt). Animal were acclimated for one week, they were fed semipurified diet until the end of experiments. Water was given ad libitum.

One week after the start, fed of the semipurified diet, the animals was divided into eight groups (each of 5 mice). Group 1 received 50 μl of corn oil by gavage and served as the control. Group 2 treated with 25 mg benz(a)pyrene 100 g^{-1} in corn oil and served as the B(a)P control (negative control). Group 3 received 25 mg $dose^{-1}$ mixture of β-carotene/ vitamin E (1:1, w/w) by gavage in 50 μl of corn oil once every two days for 2 weeks (standard control). Group 4 animal treated with B(a)P were received 20 mg $dose^{-1}$ mixture of β-carotene/ vitamin E (1:1 w/w) by gavage in 50 μl of corn oil once every two days for 2 weeks (positive control). Groups 5-8 animals treated with B(a)P were received total seven doses from different of algae lipophilic extracts by gavage once every two days for 2 weeks. Each of dosages consisted of 20 mg dissolved in 50 μl of corn oil.

Twenty four hours after the last administration, dose mice were killed by cervical dislocation and the liver and kidney were removed and quickly frozen by liquid nitrogen and stored at –40 °C for enzymes preparation.

Preparation of cytosolic fraction: The liver and kidney of mice organs were homogenized in ice cold 1.15% KCl solution using Teflon glass homogenizer. An aliquot of the homogenate was removed for soluble sulfhydryl determination. The cytosol, after 105,000 xg centrifugation for one hour, was obtained and frozen at – 40°C until used. Each sample represents one tissue from each individual animal (Zheng *et al.*, 1993).

Enzymes assays: The activity of cytosolic GST (EC, 2.5.1.18) was determined according to the method of Habig *et al.* (1974). The activity of SOD (EC, 1.15.1.1) was determined by photochemical method (Ginnopolitis and Ries, 1977). The peroxidase activity (EC 1.11.1.7) was assayed by spectrophotometrically method as described by Chance and Maehly (1955). The catalase (EC 1.11.1.6) activity was determined by the method of Hans Luck (1970).

Determination of lipid peroxidation products: The lipid peroxidation products was estimated by the formation of thiobarbituric acid reactive substances (TBARS) and quantified in term of malondialdhyde (MDA) as described by Haraguchi *et al.* (1997). The lipid peroxidation was expressed as micromoles of MDA.

Determination of glutathione (GSH): The GSH content was estimated by the acid –soluble sulfhydryl (SH) level in the tissue homogenates as described by Vecchia *et al.* (1992). Aliquot (100 μl) of tissue homogenates were precipitated with equal volumes of 5-sulfosalicylic acid (4%). The precipitate was removed by centrifugation. The supernatants (100 μl) were assayed for the presence of free SH groups by the addition of 0.9 ml of Ellmans reagent [0.1 mM 5,5`-dithiobis (2-nitrobenzoic acid)] in 0.1 M sodium phosphate buffer (pH 8.0). The absorbance was recorded

at 412 nm to calculate the GSH concentration. Complete assay mixture without the supernatants was used as the control.

Determination of protein: Protein concentration of cytosol were determined spectrophotometrically at 595 nm, using comassein blue G 250 as a protein binding dye (Bradford, 1979). Bovine serum albumin (BSA) was used as a protein standard.

Statistical analysis: Values generated for each treatment group were analyzed for statistical differences by the student's t test.

Results and Discussion

Chemical constituents of algal strain extract: The total carotenoids content in their algae strains: *Dunaliella salina*, *Chlorella ellipsoidea*, *Scenedesmus acutus* and *Scenedesmus dimorphus* were comprised 13.4, 4.69, 4.75 and 4.75 % based on dry matter, respectively. While, the tocopherols content were comprised 1.23, 2.62, 3.73 and 2.93%, respectively (Table 1). Accordingly, *Dunaliella salina*, the most algae strain possesses the ability to accumulate very large amount of carotenoids (more than 13% of the algae dry weight). On the contrary, *Scenedesmus acutus* was accumulated large amount of tocopherols compared with other algal strain. However, great variability in the gross chemical composition of microalgae has been shown as a result of the source and concentration of nitrogen used in the culture medium or other stress condition such as high light intensity and high NaCl concentration (Abalde and Fabregas, 1991 and Bar *et al.*, 1995). The carotenoids analyzed by HPLC and were found to contain at least 14 different carotenoid compounds of which, β-carotene, canthaxathin and astataxanthin were identified as major carotenoids (Table 1). In *D. salina* the β-carotene was identified as a major carotenoid (80.6 %). While, canthaxathin (5.4 %) and astaxanthin (10.2 %) were identified as minor carotenoids (<10 %). In other algae strains canthaxanthin (41.2-32.3%), β-carotene (32.3-41.2%) and astataxanthin (19.8% –31.2%) were identified as a major carotenoids. These results are in good accordance with findings of Marrin *et al.* (1998) and Orosa *et al.* (2001).

The algae strains of *Dunaliella salina*, *Chlorella ellipsoidea*, *Scenedesmus acutus* and *Scenedesmus dimorphus* chosen for study might be effective to increase detoxification and antioxidant enzyme activities toward reactive metabolites of the B[a]P. The inducing capabilities of these extracts as compared with vitamin E and β-carotene mixture was found to prevent PAHs (B(a)P, as example) carcinogenesis, were previously known to confer protection (Schwartz *et al.*, 1988 and Shklar and Schwartz, 1993).

Influence of administration of algae extracts on GST activity of B(a)P induced carcinogen in mice: Results (Table 2 and Fig. 1) demonstrated that there was a significant increase in GST enzyme activity being about 2.88 - 6.29 times higher than that of the normal control group (untreated control). This means that the increase in GST activity varied according to the type of algae strain extracts. The administrated of B(a)P (0.25% solution, 25 mg/100g mice, G2) induced significant increase in liver and kidney GST activity with 1.5 and 1.36 times, compared with untreated group (G1), respectively. The enhancement of GST activity due to administration of vitamin E and β-carotene mixture to B(a)P-mice was approximately 2 times that of the B(a)P treated mice (G3). However, the GST enzyme activity in the liver and kidney was enhanced by the administration of vitamin E and β-carotene mixture.

The enhancement of mice liver GST activity due to administration of *D. salina*, *C. ellipsoidea*, *Sc. dimorphus* and *Sc. acutus* were 4.14, 3.88, 2.40 and 1.89 times that of B[a]P treated mice, respectively. This means that administration of algal extracts caused highly increase in liver GST activity as compared with Vit. E and β-carotene mixture. Similar trend was observed in increasing the GST enzyme activity of kidney cytosols. For instance, the enhancement of GST activity due to administration of *D. salina*, *C. ellipsoidea*, *Sc. dimorphus* and *Sc. acutus* were 5.18, 5.37, 3.0 and 2.4 times that of B[a]P treated mice (G2), respectively. This means that administration of algae extracts caused highly increase in liver GST activity as compared with vitamin E and β-carotene mixture. In general, the increase of GST enzyme activity due to administration of algae lipophilic extracts were in the order of: *D. salina* > *C. ellipsoidea* > *Sc. dimorphus* > *Sc. acutus* = antioxidant mixture. These sequences indicate that the administration of *D. salina* and *C. ellipsoidea* was much better than the extracts of *Sc. dimorphus*, *Sc. acutus* and Vit. E and β-carotene mixture to increase the GST activity.

The epidemiological and plethora studies in various animals models have established unequivocally that some natural substances can influence the incidence of diseases such as cancer by modulating of detoxifying enzyme system (GST) which is responsible for the metabolic activation and deactivation of chemical carcinogens such as B[a]P (Bu-Abbas *et al.*,1995 and Burczynski and Penning, 2000). An enhancement of the activity of glutathione-S-transferase (GST) and other detoxifying enzyme with increasing glutathione concentration suggested an increase in the host's ability to detoxify xenobiotics, including carcinogen. However, the positive correlation has been established between several natural compounds of inhibitors of polycyclic aromatic hydrocarbon such as benzo[a]pyrene and induction of carcinogenesis in different animal models and their enzymes activities (Coles *et al.*, 1999 and Hu *et al.*, 1999).

Influence of administration of algal extracts on antioxidant enzyme defense system: Results (Table 3,4,5 and Fig. 1) demonstrated that there was a variation in catalase activity due to administration of algae lipophilic extracts to B[a]P – mice. Their extracts caused significantly an increase in catalase activity in both liver and kidney as compared with both control groups (G1 and G2) and Vit. E and β-carotene -B[a]P-mice (G4). For instance, *D. salina* and *Sc. acutus* were increased catalase activity in liver of P(a)B-mice by 3.67 and 3.33 times and 2.21 and 2.45 times over than that normal mice and B(a)P treated mice, respectively. On the other hand, the lipophilic extract of *Sc. acutus* and *C. ellipsoidea* was were increased catalase activity in liver and kidney of B(a)P-mice (G2) by 1.71 and 1.93 and 1.47 and 1.63 times that of in the same organ of B(a)P group. In contract, their lipophilic extracts had approximately the similar effect to enhancement of the catalase activity in the liver and kidney of the vitamins mixture group (G4). However, the administration of algae extracts induced variable increase in catalase activity depending on the algae strains. For example, *Sc. dimorphus* caused the least increase in catalase activity compared with other algal strains. The increase of catalase activity was ordered towards: *D. salina* > antioxidant mixture > *Sc. acutus* >*C. ellipsoidea* > *Sc. dimorphus*.

The activity of the enzyme peroxidase in the liver and kidney of the mice was enhanced by the administration of algae lipophilic extracts (Table 4 and Fig. 1). The enhancement of peroxidase activity in the liver and kidney were ranged from 1.3 to 3.2 and 1.79 to 3.17 times of the B(a)P group (G2). The low values was nevertheless, significantly ($P < 0.05$) different from the P(a)B group (G2). However, algae extracts of *Sc. acutus, C. ellipsoidea* and *Sc. dimorphus* did not significantly increased the peroxidase activity in both organ tissues when compared by vitamin E and β-carotene -B[a]P-mice group (G4). While, the administration of lipophilic extract of *D. salina* was significantly increased peroxidase activity in liver and kidney of B(a)P-mice over than the vitamin E and β-carotene -B[a]P-mice group (G4). However, the administration of algal extracts induced variable increase in peroxidase activity depending on the algae strains. In general, the increase in peroxidase activity was in decreasing order: *D. salina* >antioxidant mixture > *Sc. acutus C. ellipsoidea* > *Sc. dimorphus*.

The SOD activity of B[a]P-control mice (G2) did not exhibit any significant changes as compared with normal control mice (G1) (Table 5 and Fig. 1). The administration of Vit. E and β-carotene

Table 1: Antioxidant of algal strains grown under stress conditions

	Strains			
Composition	*Dunaliella salina*	*Chlorella ellipsoidea*	*Scendesmus dimorphus*	*Scendesmus acutus*
Total carotenoinds (g 100 g^{-1} D.W)	13.14	04.69	04.75	04.75
Total tocopherols (g 100 g^{-1} D.W)	01.23	02.62	02.93	03.75
Carotenoids composition % (w/w) of total carotenoids				
Astaxanthin	10.2	31.20	19.8	24.3
Canthaxanthin	05.4	32.30	41.2	35.4
β-carotene	80.6	23.15	34.0	32.3
Unidentified	03.8	13.00	05.0	09.0

Table 2: Effect of algal extracts on glutathione S-transferase(GST) in target tissues of female mice treated with penzo(a)pyrene

	Organs									
	Liver					Kidney				
Groups	Sp. activity	Ratio a	Ratio b	Ratio c	Ratio d	Sp. activity	Ratio a	Ratio b	Ratio c	Ratio d
G1: Control not treated	14.21 ±0.04	1.0				11.12 ±0.02	1.0			
G2: Negative control treated	21.62 ±0.1	1.52	1.0			15.12 ±0.17	1.36	1		
G3: Positive control vitamins mixture not treated	32.78* ±0.2	2.3	1.5	1.0		21.33* ±0.15	1.92	1.4	1.0	
G4: Positive control treated (vitamins mixture)	42.55* ±0.23	2.99	1.97	0.95	1.00	33.52* ±0.21	3.00	2.2	1.57	1.0
G5: Extract of *Sc. acutus*	40.9* ±0.2	2.88	1.89	1.22	0.96	35.93* ±0.2	3.2	2.4	1.68	1.1
G6: Extract of *Sc. dimorphus*	51.91* ±0.15	3.65	2.4	1.97	1.22	45.35* ±0.23	4.08	3.0	2.13	1.35
G7: *Chlorella ellipsodea*	83.98* ±0.2	5.91	3.88	1.97	1.97	81.2* ±0.2	7.3	5.37	3.8	2.42
G8: *Dunaliella salina*	89.42* ±0.15	6.29	4.14	2.1	2.1	78.31* ±0.23	7.04	5.18	3.7	2.34

Ratio a: Test/control Ratio b: Test/treated control Ratio c: Test/not treated with vitamins Ratio d: Test/treated with vitamins

Table 3: Effect of algal extracts on catalase in target tissues of female mice treated with penzo(a)pyrene

	Organs									
	Liver					Kidney				
Groups	Sp. activity	Ratio a	Ratio b	Ratio c	Ratio d	Sp. activity	Ratio a	Ratio b	Ratio c	Ratio d
G1: CONTROL not treated	2.91 ±0.05	1.0				1.57 ±0.09	1.0			
G2: Negative control treated	4.37 ±0.31	1.5	1.0			3.62 ±0.16	2.31	1.0		
G3: Positive control vitamins mixture not treated	6.39* ±0.17	2.19	1.46	1.0		4.79* ±0.21	3.03	1.32	1.0	
G4: Positive control treated (vitamins mixture)	9.72* ±0.15	3.34	2.22	1.52	1.0	6.66* ±0.2	4.14	1.84	1.39	1.0
G5: Extract of *Sc. acutus*	9.69* ±0.23	3.33	2.21	1.52	0.997	7.92* ±0.23	5.04	2.19	1.65	1.2
G6: Extract of *Sc. dimorphus*	7.38 ±0.2	2.54	1.7	1.15	0.76	5.31* ±0.15	3.38	1.47	1.11	0.79
G7: *Chlorella ellipsodea*	8.43* ±0.23	2.89	1.93	1.32	0.87	5.92* ±0.2	3.77	1.63	1.24	0.89
G8: *Dunaliella salina*	10.69* ±0.5	3.67	2.45	1.67	1.1	8.13* ±0.31	5.18	2.24	1.7	1.22

Ratio a: Test/control Ratio b: Test/treated control Ratio c: Test/not treated with vitamins Ratio d: Test/treated with vitamins
Specific activity: μ mol/min/mg protein * : $P < 0.05$

mixture to experimental B[a]P-mice induced significant increase in hepatic and renal SOD activity. The administration of algal extracts significantly induced the SOD activity, when compared with B[a]P–mice control group. However, SOD activity for the algal groups were higher several folds than those in the B[a]P–control group. The values for the increase of SOD activity for administration of: *D. salina*, *C. ellipsoidea*, *Sc. dimorphus* and *Sc. acutus* to B[a]P-mice were 10.03, 7.92, 6.59 and 4.28, respectively. Also, their values for increase in renal SOD activity were approximately 5.03, 4.21, 6.81 and 6.52, respectively. The comparison between the ratio of increasing hepatic SOD algae extracts and vitamin mixture led to conclusion that the *D. salina* extract cause significant increase in SOD activity than the Vit. E and β-carotene - B[a]P-mice group (G4). While, administration of *C. ellipsoidea* and *Sc. dimorphus* did not possess any significant increase in SOD activity as compared with the vitamin mixture group (G4). In general, the increase in SOD activity in the liver and kidney tissues were in decreasing order: *D. salina* > *C. ellipsoidea* = > antioxidant mixture > *Sc. acutus* > *Sc. dimorphus*.

The change in activity of liver and kidney enzymes indicated that the administration of P(a)B caused several cellular change in microsomal enzyme activity (Kodam *et al.*, 1996 and Govindwar and Adav, 1999). The algae lipophilic extracts was able to induced increase the activity of enzymatic antioxidant defenses: GST, SOD, peroxidase and catalase in liver and kidney. The active ingredients

Table 4: Effect of algal extracts on peroxidase in target tissues of female mice treated with penzo(a)pyrene

	Organs									
	Liver					Kidney				
Groups	Sp. activity	Ratio a	Ratio b	Ratio c	Ratio d	Sp. activity	Ratio a	Ratio b	Ratio c	Ratio d
G1: Control not treated	0.602 ±0.02	1.0				0.118 ±0.02	1.0			
G2: Negative control treated	0.625 ±0.05	1.04	1.0			0.284 ±.23	2.4	1.0		
G3: Positive control vitamins mixture not treated	1.24* ±0.05	2.6	1.98	1.0		0.484* ±0.05	4.1	1.7	1.0	
G4: Positive control treated (vitamins mixture)	1.06* ±0.02	1.76	2.02	1.02	1.0	0.698* ±0.05	5.91	2.46	1.44	1.0
G5: Extract of *Sc. acutus*	1.43* ±0.02	2.37	2.29	1.15	1.35	0.643* ±0.23	5.45	2.26	1.33	0.92
G6: Extract of *Sc. dimorphus*	0.851* ±0.04	1.41	1.3	0.67	0.803	0.509* ±0.31	3.46	1.79	2.98	0.73
G7: *Chlorella ellipsodea*	1.23* ±0.02	2.04	1.97	0.99	1.16	0.568* ±0.02	4.81	2.0	1.17	0.814
G8: *Dunaliella salina*	2.05* ±0.16	3.4	3.2	1.65	1.93	0.901* ±0.02	7.63	3.17	1.86	1.3

Table 5: Effect of algal extracts on superoxide dismutase (SOD) in target tissues of female mice treated with penzo(a)pyrene

	Organs									
	Liver					Kidney				
Groups	Sp. activity	Ratio a	Ratio b	Ratio c	Ratio d	Sp. activity	Ratio a	Ratio b	Ratio c	Ratio d
G1: Control not treated	2.55 ±0.05	1.0				2.13 ±0.09	1.0			
G2: Negative control treated	2.37 ±0.09	0.929	1.0			3.06 ±0.23	1.44	1.0		
G3: Positive control vitamins mixture not treated	3.27* ±0.23	1.28	1.38	1.0		2.01* ±0.31	0.94	0.97	1.0	
G4: Positive control treated (vitamins mixture)	7.96* ±0.5	3.12	3.36	2.43	1.0	4.18* ±0.23	1.96	1.37	2.08	1.0
G5: Extract of *Sc. acutus*	4.28* ±0.38	1.68	1.81	1.31	0.538	6.52* ±0.31	3.06	2.13	3.24	1.56
G6: Extract of *Sc. dimorphus*	6.59* ±0.23	2.58	2.78	2.02	0.828	6.81* ±0.16	3.19	2.22	3.39	1.63
G7: *Chlorella ellipsodea*	7.92* ±0.38	3.11	3.34	2.42	0.99	4.21* ±0.31	1.98	1.38	2.09	1.01
G8: *Dunaliella salina*	10.03* ±0.5	3.93	4.23	3.07	1.26	5.03* ±0.23	2.36	1.64	2.5	1.2

Specific activity: μ mol/min/mg protein * : $P < 0.05$
Ratio a: Test/control Ratio b: Test / treated control Ratio c: Test /not treated with vitamins Ratio d: Test / treated with vitamins

Table 6: Effect of algal extracts on Acid-soluble sulfhydryl (SH) level in target tissues of mice treated with benzopyrene

	Acid-soluble sulfhydryl level (m mol g^{-1} tissue)	
Groups	Liver	Kidney
G1: Control not treated	5.60 ±1.0	2.70 ±.6
G2: Negative control treated	2.1* ±1.2	1.8* ±1.4
G3: Positive control vitamins mixture not treated	7.1* ±1.3	4.5* ±1.2
G4: Positive control treated (vitamins mixture)	6.2* ±0.9	4.1* ±.8
G5: Extract of *Sc. acutus*	7.3* ±1.1	5.6* ±.9
G6: Extract of *Sc. dimorphus*	7.8* ±.85	5.9* ±1.1
G7: *Chlorella ellipsodea*	8.2* ±1.2	6.4* ±1.3
G8: *Dunaliella salina*	9.4* ±1.4	6.8* ±1.5

* : $P < 0.05$

Table 7: Effect of algal extracts on inhibition of microsomes lipid peroxidation in target tissues of mice treated with benzopyrene

	TBARS[a] (μM)			
Groups	Liver[a]	Inh.(%)[b]	Kidney	Inh.(%)[b]
G1: Control not treated	32.05	90.57	22.00	71.60
G2: Negative control treated	340.00	0.00	77.50	0.00
G3: Positive control vitamins mixture not treated	33.00	90.29	23.65	56.58
G4: Positive control treated (vitamins mixture)	130.50	61.62	49.00	36.77
G5: Extract of *Sc. acutus*	131.00	61.47	26.90	65.67
G6: Extract of *Sc. dimorphus*	100.65	70.39	19.05	75.48
G7: *Chlorella ellipsodea*	96.50	71.60	22.50	70.96
G8: *Dunaliella salina*	70.00	79.40	14.65	81.74

a: Thiobarbturic acid reactive substance
b: Inhibition (%) was expressed as the reduction of TBARS formation from sample compared to negative control

found in algae extract such as β-carotene, canthaxathin and astataxanthin and α-tocopherols (Vit.E) was induced of GST SOD and catalase activity in several animal modals. For instant, Palozza *et al.* (1998 and 2000) reported that the carotenoid induced changes in the activity of enzymatic antioxidant defenses (such as catalase and SOD) of the cells and increase the content of lipophilic antioxidants such as tocopherols (including, Vit.E) compounds. The dietary supplementation of rats with carotenoid modulated the increase of SOD induced by peroxyl radicals in high fat diet (Blakely *et al.*, 1988). However, the carotenoid can substantially

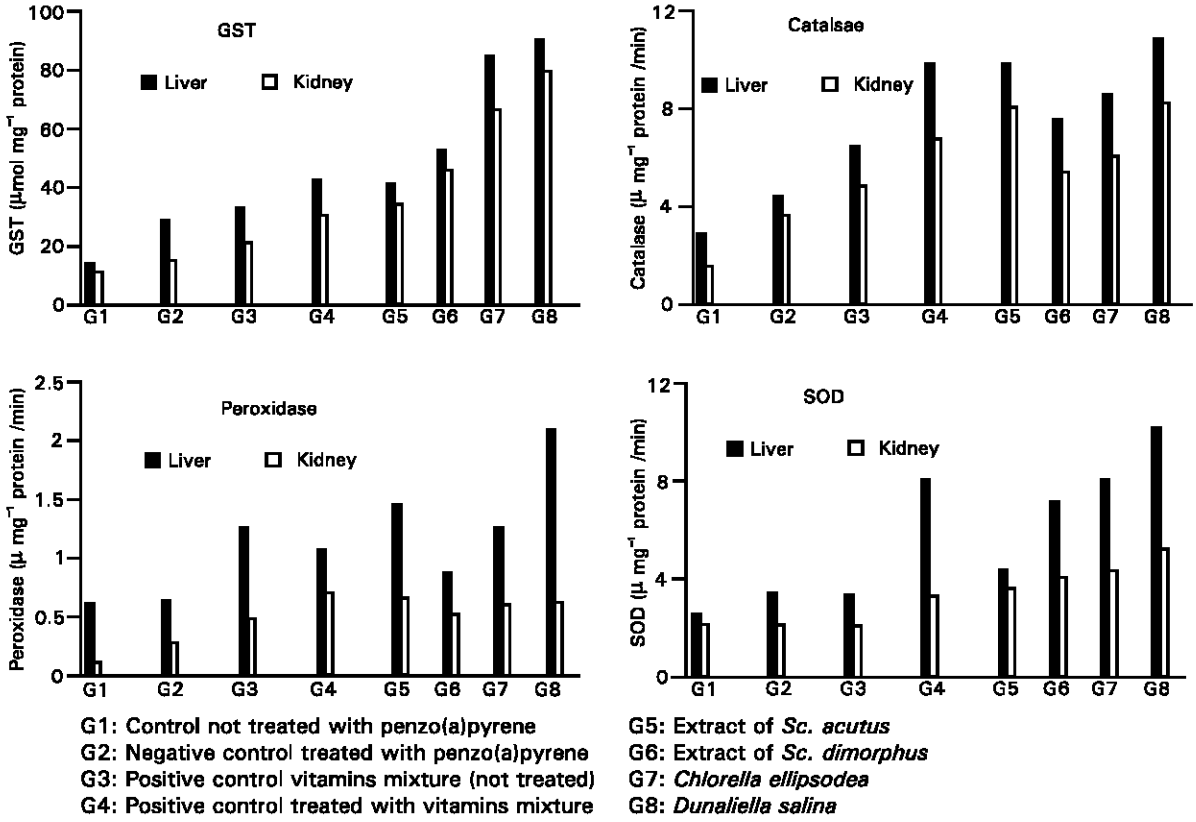

Fig. 1: Effect of algal extracts on glutathione S-transferase (GST), catalase, peroxidase and superoxide dismutase (SOD) in target tissues of female mice treated with penzo(a)pyrene

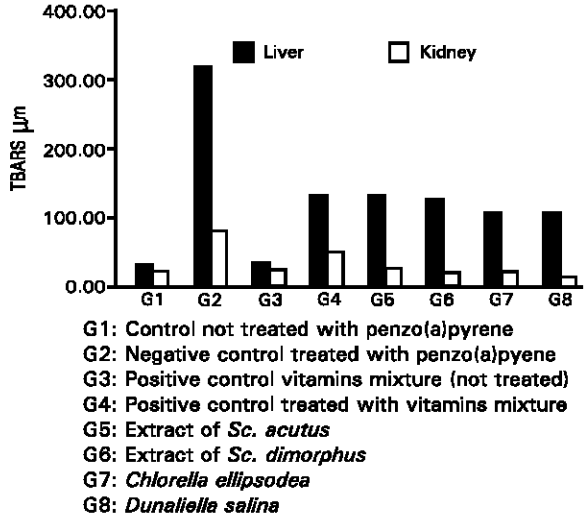

Fig. 2: Effect of algal extracts on inhibition of microsomes lipid peroxidation in target tissues of female mice treated with penzo(a)pyrene

modify the antioxidant status and the activity of enzymatic antioxidants in several experimental animal models (Lawlor and O' Brien, 1995 and 1997). The responses of enzymatic antioxidants to carotenoids treatment may be due to their different roles in oxidative processes as well as their different locations in the cells (De Leo *et al.*, 1997).

Influence of administration of algae extracts on hepatic glutathione level of B(a)P-mice: The acid-soluble sulfhydryl (SH) level, a good measure of glutathione (GSH) contents in tissues, was examined in liver and kidney of B[a]P-mice treated with algae lipophilic extracts and antioxidant mixture. The administration of B[a]P (G2) was significant decrease the GSH level of the liver (2.1 μmol g^{-1}) and kidney (1.8 μmol g^{-1}) when compared with untreated mice (G1) which their level values were 5.6 and 2.7 μmol g^{-1}, respectively (Table 6). The administration of algae extracts and vitamin mixture were induced significant increase of GSH of the hepatic tissues (ranged from 6.2 to 9.4 μmol g^{-1}) and renal (ranged from 4.1 to 6.8 μmol g^{-1}) of mice treated with B(a)P. The administration of *D. salina* extract was induced significant increase in GSH level of liver (9.4 μmol g^{-1}) and renal (6.8 μmol g^{-1}) of B(a)P-mice over than the other algae treated groups or Vit. E and β-carotene-groups (G4). However, the GSH level of liver and kidney (in parentheses) of B(a)P-mice treated with of *C. ellipsoidea*, *Sc. dimorphus* , *Sc. acutus* and Vit.E and β- carotene mixture were 8.2 μmol g^{-1} (6.4 μmol g^{-1}), 7.8 μmol g^{-1} (5.9 μmol g^{-1}), 7.3 μmol g^{-1} (5.6 μmol g^{-1}) and 6.2 μmol g^{-1} (4.1 μmol g^{-1}), respectively. It is interesting to note the lipophilic algae extracts was modify the level of reduced glutathione (GSH) which, is a limiting factor in several enzymatic activity such as superoxide dismutase (SOD) and catalase (Lawlor and O' Brien, 1997). However, it was reported that the toxicity of chemical carcinogens might increase as a result of GSH depletion (Gopalan *et al.*, 1994). It agreement with this study B(a)P reduced GSH levels in liver and kidney of mice. The administration of algal extracts was induced significant increase in GSH level, antioxidant enzymes and GST of

B[a]P- mice. This means the increase in such activity might be an adaptive mechanism of the liver and kidney to enhance the GSH conjugation capacity accumulation B(a)P in order to reduce their toxicity. Vecchia *et al.* (1992) reported that the induction of the phase II detoxifying enzyme and the level of mandatory substrate GSH, are considered favorable for the detoxification of carcinogens Such as B(a)P. However, Singh *et al.* (1999) found that the *Chlorella vulgar* was significantly elevated the sulfhydryl (-SH) and GST level in the liver of murine mice.

Influence of administration of algae extracts on B(a)P-induced lipid peroxidation. There was a higher significant values in TBARS concentration 340.0 and 77.5 μM in cytosolic fraction of liver and kidney, respectively of mice treated with B[a]P (G2) when compared with untreated mice (G1) (32 and 22 μm, respectively) (Table 7 and Fig. 2). The algae extracts and antioxidant mixture showed a significant decreased in the TBARS level in both tissues. However, the concentration (in parentheses) and percent of reduction (%) in TBARS level in liver of B(a)P-mice treated with of *D. salina, C. ellipsoidea, Sc. dimorphus and Sc. acutus* were approximately 70 μM (79%), 96 μM (72%), 100 μM (70%) and 131 μM (61%), respectively compared with liver of mice treated by B(a)P only (G2) (Table 7). It is interesting to note the lipophilic algae extracts were modify *in vivo* lipid peroxidation as measured by TBARS production. Results from this study showed that there was a significant increase in TBARS after B(a)P administration, suggesting that B(a)P induced the lipid peroxidation. However, the carcinogen such as aflatoxin or B(a)P induced cellular oxidative damage and play an important role in the cytotoxicity and carcinogenesis (Farber *et al.*, 1990 and Shen *et al.*, 1997).

In this study, the algae extracts, Vit.E and β-carotene mixture was significantly inhibited the B(a)P-induced hepatic and renal lipid peroxidation. Also, the results was suggested that the modulation of the endogenous antioxidant enzyme (SOD, catalase and peroxidase) is still able to prevent the oxidative stress caused by B(a)P (carcinogen-induced cellular oxidative damage) of on cell membranes (Farber *et al.*, 1990 and Palozza *et al.*, 1998, 2000). However, the lack of antioxidant defense including enzyme and non-enzyme system leads to increase the lipid peroxidation and deleterious effects (Yagi, 1987). In contrast, the increase of cellular enzymes that regulate the cells oxidative stress such as superoxide dismutase (SOD), catalase or glutathione S−transferase (GST) and cellular antioxidants (such as: glutathione, carotenoids or Vit. E) significantly induced carcenogenses regression (Buring and Hennekens, 1997). The inhibition of algal lipophilic extracts and Vit.E-β-carotene mixture on B(a)P-induced hepatic and renal lipid peroxidation may indicate the possible mechanism of its protection. Previous studies on the protective effects of antioxidants, such as β-carotene and vitamins A and E against the cytotoxicity and genotoxicity of chemical carcinogen (B(a)P and aflatoxins) mostly focused on the metabolism and detoxification of carcinogen or the formation of B(a)P or AFB1-DNA adducts (Shen *et al.*, 1997). Vit.E has been found to be an effective antioxidant and can inhibit lipid peroxidation by breaking the chain reaction initiated by $^{\bullet}OH$ radical through the so-called Fenton reaction (Farber *et al.*, 1990). Singh *et al.* (1998) found that the administration of *Chlorella vulgar* was significant inhibited the lipid peroxidation product (as MDA) in hepatic tissue of murine mice.

Epidemiological studies as well as a plethora studies in many animals models have established unequivocally that diet can influence the incidence of diseases such as cancer by modulating of the enzyme system responsible for the metabolic activation of carcinogens such as aromatic hydrocarbons for example, B[a]P and benzo[a]anthracene (Shklar and Schwartz 1993 and Bu-Abbas *et al.*,1995). As algae extracts and their major constituent (vitamin E and β-carotene) have been shown to possess anticarcinogenic potential (Singh *et al.*, 1998 and 1999). The results obtained in this study clearly are capable of inducing increased activity of hepatic and renal detoxifying enzyme system on the Phase II of conjugation system. Also, the chemical carcinogens are always associated with the alteration of the oxidant−antioxidant status (Gerber *et al.*, 1996) and induce lipid peroxidation which increase the levels of peroxidation products (electrophilic substrate). Therefore, the enzyme activity acts on lipid peroxidation products (electrophilic substrate for GST) was found to be significantly increased in cells to prevents continuing damage to functional and intact cell constituents (Zimnik *et al.*, 1997). Shklar and Schwartz (1993) mentioned that the mixture of β-carotene, reduced glutathione, Vit.E and ascorbic acid were very effective in preventing of carcinogenesis in cancer model. Also, the cellular enzymes that regulate the cell's oxygen state, such as superoxide dismutase, catalase, or glutathione S-transferase play a role in preventing of carcinogenesis in cancer mode (Shklar and Schwartz 1993 and Waxman, 1990).

The algae extracts was more effective than the individual components of the mixture as a cancer chemopreventive and antioxidant agents, because the carotenoids and tocopherols act together as antioxidants with synergistic effect to inhibit tumor cell growth (Shklar and Schwartz, 1993 and Mathew *et al.*, 1995). Moreover, *Dunaliella* extracts significantly inhibit the spontaneous mammary tumorigenesis of both breeding and virgin mice (Nagasawa *et al.*, 1989). *Spirulina* extract was found to inhibit buccal cancer in animal modals Mathew *et al.* (1995). Schwartz and Shklar (1987) and Schwartz *et al.* (1992) and they mentioned that the extract of *Spirulina* and *Dunaliella* significantly induced tumor regression, and stimulated large number of tumor necrosis factor alpha (TNF-α), which is responsible for the tumor regressing and degeneration. On the other hand, extract of *Spirulina* and *Dunaliella* resulted in regression of 7,12 dimethylbenz −(α) anthracene −induced hamster buccal squamous cell carcinomas (Schwartz *et al.*, 1988 and Schwartz, 1997). However, other algae extracts such as *Chlorella vlugaris* and *P. Mathamensis* showed high antitumor activity both *in vivo* and *in vitro* studies (Noda *et al.*, 1990 and Zhang *et al.*, 1995). A glycoprotein extract derived from the unicellular green alga *Chlorella vulgaris* exhibited a pronounced antitumor effect against both spontaneous and experimentally induced metastasis in mice (Tanaka *et al.*, 1998). From aforementioned results it could be concluded that, the green algae *Chlorella ellipsoidea, Scenedesmus acutus* 276−3a, *Scenedesmus dimorphus and Dunaliella salina* accumulated massive amount of antioxidant: carotenoids and Vit. E (tocopherols) when grown under combined stress conditions: high NaCl concentration, high light intensity and nitrogen deficiency. The accumulated antioxidant substances in algae cells are presented in higher concentrations than conventional food, traditionally considered rich in these compounds (ElBaz *et al.*, 2002). Their algae antioxidant compounds can play an important role as antioxidant to inhibited the cellular damage oxidative induced by carcinogen (B(a)P), which its play important role in the cytotoxicity and carcinogenesis. Also, the lipophilic extracts of algae can protect our body from harmful effects of reactive oxygen species and lipid peroxidation products caused by some carcinogens and mutagens may found in human diet, involved in the pathophysiology of many human diseases (Wang *et al.*, 1989). Consequently, the algae extracts may considered as potential chemoprevention agents. Also, the data obtained in this study, showed that the lipophilic substances of algae strains can be amenable to manipulation by varying culture conditions as well as by improvement of strains.

References

Abalde, J. and J. Fabregas, 1991. β-carotene ,vitamin C and vitamin E content of the marine microalga *Dunaliella tertiolecta* culture with different nitrogen sources. Bioresources Technol., 38: 121-125.

Abdel-Baky, H. H., 1999. Biochemical studies on some algae as a source of antioxidant vitamins. Thesis, Ph. D, Faculty of Agriculture, Biochem. Dept., Cairo University, Egypt.

AOAC, 1995. Official Methods of Analysis. Association of Official Analytical Chemists, 16th. edition., K. Hlrich. Arlington, Virginia, USA.

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

Blakely, S.R., L. Slaughter, J. Adkins and E.V. Knight, 1988. Effects of β-carotene and retinyl palmitate on corn oil-induced superoxide dismutase and catalase in rats. J. Nutr., 118: 152-158.

Bradford, M.M., 1979. A rapid and sensitive methods for the quantitation of microgram of protein utilizing the principle of protein–day binding. Anal. Biochem., 72:248-254.

Bu-Abbas, A., M.N. Clifford, C. Ioannides and R. Walker, 1995. Stimulation of rat hepatic Udp-glucuronosyl transferase activity following treatment with green tea. Food Chem. Toxic., 33: 27-30.

Burczynski, M.E., R.G. Harvey and T.M Penning, 1998. Expression and characterization of four recombinant human dihydrodiol dehydrogenase isoform: Oxidation of trans-7, 8 dihydroxy-7, 8 dihydrobenzo [a] pyrene to the activated O-quinone metabolite benzo[a]pyrene –7, 8-dione. Biochemistry, 37: 6781-6790.

Burczynski, M.E., H.K. Lin and T.M. Penning, 1999. Isoform specific induction of a human ald-keto reductase, electrophiles and oxidative stress implication for alternative pathway of PAH activation catalyzed by human dihydrodiol dehydrogenase. Cancer Res., 59: 607-614.

Burczynski, M. E. and T.M. Penning, 2000. Genotoxic polycyclic aromatic hydrocarbon ortho-quinones generated by aldo-keto reductase induce CYP1A1 via nuclear translocation of the aryl hydrocarbon receptor. Cancer Res., 60: 908-915.

Buring, E. J. and H. C. Hennekens, 1997. Cardiovascular disease J. Nut. Rev., 55: S53 – S60.

Ceutti. P. A., 1985. Pro-oxidant states and tumor promotion. Sciences (Washington DC), 227: 375-381.

Chance, B. and A.C. Maehly, 1955. Assay of catalase and peroxidase. 764. In: S.P. Colowic and N.O. Kaplan (Eds), Methods of Enzymology. Vol. 2 Academic Press, New York.

Coles, B., M. Yang, W. Lang and F. Kadlubar, 1999. Variation of glutathione –S-transferase expression in lung . Proceeding Am. Assoc. Cancer Res., 40:343.

De Leo, M.E., M. Landriscina, H.B. Palazzo, S. Borrello and T. Galeotti, 1997. Iron modulation of LPS-induced manganese superoxide dismutase gene expression in rat tissues. FEBS Lett., 403: 131-135.

Edes, E.T., M.S. Kwan, S.C. Buckiey and H. W. Thornton, 1991. Tissue vitamin arepletion is impaired by exposure to carcinogen. Int. J. Cancer, 50: 99 – 102.

ElBaz, F.K., A.M. Aboul-Enien, G.S. El-Baroty, A. Youssef, H.H. Abdel-Baky, 2002. Accumulation of antioxidant vitamins in *Dunaliella salina*. OnLine J. Biol. Sci., 2: 220-223.

Farber, J.L., M.E. Kyle and J.B Coleman, 1990. Biology of disease: Mechanisms of cell injury by activated oxygen species. Lab. Invest., 62: 670-679.

Frank S., T. Renner and G. Scherer, 1998. Determination of albumin adducts of (+)-anti-benzo[a]pyrene-diol-expoxide using an high performance liquid chromatographic column switching technique for sample preparation and gas chromatography-mass spectrometry for the final detection. J. Chromato. B., 713: 331-337.

Gerber, M., C. Astre, C. Segala and J. Saintoto, 1996. Oxidant antioxidant status alterations in cancer patients relationship to tumor progression. J. Nutr., 126: 1201S–1207 S.

Ginnopolitis, N.C. and S.K. Ries, 1977. Superoxide dismutase occurrence in higher plants. Plant Phys., 59:309-314.

Gopalan, P., S. Hirumn and P.D. Lotlikar, 1994. Effect of glutathione levels on aflatoxin B_1-DNA binding in livers and kidney of male rats and hamsters pretreated with buthionine sulfoxamine and diethylmaleate. Cancer Lett., 76: 25-30.

Govindwar, S.P. and S.S. Adav, 1999. Effect of aflatoxin B_1 on the hepatic microsomal mixed function oxidase system during phenbarbitol and benzo[a]pyrene treatment in chickens. Vet. Human Toxicol., 41: 210-212.

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

Hans Luck, 1970. Method of Enzymatic Analysis Verlag Chemie Gmb H.(Ed.), pp: 885-888. Weinheim Bergstr, Academic Press, New York and London.

Haraguchi, H., H. Ishikawa and I. Kubo, 1997. Antioxidative action of di-terpenoids from *Podocarpus nagi*. Planta Medica, 63: 213–215.

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

Hu, X., A. Seidel and S.V. Singh, 1999. Activity of alpha class murine glutathione-S-transferase to wards 11,12 di(OH)-13,14 oxy-11,12,14-tetra-hgdrobenzo[g]chrysens. Proceeding Am. Assoc. Cancer Res., 40: 345.

Kada, T., T. Inoue and M Namiki, 1981. Environmental desmuutagens, In Environmental Mutageneses, Carcinogenesis and Plant Biology, Vol.1. Kelowski, E. J., Jr., (Ed.), Praeger Press, New York, pp: 134.

Kodam, K.M., S.S. Adav and S.P. Govindwar, 1996. Effect of sulfamethazine on phenbarbitol and benzo[a]pyrene induced hepatic microsomal mixed function oxidase system in rats. Toxicol. Lett., 87: 25-30.

Lawlor, S.M. and N.M. O'Brien, 1995. Modulation of oxidative stress by β-carotene in chicken embryo fibroblasts. Br. J. Nutr., 73: 841-850.

Lawlor, S.M. and N.M. O'Brien, 1997. Modulation of parquet toxicity by β-carotene at low oxygen partial pressure in chicken embryo fibroblasts. Br. J. Nutr., 77: 133-140.

Marrin, N., F. Morales, C. Lodeiros and E. Tamigneanx, 1998. Effect of nitrate concentration on growth and pigment synthesis of *Dunaliella salina* cultivated under low illumination and preadapted to different salinities. J. Appl. Phyco., 10: 465-411.

Mathew, B., R. Sankaranaryanan, P. Padmanabhan, 1995. Evaluation of chemoperevention of oral cancer with *Spirulina fusiformis*. Nutr. Cancer, 24: 197 Nagasawa H.K., R. Konishi, N. Sensui, K. Yamamoto and A. Ben–Amotz, 1989. Inhibition by β-carotene rich algae *Dunaliella* of spontaneous mammary tumorigenesis in mice. Anticancer Res., 9: 71–76.

Noda, H.H. Amano, K. Arashima and K. Nisizawa, 1990. Antitumor activity of marine algae. Hydrobiology, 204 /205: 577–584.

Orosa, M., J.F., Valero, C. Herrero and A. Abalde, 2001. Comparison of accumulation of astaxanthin Haematococcus pluvial is and other green microalga under N-starvation and high light conditions. Biotechn. Lett., 23: 1079-1085.

Palozza, P., G. Calviello, S. Serini, P. Moscato and G.M. Bartoli, 1998. Supplementation with canthaxanthin affects plasma and tissue distribution of α and γ-tocopherol in mice. J. Nutr., 128: 1989-1994.

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. Nutr. Interact. Toxicol., 1303-1308.

Room, M.W., S. Netke and C. Tsao, 1998. Modulation of drug metabolizing enzyme in Guinea Pig liver by high intakes of ascorbic acid. Inter. J. Vit. Nutr. Res., 68:45-47.

Ruch, R. J., S.J. Cheng and J.E. Klaunig, 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10: 1003-1008.

Schwartz, L. J., 1997. The dual role of nutrients as antioxidants and prooxidants : their effects pn tumor cell growth. Am. Instit. Nutr., 1221S–1227, S.

Schwartz, J. and G. Shklar, 1987. Regression of experimental hamster cancer by β carotene and algae extracts, J. Oral Maxillofac Surg., 45: 510 – 515.

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

Schwartz, J.L., V. Tanaka, V. Khandekar, T.S. Herman and B.A. Teicher, 1992. β-carotene and /or vitamin E as modulators of alkylating agents in SCC-25 human squamous carcinoma cells. Cancer Chemother. Pharmacology, 29: 207-213.

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.

Singh, A., S.P. Singh, R. Bamezai, 1998. Perinatal influence of *Chlorella vulgaris* (E-25) on hepatic drug metabolizing enzymes and lipid peroxidation. Anticancer Res., 18: 1509-1514.

Singh, A., S.P. Singh and R. Bamezai, 1999. Inhibitory potential of *Chlorella vulgaris* (E-25) on mouse skin papillomagenesis and xenobiotic detoxication system. Anticancer Res.,19: 1887-1891.

Shen, H.M., S.E. Chia, A.L. Nizynew, B.L. Lee and C.N. Ong, 1997. Detection of oxidative DNA damage in human sperm and the association with cigarette smoking. Reprod. Toxicol., 11: 67580.

Shklar, G. and J. Schwartz, 1993. Oral cancer inhibition by micronutrients. The experimental basis for clinical trials. Oral Oncol. Eur. J. Cancer, 24 B: 9-16.

Tanaka, K., A. Yamada, K. Noda, T. Hasegawa, M. Okuda, Y. Shoyama and K. Nomoto, 1998. A novel glycoprotein obtained from Chlorella vulgaris strain CK22 shows antimetastatic immunopotentiation. Cancer Immunol. Immunother., 45: 313-320.

Van–Ness, P.K., E.M. Thamas, K.B. Theo and L.E. David, 1998. Identification of amino acid residues essential for high aflatoxin B_1–8,9 –epoxide conjugation activity in alpha class glutathione S- transferase through site –Directed mutagenesis. Toxicol. Appl. Pharmacol., 152: 166 – 174.

Vecchia, L.C., E. Negri, S. Franceschi, D.B. Avanzo and P. Boyle, 1992. Tea consumption and cancer risk. Nutr. Cancer, 17: 27-31.

Wang, Z.Y., W.A. Kan, D.R. Bickers and H. Mukhtar, 1989. Protection against polycyclic hydrocarbon induced skin tumor inhibition in mice by green tea phenols. Carcinogenesis, 10: 411-415.

Waxman, D.J., 1990. Glutathione S-transferase: Role in alkylating agent resistance and possible target for modulation chemotherapy. A review Cancer Res., 30: 6449-6454.

Yagi, K., 1987. Lipid peroxides and human disease J. Chem. Phys. Lipids, 45: 337-341.

Zhang, L.X., P. Acevedo, H. Guo and J.S. Bertram, 1995. Up regulation of gap junctional communication and connection 43 gene expression by carotenoids in human dermal fibroblasts but not in human keratinocytes. Mole. Carcinogenesis, 12: 50–58.

Zheng, G., M. Patnick and K.T. Luke, 1993. Potential anticarcinogenic natural products isolated from lemongrass oil and galanga root oil. J. Agric. Food Chem., 41: 154–156.

Zimnik, L., S. Awasthi, K.S. Srivastava and P. Zimnika, 1997. Increased resistance to oxidative stress in transfected culture cells over expressing glutathione S- transferase mGSTA 4 –4. Toxicol. Appl. Pharmacol., 143: 221–229.

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