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Anticarcinogenic Activity of Algal Extracts

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A total 14 vitamin algal extracts obtained from 7 strains (*Dunaliella salina*, *Scenedasmus dimorphus* (mutant), *Chlorella* (mutant), *Scenedasmus dimorphus* (normal), *Chlorella* (normal), *Scenedasmus acutus* (mutant) and *Scenedasmus acutus* (normal) grown under different environmental conditions, were tested for their ability to induce increased activity of the detoxifying enzyme system glutathione-S-transferase (GST) in several target tissues of female mice. In the normal algal extracts, increase of GST activity was ranged from 3 to 4.27, 1.99 to 2.77, 2.1 to 2.99 and 1.6 to 2.4 while the extracts obtained from the mutant algae, increase of GST activity was ranged from 4.29 to 6.79, 3.34 to 5.81, 3.64 to 4.37 and 2.31 to 3.19 times in the liver, small intestine, large intestine and lung, respectively as compared to control group. The vitamin extracts of *D. salina* were increased GST activity with 7.2, 6.21, 5.63 and 2.91 times than the control group in liver, small intestine, large intestine and lung tissues, respectively. Consequently, the vitamin extracts were evaluated to induce GST activity in different organs tissues of tumorous mice. The vitamin extracts of *Dunaliella* grown under stress conditions showed the most active extract that induced GST enzyme activity over all control groups including non-tumorous, tumorous (negative) and positive control groups (standard vitamins mixture). In liver, the GST activity was increased over the control groups by 8.12, 6.0 and 3.0, respectively. The data indicating that vitamin algal extracts were increase GST activity in tumorous tissues over than tumorous control group in all tissues examined suggested a correlation between the GST-induced ability in tumorous and inhibitory of tumorigeneses. Since, the ability to induce an increase in the detoxifying enzyme activity by natural compounds has been found to correlate with their activity in the inhibition of tumorigeneses. Therefore, algae extracts may be considered as a potential chemopreventive agent.

Key words: Anticarcinogenic, vitamins C, tocopherol, per-oxidation, carotenoids, microalgae

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Introduction

In last few years, there was an increase attention directed towards the association of non-nutritive component in foods and protection against chronic diseases including some forms of cancer and aging. Chemoprevention refers to the use of natural or synthetic chemical compounds to reverse, suppress or prevent progression to invasive some long-term diseases like cancer or cardiovascular disorders.

Vitamins, minerals or other food constituents are among the most ideal chemoprevention agents (Kim *et al.*, 1990). The mechanisms for action of chemopreventers is complicated and still need more attention. However, the most chemopreventers act primarily as antioxidant. There are several other mechanisms, more specific or site oriented that can complement the total beneficial potential of chemopreventers (Wattenberg, 1985). GST enzyme catalyzes the conjugation of glutathione with many environmental and electrophilic molecules (including metabolites of mutagens and carcinogens) to form less toxic and water-soluble substances which can readily excreted (Lam and Zheng, 1991; Sheweita, 1998). An enhancement of the activity of GST suggests an increase in the hosts ability to detoxify xenobiotics including carcinogens (Lam and Hasegawa, 1989; Van Ness *et al.*, 1998). Thus, any substance that can increase activity of GST and other detoxifying enzymes may be a potential anticarcinogen which act as potential inhibitors of chemically induced tumorigenesis.

The correlation between the induction of increased GST activity and the inhibition of carcinogenesis has been well documented. For instance, the GST and GSH reduced the covalent binding of epoxides (the ultimate metabolites of well-known chemical carcinogens, such as aflatoxin-β and (α) pyrene with DNA and other macromolecules. Such reduction in DNA binding was found to be effective in decreasing the toxicity and hepatocarcinogenicity caused by these compounds (Gould, 1995; Sheweita, 1998). A number of known compounds that elicit GST enzyme activity have been found to inhibit chemically induced tumorigenesis in laboratory. Therefore, any compound that induce the activity of this detoxifying enzyme system may act as potential inhibitors for chemically induced tumorigenesis and can be used as a method for detecting potential inhibitors of carcinogenesis (Zheng *et al.*, 1992 and 1993; Awasthi *et al.*, 1995; Zimnik 1997; Morris *et al.*, 1998). Some of chemopreventive agents are naturally occurring as secondary metabolites in plants, which may be useful in reducing the incidence of cancer in humans (Lam and Hasegawa, 1989). Dietary components such as cruciferous vegetables spices and their constituents (e.g., curcumin, turmeric, diallyl sulfide, diallyl disulfide, S-allyl cysteine, diallyl polysulfides, d-limonene and carvone) have been shown to exert protective effects against the induction of cancer by chemical carcinogenesis (Gould, 1995; Starvic, 1994; Schwartz, 1997). Many natural antioxidant vitamins that also induce GST activity are considered as potential inhibitors for chemical carcinogenesis (Lam and Zheng, 1991; Zheng *et al.*, 1992).

Consequently, there is an urgent need for potential chemopreventive agents from a natural source. The study was therefore, conducted to determine the induction of GST activity by vitamin algal extracts in the liver, lung, large intestine and small intestine of female Swiss mice and tumorous mice.

Materials and Methods

Algal source: *Dunaliella salina* and *Chlorella ellipsoidea* were obtained from the Cultures Collection of W.H. Thomas, La Jolla, CA, U.S.A. and University of Gottingen, Germany, respectively. *Scenedesmus acutus* 276-3a and *Scenedesmus dimorphus* were obtained from the Culture Center of Algae and Protozoa, Cambridge, U.K. Mutant strains of *Chlorella ellipsoidea, Scenedesmus acutus* 276-3a and *Scenedesmus dimorphus* were generated from the parent strains and propagated in National Research Center (NRC, Dokki, Egypt) as described by Abd El-Baky (1999).

Growth conditions: Algae cells were cultivated in specific growth medium under stressed environmental conditions in Algae Laboratory, National Research Center, during the spring and summer 1999 as reported by Abd El-Baky (1999) and El-Baz *et al.* (2002).

Extraction of antioxidant vitamins: The lipophilic antioxidant vitamins containing carotenoids and tocopherol were extracted from the algae cells with acetone: ethanol (7:3, v/v) according to Anonymous (1995) methods. Vitamin C (ascorbic acid) was extracted from algae cells by m-phosphoric acid (2%, w/v) as reported by Augustin *et al.* (1985).

Preparation of algal model systems: A known weight of algal ascorbic acid extracts (66.6 mg) was added to a test tube (3 ml) contained algal carotenoids and tocopherol extracts (133.2 mg), then, corn oil (1 ml) was added. The contents were agitated using a Julabo Ultrasonic bath (40 KHz) for 15 min. This system was stable for at least 4 h. The algal extracts modal system was prepared freshly before administration into animals. The final concentration of algal vitamins extracts in each modal system were 20 mg 100 μl^{-1} corn oil. To prepare the standard vitamins mixture, 66.6 mg from each β-carotene, ascorbic acid and α- tocopherol were added to 1 ml corn oil (20 mg 100 μl^{-1} corn oil).

Experimental animals: Female Swiss mice, 7 week aged, weighing 23-27 g were obtained from Ophthalmology Research Institute (ORI), Giza, Egypt. All animals were kept in temperature-controlled room and the photoperiod was set at 12 h light and 12 h dark (7 am to 7 pm) in experimental animal house at ORI. During the experimental period, the animals were fed semipurified diet. This diet was similar in composition as described by Venkatarman *et al.* (1980) except that the antioxidant vitamins were removed and the sucrose was replaced by a 1:1 mixture of starch and sucrose. The water was given ad libitum. One week after the start of this fed semipurified diet, the animals were divided into two major groups as follows:

Evaluation of vitamin algal extracts model system on normal mice

Eighty mice were divided into two control groups (negative and positive control groups) and experimental animal groups (14 groups) with five mice per groups. The experimental groups were given total three doses (20 mg dose^{-1}) of algal extracts suspended in corn oil (100 μl per dose) by oral intubation once every two days.

The negative control group was given corn oil only (100 μl per dose). The other positive control group was given 20 mg standard vitamin mixture containing β-carotene: tocopherol: ascorbic acid 1:1:1, (w/w/w) suspended in (100 μl) corn oil.

Experimental animal groups: Mice were classified into follows groups according to their given experimental conditions.

G1:	Negative control mice given corn oil only,
G2:	Positive (given standard vitamins mixture),
G3 and G4:	Extract of *D. salina* grown under control and stressed conditions (N deficiency, high salt concentration and high light intensity), respectively.
G5 and G6:	Extract of *Chl. ellipsodea* grown under control and stressed conditions, respectively.
G7 and G8:	Extract of *Chl. ellipsodea* mutant cells grown under control and stressed conditions, respectively.
G9 and G10:	Extract of *Sc. dimorphus* grown under control and stressed conditions, respectively.
G11 and G12:	Extract of *Sc. dimorphus* mutant cells grown under control and stressed conditions, respectively.
G13 and G14:	Extract of *Sc. acutus* grown under control and stressed conditions, respectively.

G15 and G16: Extract of *Sc. acutus* mutant cells grown under control and stressed conditions, respectively.

After 24 h at the last dose, the mice were killed by cervical dislocation and the lung, liver and mucosa of the proximal one −third of the small intestine, large intestine were removed and quickly frozen by liquid nitrogen then stored at -30°C for enzyme GST preparation.

Evaluation of vitamin algal extracts model system on tumorous mice: The vitamin algal extracts model system possess high activity as potential anticarcinogenic agent and were evaluated again by using tumorous mice.

Tumor transplantation

Tumor cell line: The original tumor cells were obtained from Cell Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt. The tumor cells line were maintained in Department of Biochemistry, Faculty of Agriculture, Cairo University. The mice were injected (i.p) with aliquot 0.2 ml (for each mouse) of a 10% suspension of minced tumor cell line ($2x10^6$ cells) saline. After 3 days of tumor transplantation, all animals had obvious gross tumors in peritoneal region. At this point the experimental animals were divided into seven equal groups, to study the influence of algal extracts on glutathione-S-transferase (GST) of tumorous mice. Animal groups were given a total of three doses of the vitamin algal extracts suspended in corn oil by oral intubation once after every two days. After twenty four hours from last intubation all animals were killed by cervical dislocation and the lung, liver, small and large intestinal mucosa were removed and stored quickly in liquid nitrogen then stored at −30°C.

Organs weight: Organs were weighed only in tumorous mice. After the mice were killed the liver, lung, small intestine and large intestine were removed and washed with ice cooled 0.15 M KCl solution (pH 7.4) and blotted between two sheets of filter paper, then any adhering or fat tissues were removed before being weighed.

Preparation of cytosolic fraction: The organs were homogenized in ice cold 1.15% KCl saline solution using Teflon glass homogenizer. The homogenate was filtered through a cold double layer of gauze and then centrifuged at 1600 xg for 10 min at 4°C to isolate the nuclear and broken cells. The supernatant was centrifugated at 12000 xg for 20 min at 4°C and pellets were discarded. The supernatants obtained from 12000 xg were subjected to ultracentrifugation at 105,000 xg for 60 min at 4°C. The final supernatant was collected as the cytosolic fraction and was kept at -40°C. Each sample represented one tissue from each individual animal (Zheng *et al.*, 1993). The enzyme assay (GST) was done within 24 h. from homogenization of organ samples.

Determination of glutathione−S−transferase (GST) activity: The activity of cytosolic GST was determined according to the method of Habig *et al.* (1974), using 1-chloro-2, 4 dinitrobenzene (CDNB) as a substrate. Assays were performed using 1 mM CDNB and 20 μl of cytosol (sample) at 30°C in 0.1 M phosphate buffer (pH 6.5) in the presence of 5 mM reduced glutathione (GSH). The absorbance was recorded from 1 to 15 min using LKB spectrophotometer at 340 nm. Complete assay mixture without the cytosolic enzyme was used as blank. The extinction coefficient of CDNB−glutathione product of 9.6 at a GSH concentration value was 1 mM, it was used to convert the change in absorbance values (Δ A min^{-1}) into concentration.

Activity and specific activity: The formula to determine activity is given as:

$$\text{Activity} = \Delta A_{340\,nm} / \varepsilon (9.6) \times 1000 = \mu M\ min^{-1}$$

Where,

$\Delta A340_{nm}$ = Change in absorbance min^{-1} at 340 nm

ε = Molar extinction co-efficient of CDNB-GSH product = 9.6

Specific activity = Activity/protein (mg) = μM/min mg^{-1} protein (Habig *et al.*, 1974).

Determination of protein: Protein concentration of cytosol were determined by spectrophotometric method at 595 nm (Bradford, 1976) using comassein blue G as a protein binding dye. Bovine serum albumin (BSA) was used as a standard.

Statistical analysis: Data were statistically analyzed using student t- test accordance to the method established by Little and Hills (1978).

Results and Discussion

Effect of vitamin algal extract on GST activity: The vitamin extracts of D. *salina* grown under stress conditions showed more potent of GST activity (Table 1) as compared with control group animals (mice given corn oil only). Cytosols of liver, small and large intestinal mucosa and lung showed high GST activity (7.2, 6.21, 5.63 and 2.91 times higher than the control group). Also, the level of GST activity in the same organs were increased by 3.1, 4.34, 3.33 and 2.26 times when compared with positive control group (mice given standard vitamins mixture).

Table 1: Effect of vitamin extracts of *D. salina* on glutathione-S-transferase activity in target tissues of female mice

Groups	Act. μmol min^{-1}	Sp. act. μmol/min/mg prot.	Ratio a	Ratio b
Liver				
G1: Negative control (oil)	0.652	1.073 ±0.1		
G2: Positive control (vitamin)	1.315	2.50* ±0.15	2.33	
G3: Ext. of *Dun.* grown under control conditions	1.29	2.43* ±0.18	2.26	1.04
G4: Ext. of *Dun.* grown under stress cond.	5.09	7.71* ±0.49	7.18	3.1
Small intestine				
G1: Negative control (oil)	0.09	0.322 ±0.02		
G2: Positive control (vitamin)	0.11	0.461* ±0.04	1.43	
G3: Ext. of *Dun.* grown under control conditions	0.16	0.59* ±0.04	1.84	1.3
G4: Ext. of *Dun.* grown under stress cond.	0.056	1.97* ±0.38	6.21	4.34
Large intestine				
G1: Negative control (oil)	0.069	0.245 ± 0.02		
G2: Positive control (vitamin)	0.077	0.415* ± 0.01	1.69	
G3: Ext. of *Dun.* grown under control conditions	0.118	0.459* ±0.03	1.87	1.1
G4: Ext. of *Dun.* grown under stress cond.	0.363	1.38* ±0.28	5.63	3.33
Lung				
G1: Negative control (oil)	0.051	0.212 ± 0.02		
G2: Positive control (vitamin)	0.048	0.273* ± 0.04	1.29	
G3: Ext. of *Dun.* grown under control conditions	0.073	0.313* ±0.38	1.48	1.14
G4: Ext. of *Dun.* grown under stress cond.	0.165	0.618* ±0.09	2.91	2.26

Table 2: Effect of vitamin extracts of *Sc. dimorphus* (normal and mutant cells) on glutathione-S-transferase activity in target tissues of female mice

Groups	Act. μmol min^{-1}	Sp. act. μmol/min/ mg prot.	Ratio a	Ratio b
Liver				
G1: Negative control (oil)	0.652	1.073 ± 0.12		
G2: Positive control vitamin	1.315	2.503* ± 0.15	2.33	
G9: Ext. of *Sc.* grown under control cond.	1.043	1.94* ± 0.07	1.81	0.83
G10: Ext. of *Sc.* grown under stress cond.	2.98	4.58* ± 0.16	4.27	1.96
G11: Ext. of *Sc.* mut. grown under cont.	1.56	2.4* ± 0.15	2.2	1.03
G12: Ext. of *Sc.* mut. grown under stress	4.23	7.27* ± 0.37	6.79	3.12
Small intestine				
G1: Negative control (oil)	0.09	0.322 ± 0.02		
G2: Positive control vitamin	0.11	0.461* ± 0.041	1.43	
G9: Ext. of *Sc.* grown under control cond.	0.12	0.421* ±0.006	1.3	0.91
G10: Ext. of *Sc.* grown under stress cond.	0.27	0.89* ±0.03	2.77	1.93
G11: Ext. of *Sc.* mut. grown under cont.	0.18	0.62* ±0.02	1.93	1.35
G12: Ext. of *Sc.* mut. grown under stress	0.55	1.87* ±0.17	5.81	4.1
Large intestine				
G1: Negative control (oil)	0.069	0.245 ± 0.02		
G2: Positive control vitamin	0.077	0.415* ± 0.01	1.69	
G9: Ext. of *Sc.* grown under control cond.	0.084	0.35* ±0.07	1.43	0.84
G10: Ext. of *Sc.* grown under stress cond.	0.183	0.72* ±0.07	2.94	0.73
G11: Ext. of *Sc.* mut. grown under cont.	0.117	0.594* ±0.08	2.24	1.43
G12: Ext. of *Sc.* mut. grown under stress	0.269	1.07* ±0.95	4.37	2.58
Lung				
G1: Negative control (oil)	0.051	0.212 ±0.021		
G2: Positive control vitamin	0.048	0.273* ±0.044	1.29	
G9: Ext. of *Sc.* grown under control cond.	0.057	0.27* ±0.004	1.29	1.04
G10: Ext. of *Sc.* grown under stress cond.	0.122	0.50* ±0.08	2.4	1.86
G11: Ext. of *Sc.* mut. grown under cont.	0.06	0.408* ±0.17	1.9	1.49
G12: Ext. of *Sc.* mut. grown under stress	0.122	0.67* ±0.04	3.19	2.47

Activity : μmol min^{-1} Specific activity: μmol min^{-1} (mg of protein $)^{-1}$
Ratio a: Test/control Ratio b: Test/positive control
Values represents mean of five mice ± S.D *:$P < 0.05$

Table 3: Effect of vitamin extracts of *Ch. ellipsoidea* (normal and mutant cells) on glutathione-S-transferase activity in target tissues of female mice

Groups	Act. μmol min^{-1}	Sp. act. μmol/min/ mg prot.	Ratio a	Ratio b
Liver				
Control (oil)	0.652	1.073 ± 0.12		
G2: Positive control (standard)	1.315	2.503* ± 0.15	2.33	
G5: Ext. of *Chl.* grown under control cond.	0.877	1.53* ± 0.07	1.43	0.611
G6: Ext. of *Chl.* grown under stress cond.	1.92	3.4* ± 0.18	3.17	1.35
G7: Ext. of *Chl.* mutant grown under control	1.41	2.5* ± 0.33	2.33	1.0
G8: Ext. of *Chl.* mutant grown under stress	2.17	5.04* ± 0.27	4.69	2.01
Small intestine				
Control (oil)	0.09	0.322 ± 0.02		
G2: Positive control (standard)	0.108	0.461* ± 0.04	1.43	
G5: Ext. of *Chl.* grown under control cond.	0.122	0.418* ± 0.011	1.3	0.91
G6: Ext. of *Chl.* grown under stress cond.	0.2	0.8* ± 0.01	2.52	1.76
G7: Ext. of *Chl.* mutant grown under control	0.115	0.516* ± 0.18	1.6	1.11
G8: Ext. of *Chl.* mutant grown under stress	0.207	1.071* ± 0.17	3.34	2.34
Large intestine				
Control (oil)	0.069	0.245 ± 0.02		
G2: Positive control (standard)	0.077	0.415* ± 0.01	1.69	
G5: Ext. of *Chl.* grown under control cond.	0.066	0.293* ± 0.01	1.2	0.71
G6: Ext. of *Chl.* grown under stress cond.	0.148	0.701* ± 0.14	2.86	1.7
G7: Ext. of *Chl.* mutant grown under control	0.09	0.401* ± 0.24	1.64	0.966
G8: Ext. of *Chl.* mutant grown under stress	0.237	1.0* ± 0.02	4.11	2.41
Lung				
Control (oil)	0.051	0.212 ± 0.021		
G2: Positive control (standard)	0.048	0.273* ± 0.044	1.29	
G5: Ext. of *Chl.* grown under control cond.	0.054	0.23* ± 0.031	1.1	0.85
G6: Ext. of *Chl.* grown under stress cond.	0.071	0.34* ±0.034	1.6	0.85
G7: Ext. of *Chl.* mutant grown under control	0.055	0.267* ±0.018	1.62	0.978
G8: Ext. of *Chl.* mutant grown under stress	0.097	0.55* ±0.016	2.6	2.02

Table 4: Effect of vitamin extracts of *Sc. acutus* (normal and mutant cells) on glutathione-S-transferase activity in target tissues of female mice

Groups	Act. μmol min^{-1}	Sp. act. μmol/min/ mg prot.	Ratio a	Ratio b
Liver				
G1: Negative control (oil)	0.652	1.073 ±0.12		
G2: Positive control vitamin	1.315	2.5* ±0.15	2.33	
G13: Ext. of *Sc.acu.* grown under control cond.	0.925	1.69* ±0.17	1.57	0.68
G14: Ext. of *Sc.acu.* grown under stress cond.	2.11	3.25* ±0.24	3.03	1.3
G15: Ext. of *Sc. acu.* mutant grown under control	1.69	2.4* ±0.15	2.24	0.96
G16: Ext. of *Sc. acu.* mutant grown under stress	3.59	6.0* ±0.44	5.59	2.39
Small intestine				
G1: Negative control (oil)	0.09	0.322 ±0.02		
G2: Positive control vitamin	0.108	0.46* ±0.041	1.43	
G13: Ext. of *Sc.acu.* grown under control cond.	0.102	0.39* ±0.005	1.22	0.85
G14: Ext. of *Sc.acu.* grown under stress cond.	0.18	0.64* ±0.21	1.99	1.4
G15: Ext. of *Sc. acu.* mutant grown under control	0.191	0.67* ±0.032	2.09	1.46
G16: Ext. of *Sc. acu.* mutant grown under stress	0.449	1.43* ±0.082	4.44	3.1
Large intestine				
G1: Negative control (oil)	0.069	0.25 ±0.025		
G2: Positive control vitamin	0.077	0.41* ±0.017	1.68	
G13: Ext. of *Sc.acu.* grown under control cond.	0.085	0.31* ±0.012	1.24	0.74
G14: Ext. of *Sc.acu.* grown under stress cond.	0.095	0.531* ±0.008	2.1	1.26
G15: Ext. of *Sc. acu.* mutant grown under control	0.115	0.56* ±0.032	2.3	1.36
G16: Ext. of *Sc. acu.* mutant grown under stress	0.245	0.91* ±0.017	3.64	2.2
Lung				
G1: Negative control (oil)	0.051	0.212 ±0.021		
G2: Positive control vitamin	0.048	0.273* ±0.044	1.29	
G13: Ext. of *Sc.acu.* grown under control cond.	0.034	0.23* ±0.011	1.15	0.85
G14: Ext. of *Sc.acu.* grown under stress cond.	0.067	0.45* ±0.04	2.14	1.7
G15: Ext. of *Sc. acu.* mutant grown under control	0.058	0.368* ±0.062	1.76	1.37
G16: Ext. of *Sc. acu.* mutant grown under stress	0.059	0.48* ±0.028	2.31	1.79

The GST activity induced by vitamin extract of *D. salina* grown under optimum conditions were 2.26, 1.84, 1.87 and 1.48 times than the control group in the same tissue (Table 1). The GST activity showed similar values, 0.97 –1.30 when compared with positive control group (T/positive).

The vitamins extract of *Sc. dimorhpus* including native or mutant strain grown under different conditions enhanced the activity of GST in all the tissues under examined (Table 2). Vitamins extract of mutant strain grown under stress condition were induced GST enzyme activity significantly and was higher than the normal and positive control groups in all tissue ranged from 6.79 to 3.19 and 3.12 to 2.47, respectively. On the other hand, the vitamin mixture of *Sc. dimorphus* normal cells grown under stress conditions showed a significant potential as GST inducers in the liver (T/C 4.27), small intestine mucosa (T/C 2.77), large intestine mucosa (T/C 2.94) and lung (T/C 2.4).

The induced activity of GST by *Chl. ellipsoidea* vitamins extract was appeared to vary according to type of strain and growth conditions (Table 3). Mutant strain grown under stress conditions increased the GST activity in all the tissues examined and the values (T/C) were 4.69, 3.34, 4.11 and 2.6 times greater than that of normal control group. Also, the activity was at least 2 fold higher than vitamin mixture control group (positive control) in all the tissues examined. The native strain grown under stress conditions induced GST activity in liver tissue with value 3.1(T/C) and with 2.52, 2.86 and 1.6 in the large, small intestinal mucosa and lung, respectively. However, *Chlorella* vitamins extract did not cause differences in induction activity of GST activity in all examined tissues, except large intestine when compared with positive control group. The vitamin mixture obtained from *Chlorella* grown under optimum conditions had no significant induction for GST when compared with the control group and its activity was lower than GST activity obtained from positive control (Table 3). *Scenedesmus acutus* vitamin extract in both native and mutant strain grown under stress conditions showed higher GST activity in all the tissues examined specially in liver when compared with control group. The mutant strain of *Sc. acutus* grown under stress conditions was higher in GST with 5.59, 4.11, 3.64 and 2.31 fold when compared with control groups (in all tissues examined). Also its activity was higher than the positive control group at least 2 times (Table 4).

Data elucidated the vitamin extracts obtained from different algal strains had a significant ($P<0.05$) induction of GST activity. The vitamin extracts appeared to increase GST activity greater than 2-7 times over than control groups in the liver tissues and 3-5 times in small and large intestine mucosa and lung, all vitamin extracts increased GST activity by about 1.6 times than that of control groups. Also, the induced GST activity by vitamin extract obtained from D. *salina* and other mutant strains grown under stress conditions were significantly greater than that of positive control group.

The *D. salina* vitamin extracts grown under stress conditions appear to be the highest in GST–inducing activity, among other algal extract. This may be due to the ability of this strain to accumulate a highest amount of β-carotene (13%). The accumulation of β-carotene in *D. salina* grown under stress conditions was in accordance with that reported by Ben-Amotz *et al.* (1991), Gomez-Pinchetti *et al.* (1992), Avalos *et al.* (1993), Morelli *et al.* (1993) and Zhang *et al.* (1997).

Algal extracts of mutant strains showed a high GST activity which may be due to the high amount of ascorbic acid in algal strain cells (4-5%) (Happette and Poulet, 1990; Abalde and Fabregas, 1991; Running *et al.*, 1994; Brown, 1995; Merchie *et al.*, 1995). Thus, the constituent of vitamin extracts appears to be responsible for high GST activity in mice.

Effect of vitamins algal extracts on tumorous mice: The data (Table 5) showed that the organs weight of tumorous mice of negative control was higher than other groups administrated by algal vitamin extracts (3 doses) or vitamin mixture (positive control). The differences were more obvious in small, large intestine and lung than in liver.

The vitamin extract of *D. salina* was the most active extract that induced GST enzyme activity and was significantly ($P<0.05$)

Table 5: Effect of vitamin extracts of green algae on the organs weight (g) of tumorous mice

Groups	Liver	small intestine	Large intestine	Lung
G1: Control non tumorous	1.246±0.25	0.173±0.16	0.781±0.06	0.821±0.07
G2: Positive control tumorous (vitamins mixture)	1.44±0.32	0.289±0.18	0.843±0.09	0.928±0.15
G3: Negative control tumorous	2.03±0.12	1.393±0.35	0.997±0.37	1.280±0.38
G4: Positive control tumorous (carotene from 0.00 time)	1.831±0.32	1.102±0.24	0.895±0.15	1.213±0.15
G5: Ext.of *Chlorella* normal cells (stress)	1.36±0.19	0.145±0.22	0.513±0.28	0.613±0.17
G6: Ext.of *Chlorella* mutant cells (stress)	1.68±0.21	0.161±0.25	0.499±0.08	0.614±0.09
G7: Ext.of *Sc. dimorphus* normal cells (stress)	1.546±0.12	0.240±0.05	0.94 ±0.11	0.703±0.13
G8: Ext.of *Sc. dimorphus* mutant cells (stress)	1.29±0.15	0.168±0.23	0.701±0.12	0.697±0.07
G9: Ext.of *Sc. acutus* normal cells (stress)	1.48±0.15	0.201±0.09	0.616±0.13	0.831±0.11
G10: Ext.of *Sc. acutus* mutant cells (stress)	1.631±0.08	0.203±0.02	0.831±0.07	0.932±0.09
G11: Ext.of *D. salina* normal cells (stress)	1.21±0.23	0.151±0.08	0.671±0.12	0.731±0.13

Each value represent the mean of five mice±S.D

Table 6: Effect of vitamin extracts of *D. salina* on glutathione-S-transferase activity in tissues of tumorous female mice

Groups	Act. μmol min^{-1}	Sp. act. μmol/min/ mg prot.	Ratio a	Ratio b	Ratio c
Liver					
G1: Control non tumorous	0.816	1.16 ±0.04			
G2: Negative control tumorous	1.13	1.62 ±0.1	1.39		
G3: Positive control tumorous (vitamin standard)	1.89	3.5 ±0.17	3.03	2.2	
G10: Ext.of *D. salina* N. starvation	5.08	9.42* ±0.36	8.12	5.8	2.7
G11: Carotene from 0 time	1.52	2.79 ±0.53	1.4	1.7	0.8
Small intestine					
G1: Control non tumorous	0.104	0.387 ±0.02			
G2: Negative control tumorous	0.112	0.435 ±0.01	1.33		
G3: Positive control tumorous (vitamin standard)	0.194	0.784 ±0.05	2.03	1.8	
G10: Ext.of *D. salina* N. starvation	0.713	2.813* ±0.41	7.27	6.5	3.59
G11: Carotene from 0 time	0.153	0.654 ±0.2	1.68	0.5	0.83
Large intestine					
G1: Control non tumorous	0.082	0.252 ±0.02			
G2: Negative control tumorous	0.091	0.334 ±0.01	1.33		
G3: Positive control tumorous (vitamin standard)	0.149	0.703 ±0.05	2.79	2.1	
G10: Ext.of *D. salina* N. starvation	0.635	2.403 ±0.2	9.54	7.2	3.42
G11: Carotene from 0 time	0.105	1.65 ±0.26	0.42	1.25	0.59
Lung					
G1: Control non tumorous	0.066	0.268 ±0.01			
G2: Negative control tumorous	1.13	1.62 ±0.1	1.4		
G3: Positive control tumorous (vitamin standard)	0.06	0.389 ±0.27	1.5	1.31	
G10: Ext.of *D. salina* N. starvation	0.465	1.72* ±0.21	6.4	5.8	4.42
G11: Carotene from 0 time	0.061	0.284 ±0.15	1.1	0.96	0.73

Activity: μmol min^{-1} Specific activity: μmol min^{-1} (mg of protein)$^{-1}$
Ratio a: Test/control non tumorous
Ratio b: Test/negative control tumorous
Ratio c: Test/positive control
Values represents mean of five mice± S.D * : P<0.05

Table 7: Effect of vitamin extracts of *Sc. dimorphus* (normal and mutant cells) on glutathione-S-transferase activity in target tissues of tumorous female mice

Groups	Act. μmol min^{-1}	Sp. act. μmol/min/ mg prot.	Ratio a	Ratio b	Ratio c
Liver					
G1: Control non tumorous	0.816	1.16 ±0.04			
G2: Negative Control tumorous	1.13	1.62 ±0.1	1.39		
G3: Positive control tumorous (vitamin mixture)	1.89	3.5 ±0.17	3.03	2.16	
G6: Ext. of *Sc.dim.* normal cells N. starvation	2.45	5.38* ±0.2	4.62	3.31	1.53
G7: Ext. of *Sc.dim.* mutant cells N. starvation	3.25	8.1* ±0.53	6.98	5	2.3
Small intestine					
G1: Control non tumorous	0.104	0.387 ±0.02			
G2: Negative Control tumorous	0.112	0.435 ±0.01	1.33		
G3: Positive control tumorous (vitamin mixture)	0.194	0.784 ±0.05	2.03	1.8	
G6: Ext. of *Sc.dim.* normal cells N. starvation	0.571	1.85* ±0.25	4.78	4.25	2.36
G7: Ext. of *Sc.dim.* mutant cells N. starvation	0.797	2.8 ±0.2	6.4	5.7	3.17
Large intestine					
G1: Control non tumorous	0.082	0.252 ±0.02			
G2: Negative Control tumorous	0.091	0.334 ±0.01	1.33		
G3: Positive control tumorous (vitamin mixture)	0.149	0.703 ±0.05	2.79	2.11	
G6: Ext. of *Sc.dim.* normal cells N. starvation	0.649	1.7* ±0.2	6.8	5.1	2.4
G7: Ext. of *Sc.dim.* mutant cells N. starvation	0.907	2.4 ±0.26	6.0	7.0	3.3
Lung					
G1: Control non tumorous	0.066	0.268 ±0.01			
G2: Negative Control tumorous	1.13	1.62 ±0.1	1.39		
G3: Positive control tumorous (vitamin mixture)	0.06	0.389 1.45 ±0.27		1.31	
G6: Ext. of *Sc.dim.* normal cells N. starvation	0.373	1.08* ±0.55	4.02	3.67	2.8
G7: Ext. of *Sc.dim.* mutant cells N. starvation	0.321	1.4* ±0.15	5.26	4.76	3.6

Table 8: Effect of vitamin extracts of *Chl. ellipsoidea* (normal and mutant cells) on glutathione- S-transferase activity in target tissues of tumorous female mice

Groups	Act. μmol min^{-1}	Sp. act. μmol/min/ mg prot.	Ratio a	Ratio b	Ratio c
Liver					
G1: Control non tumorous	0.706	0.816 ±0.043	1.16		
G2: Negative control tumorous	0.699	1.13 ±0.1	1.62	1.39	
G3: Positive control tumorous (vitamin mixture)	1.89	3.51* ±0.17	3.03	2.16	
G6: Ext. of *Chlorella* normal cells N. starvation	3.27	5.97* ±0.2	5.15	3.68	1.71
G7: Ext. of *Chlorella* mutant cells N. starvation	2.72	6.31* ± 0.22	5.45	3.9	1.8
Small intestine					
G1: Control non tumorous	0.104	0.387 ±0.02			
G2: Negative control tumorous	0.112	0.431 ±0.01	1.12		
G3: Positive control tumorous (vitamin mixture)	0.194	0.78* ±0.05	2.6	1.8	
G6: Ext. of *Chlorella* normal cells N. starvation	0.453	1.15* ±0.088	1.05	2.63	0.58
G7: Ext. of *Chlorella* mutant cells N. starvation	0.378	1.55 ±0.054	4.0	3.6	2.0
Large intestine					
G1: Control non tumorous	0.082	0.25 ±0.02			
G2: Negative control tumorous	0.0916	0.334 ±0.012	1.33		
G3: Positive control tumorous (vitamin mixture)	0.149	0.703* ±0.052	2.81	2.11	
G6: Ext. of *Chlorella* normal cells N. starvation	0.249	0.887* ±0.045	3.52	2.66	1.3
G7: Ext. of *Chlorella* mutant cells N. starvation	0 .392	1.25* ±0.12	4.96	3.74	1.8
Lung					
G1: Control non tumorous	0.066	0.262 ±0.013			
G2: Negative control tumorous	0.064	0.294 ±0.019	1.12		
G3: Positive control tumorous (vitamin mixture)	0.060	0.385 ±0.027	1.47	1.31	
G6: Ext. of *Chlorella* normal cells N. starvation	0.185	0.585* ±0.033	2.23	1.99	1.5
G7: Ext. of *Chlorella* mutant cells N. starvation	0.236	0.722* ±0.022	2.75	2.45	1.9

Table 9 : Effect of vitamin extracts of *Sc. acutus* (normal and mutant cells) on glutathione-S-transferase activity in target tissues of tumorous female mice

Groups	Act. μmol min^{-1}	Sp. act. μmol/min/ mg prot.	Ratio a	Ratio b	Ratio c
Liver					
G1: Control non tumorous	0.816	1.16 ±0.04			
G2: Negative control tumorous	1.13	1.62 ±0.1	1.39		
G3: Positive control tumorous (vitamin standard)	1.89	3.5 ±0.17	3.03	2.16	
G8: Ext. of *Sc. acut.* normal cells N. starvation	2.146	4.06* ±0.2	3.5	2.51	1.15
G9: Ext. of *Sc. acu.* mutant cells N. starvation	3.16	6.58* ±0.42	5.67	4.10	1.87
Small intestine					
G1: Control non tumorous	0.104	0.387 ±0.02			
G2: Negative control tumorous	0.112	0.435 ±0.01	1.33		
G3: Positive control tumorous (vitamin standard)	0.194	0.784 ±0.05	2.03	1.8	
G8: Ext. of *Sc. acut.* normal cells N. starvation	0.307	1.33* ±0.15	3.44	3.1	1.7
G9: Ext. of *Sc. acu.* mutant cells N. starvation	0.4	1.9* ±0.2	4.9	4.36	2.42
Large intestine					
G1: Control non tumorous	0.082	0.252 ±0.02			
G2: Negative control tumorous	0.091	0.334 ±0.01	1.33		
G3: Positive control tumorous (vitamin standard)	0.149	0.703 ±0.05	2.79	2.11	
G8: Ext. of *Sc. acut.* normal cells N. starvation	0.367	1.07* ±0.12	4.28	3.2	1.52
G9: Ext. of *Sc. acu.* mutant cells N. starvation	0.342	1.13* ±0.12	4.52	3.38	1.6
Lung					
G1: Control non tumorous	0.066	0.268 ±0.01			
G2: Negative control tumorous	1.13	1.62 ±0.1	1.39		
G3: Positive control tumorous (vitamin standard)	0.06	0.389 ±0.27	1.45	1.31	
G8: Ext. of *Sc. acut.* normal cells N. starvation	0.136	0.45* ±0.13	1.6	1.53	1.1
G9: Ext. of *Sc. acu.* mutant cells N. starvation	0.141	0.5* ±0.15	1.91	1.7	1.3

higher than other the control groups (including negative and positive tumorous mice, both of positive control with vitamins mixture standard and β-carotene standard). The GST activity was greater than 5.81 and 2.69 time in liver of negative and positive control, respectively (Table 6).

Both vitamin extracts of mutant and native cells of *Sc. dimorphus* grown under stress condition showed significantly ($P<0.05$) induced GST activity when compared with all control groups (Table 7). However, the mutant vitamin extracts increased greater GST activity than the vitamin extract of normal strain. The vitamins extract of mutant cells increased GST activity in liver with values 6.98, 5.1 and 2.3 in normal, tumorous and positive control mice, respectively. While in the normal cells, vitamins extract increased their activity with 4.62, 3.31 and 1.53, respectively. Similar results were also found in the other target tissues.

Vitamin extracts of mutant and normal *Chloerlla* and *Sc. acutus* strain induced GST activity in all tissue greater than controls groups (Table 8 and 9). However, their activity was less than that obtained by *Dunaliella* and *Sc. dimorphus*.

In general, the induced activity of GSTs in tumorous mice were higher than the normal control (non-tumorous, mice). Furthermore, the GST activity in tumorous mice administrated by vitamin algae extracts were greater than of non-tumorous mice (negative control). The activity was dependent on algae strains grown under stress conditions. The effectiveness of induced GST activity by vitamin algal extracts obtained was in the following descending order: *Dunallela salina* > *Sc. dimorphus* mutant extract > *Chlorella* (mutant) > *Sc. acutus* (mutant) > *Sc. dimorphus* (normal) > *Chlorella* (normal) > *Sc. acutus* (normal).

Tumor cells must attach themselves to the basement membranes,

through which they migrate and modify the process (Premalatha *et al.*, 1995). Therefore, the cancer cells caused damage in membrane of some organs and tissues which induce lipid peroxidation in the membrane and accelerates the disorder in structure and function of these membrane. Consequently, the lack of antioxidant defense including enzyme and non-enzyme system leads to increase the lipid peroxidation and deleterious effects (Yaji, 1987). In contrast, the increase of cellular enzymes that regulate the cells oxidative stress such as superoxide dismutase (SOD), catalase or glutathione–S–transferase and cellular antioxidants such as glutathione, carotenoids (with or without pro-vitamin A activity), ascorbic acid or tocopherol significantly induced cancer regression also stimulated large number of tumour necrosis factor alpha-positive macrophages (TNF-α) which may be mechanism for the tumor degeneration and regression (Jenkins *et al.*, 1993; Chew, 1995; Buring and Hennekens, 1997).

The induced activity of glutathione-S-transferase after tumor transplantation in mice was significantly greater than the non-tumor mice. The tumors were always associated with the alteration of the oxidant–antioxidant status (Gerber *et al.*, 1996). The tumors induced lipid peroxidation, which increased the levels of peroxidation products (electrophilic substrate). Therefore, the enzyme activity acts on lipid peroxidation products (electrophilic substrate for GST) was significantly increased in cellular to prevent continuing damages to functional and intact cell constituents (Zimniak *et al.*, 1997).

Therefore, algae extracts induced the GST activity in tumor mice to inhibit some xenobiotics formed by tumor cells. The algal extracts contained some antioxidant vitamins, β-carotene, E, C and other carotenoids which appeared to enhance macrophage cytotoxicity of cell tumor targets and prevent tumor cell growth (Davison *et al.*, 1993; Schwartz *et al.*, 1997). The vitamins algal extracts under studies, significantly increased the GST activity in tumor transplanted mice over the non tumor mice. This was in good agreement with the results reported by Schwartz *et al.* (1987 and 1988) that the extract of *Spirulina* and *Dunaliella* significantly induced tumor regression and stimulated large number of tumor necrosis factor (TNF-α), which is responsible for the tumor regressing and degeneration. Mathew *et al.* (1995) added that *Spirulina* extract was found to inhibit buccal cancer in animals. 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 1997).

The algae extracts contained carotenoids (with or without provitamin A activity), ascorbic and α-tocopherol which had anticarcinogenic and antioxidant activities. The mixture of algal extracts was more effective than the individual components of the mixture as a cancer chemopreventive and antioxidant agents because β carotene and vitamin E act together as antioxidants with synergistic effect to inhibit tumor cell growth. Also, a synergistic action of vitamin E and C has been demonstrated on a basis of a free radical interaction between them (Shklar and Schwartz 1993; Mathew *et al.*, 1995). *Dunaliella* extracts significantly inhibited the spontaneous mammary tumorigenesis of both breeding and virgin mice (Nagasawa *et al.*, 1989).

In general, the chemoprevention of carcinogenesis with algae extracts mainly depend on the chemical constituent of algae extracts which are rich in micronutrients such as carotenoids (with or without provitamin A activity), vitamin E and tocopherol (Fujii *et al.*, 1993; Shklar and Schwartz, 1993). In addition, other liposoluble constituents present in algae extracts possessed anti-tumor activity such as 7 Z,10 Z–hexadecodienoic acid from *Chl. vlugaris* (Morimoto *et al.*, 1995) and mathamensilipin isolated from *P. mathamensis* (Gerwick *et al.*, 1994). Also the aqueous extract of some algae contained some bioactive compounds which showed high anti-tumor activity both *in vivo* and *in vitro* studies (Noda *et al.*, 1990; Zhang *et al.*, 1995).

From aforementioned results, it could be concluded that the green algae *Chl. ellipsoidea, Scenedesmus acutus* 276–3a, *Sc. dimorphus* and *D. salina* accumulated massive amount of antioxidant vitamins carotenoids, ascorbic acid and tocopherol when grown under nitrogen starvation medium high light intensity and high salinity. In addition, the generated mutant strains treated with camphor can be a potential candidate for accumulation of vitamins when grown under stress conditions. The accumulated vitamins are presented in higher concentrations than conventional food and traditionally considered rich in these vitamins.

The algal vitamins play an important role as an antioxidant to inhibit lipid peroxidation. Therefore, antioxidant activity of the algal vitamins can protect our body from harmful effects of reactive oxygen species and lipid peroxidation products, involved in the pathophysiology of many human diseases. Also, the antioxidant vitamins of algal extracts induced the glutathione-S-transferase activity in many tissues of both tumorous and non tumorous mice and consequently the algae extracts may considered as potential chemopreventation agent. The data obtained showed that the antioxidant vitamins of algae strains can be amenable to manipulation by varying culture conditions as well as by improvement of strains (mutant).

In conclusion, vitamin extracts exhibited an increase in the activity of detoxifying enzyme system (GST) which believed as group of the characteristic anticarcinogens. Thus these extracts show promise as potential chemopreventive agents. However, chemoprevention is frequently described as a clinical strategy to block or reverse carcinogenesis before the development of invasive cancer.

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