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Protective effect of *Dunaliella salina* (Volvocales, Chlorophyta) against experimentally induced fibrosarcoma on wistar rats

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Summary

The β-carotene-yielding microalga, *Dunaliella salina* (Dunal) Teod. maintained in De Walne's medium was harvested and lyophilized. Fibrosarcoma was induced in rats by 20-methylcholanthrene. 0.5 g and 1.0 g of lyophilized *D. salina* powder was administered to the rats orally through carboxy methyl cellulose. Cisplatin was administered along with vitamin E to compare the protective effect of *D. salina* against fibrosarcoma. Administration of *D. salina* decreased the levels of cholesterol and lactate dehydrogenase as well as the activities of catalase, superoxide dismutase, serum aspartate aminotransaminase, serum alanine aminotransferase, when compared to control. A significant reduction in the levels of hepatic and renal RNA and DNA was observed in the sarcoma rats when treated with *D. salina* powder. Histopathological studies of tumor tissues showed regenerative and regressive changes. β-carotene globules isolated from the powder of *Dunaliella salina* confirmed the presence of 9-*cis*-β-carotene and all-*trans*-β-carotene.

Introduction

Cancer is a much dreaded disease, not because it kills, but because of the way in which it kills. Tremendous advances have been made in the knowledge and treatment of cancer. The discovery of new cancer therapeutic agents remains critically important (Driscoll, 1984; Chabner and Shoemaker, 1989). Many of the currently used antineoplastic agents are natural products initially isolated from plants. In our present investigation, β-carotene-yielding halotolerant alga, *Dunaliella*

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salina was used to treat fibrosarcoma (Raja et al., 2003).

D. salina is a unicellular, naked biflagellate green alga. The alga is being commercially well exploited for its natural β-carotene, glycerol and also for fine chemicals. *Dunaliella bardawil* promotes the growth of normal mammary gland cells, but inhibits neoplastic cells (Fujii et al., 1993).

β-carotene is well known for its antioxidant and anticancer properties (Burton, 1956; Krinsky, 1988). Epidemiological studies have consistently demonstrated that individuals who have higher serum β-carotene levels have a lower risk of cancer (Williams et al., 2000). β-carotene is reported to be potent to remove the free radicals and lipid antioxidants (Bitterman et al., 1994). β-carotene has far-reaching applications ranging from nutritional supplements to chemotherapeutic agents in cancer therapy (Simpson and Chichester, 1982; Borowitzka and Borowitzka, 1988). Supplementation of β-carotene with vitamin E is known to reduce the risk of prostate cancer (Wald et al., 1984; Bertram et al., 1991; Albanes et al., 1996; Raja, 2003). It has been implied that the antiperoxidative effect of 9-*cis*-β-carotene is important for its ability to prevent malignant and cardiovascular diseases (Levin et al., 1997). Cis-Pt is an antineoplastic drug administered against numerous animal and human tumors. The pharmacodynamic distribution and toxicity of the drug has been well established (Ward and Fauviek, 1976; Litterest et al., 1979; Hemaiswarya and Doble, 2006).

Materials and methods

Animals and diet

Male albino rats of the Wistar strains of 100–120 g procured from the inbred stock of the Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences Univerisity, Madhavaram (Chennai), were used for this experiment. They were housed in well-ventilated polyurethane cages with a 12 h light/dark cycle; animals received a standard rat chow marketed by M/s Hindustan Lever limited, Mumbai, under the name 'Gold Mohur rat feed' and tap water was given as liquid source. Seven groups of each consisting six animals were maintained.

All procedures complied with the standards for the care and use of animal subjects as stated in the guidelines laid by Institutional Animal Ethical Committee (IAEC number-02/002/2002), University of Madras, Guindy Campus, Chennai 600 025.

Chemicals

20 MC was purchased from Sigma Chemical Company (St. Louis, MO) and 0.2% solution was prepared with DMSO and stored at $-20\,^{\circ}C$.

The drug Cis-Pt was purchased from Genie, Bangalore (India). Other chemicals used for this experiment were of analytical grade.

Experimental induction of fibrosarcoma

Fibrosarcoma was induced by a single intraperitoneal injection of 0.2% solution of 20 MC into thigh region of the rats (Chandrasekaran and Nagarajan, 1983).

Groups	Experimental condition
I	Control rats received 0.9% saline.
II	Cancer induced rats administered orally 500 mg of *D. salina*/kg body wt.
III	Cancer induced rats administered orally 1.0 g of *D. salina*/kg body wt.
IV	Cancer induced rats administered orally with vitamin E (400 mg/kg body wt. orally)+Cis-Pt (600 mg/kg body wt by i.p).
V	Cancer induced rats received 0.9% saline only.
VI	Control rats administered orally 500 mg of *D. salina*/kg body wt.
VII	Control rats – administered orally 1.0 g of *D. salina*/kg body wt.

Algal source

D. salina was obtained from the Centre for Advanced Studies in Botany, University of Madras, Chennai, and maintained in De Walne's medium in a 12 h/12 h light/dark regime at $24\pm1\,^{\circ}C$ under 30 $\mu E/m^2$ s irradiance (Orset and Young, 1999; Raja et al., 2006).

Preparation of algal sample

The optimally grown 18-day-old culture of *D. salina* was harvested by centrifugation at $1000\times g$ for 15 min (Ben-Amotz et al., 1982). The pellets were washed with distilled water to remove the salts, lyophilized and stored at $4\,^{\circ}C$ in an airtight container covered with a black paper to avoid light contact since β-carotene is sensitive to light (El-Tinay and Chichester, 1970).

Dosages

0.5 and 1.0 g of *D. salina* and CM cellulose were mixed at a ratio of 1:1 and given orally to experimental rats every alternate day for a period of 45 days.

Biochemical assays

At the end of experimental regime, rats were fasted overnight and sacrificed by cervical decapitation. The blood sample was collected using ethylene diamine tetra acetic acid as anticoagulant for the analysis of serum AST, serum ALT and LDH. The liver and kidney were dissected out and washed in ice-cold saline immediately to estimate SOD (Misra and Fridovich, 1972), CAT (Takahara et al., 1960), cholesterol (Parekh and Jung, 1970), serum AST and serum ALT (Mohur and Coore, 1975), LDH (King, 1965), DNA (O'keefe and Cuatrecases, 1974; Burton and Ingold, 1984) and RNA (Rawal et al., 1977).

Histological studies

A small portion of tumor tissue was taken from the thigh region of rats and washed with ice-cold saline. They were fixed with 10% formal saline and embedded in paraffin wax. Using a microtome, they were sectioned and then stained with hematoxylin and eosin. The sections were mounted and observed for morphological changes under light microscopy (Shirwaikar et al., 2003).

Statistical evaluation

The data were statistically analyzed and the values are expressed as mean $\pm$ SD. The Student's '*t*' test and '*P*' values are also indicated (Fisher, 1990).

Isolation and estimation of β-carotene isomers

β-carotene isomers were extracted from the lyophilized sample of *D. salina* using 80% acetone (Raja, 2003). The algal powder of about 0.2–0.4 mg was resuspended in 30 mM NaCl in 200-fold volume excess, mixed and centrifuged at 12,000 $\times$ *g* for 10 min. The supernatant was discarded and the pellet resuspended in distilled water was mixed and centrifuged again at 12,000 $\times$ *g* for 15 min. It was further mixed with one-forth its volume of a solution containing 50% sucrose, 10 mM Tris-Cl, pH 8.0. On top of the mixture, 0.5 mL of 10 mM Tris-Cl pH 8.0 was layered and the preparation was centrifuged at 48,000 $\times$ *g* for 2 h. The purified β-carotene globules were collected from the top layer. Visible absorption spectrum was taken at 443 and 475 nm to confirm the presence of 9-*cis* and all-*trans*-β-carotene isomers (Ben-Amotz et al., 1982).

Results

Table 1 shows the activities of the marker enzymes namely, LDH, serum AST and serum ALT of the control and experimental groups of rats. The activities of these marker enzymes increased significantly in tumor induced rats of Group V when compared to Group I. The Groups VI and VII rats showed similar activities of marker enzymes as that of control rats. *D. salina*-treated sarcoma rats (Group III) showed a significant reduction ($P<0.001$) in the activities of these marker

Table 1. Activities of serum marker enzymes on the experimental groups of rats

Groups	Parameters[a]		
	AST (IU/L)	ALT (IU/L)	LDH (IU/L)
I Control	38.5 $\pm$ 2.31	12.6 $\pm$ 0.63	327.60 $\pm$ 16.4
II Fibrosarcoma+*Dunaliella salina* 500 mg	48.7 $\pm$ 2.9[b]	19.4 $\pm$ 0.78[b]	380.39 $\pm$ 15.2[b]
III Fibrosarcoma+*Dunaliella salina* 1.0 g	43.3 $\pm$ 2.2[b]	16.0 $\pm$ 1.12[b]	347.88 $\pm$ 20.9[b]
IV Fibrosarcoma+vit E+Cisplatin	42.6 $\pm$ 1.7[c]	14.7 $\pm$ 0.85[c]	339.22 $\pm$ 20.4[b]
V Fibrosarcoma induced	64.8 $\pm$ 3.9[b]	27.5 $\pm$ 1.93[b]	454.14 $\pm$ 22.7[b]
VI *Dunaliella salina* 500 mg	39.0 $\pm$ 1.9[NS]	13.0 $\pm$ 0.78[NS]	314.7 $\pm$ 22.0[NS]
VII *Dunaliella salina* 1.0 g	37.2 $\pm$ 2.6[NS]	12.1 $\pm$ 0.73[NS]	307.62 $\pm$ 18.5[NS]

[a]Results are mean $\pm$ SD, $n = 6$. Comparisons are made with Group I vs. Group V, Group I vs. Group VII, Group I vs. Group VI, Group II vs. Group V, Group IV vs. Group V and Group III vs. Group IV. Student's '*t*' test.
[b]$P<0.001$; [NS] – non-significant.
[c]$P<0.01$.

enzymes compared to the untreated sarcoma group.

Figure 1 shows the activities of SOD, catalase and the levels of cholesterol in the control and experimental groups of rats in liver and kidney tissues. The activities of SOD and CAT were significantly low in fibrosarcoma rats when compared with those in control animals, whereas Groups II and III animals significantly showed increase in the activities of (especially in Group III) SOD. The levels of cholesterol in kidney and liver significantly increased in sarcoma rats when compared with control rats (Fig. 1). The sarcoma rats (Group III) with 1.0 g of *D. salina* showed a significant reduction in the levels of cholesterol when compared with untreated fibrosarcoma rats (Group V).

Table 2 depicts the levels of RNA and DNA in liver ($P<0.001$) and kidney tissues ($P<0.001$) of normal and experimental group of rats. Increased levels of hepatic and renal DNA ($P<0.01$) and RNA ($P<0.01$) were observed in sarcoma rats. Significant reduction of these levels was noted in *D. salina*-treated sarcoma rats. Treatment with *D. salina* did not elicit any significant change in the levels of DNA and RNA in the treated control rats.

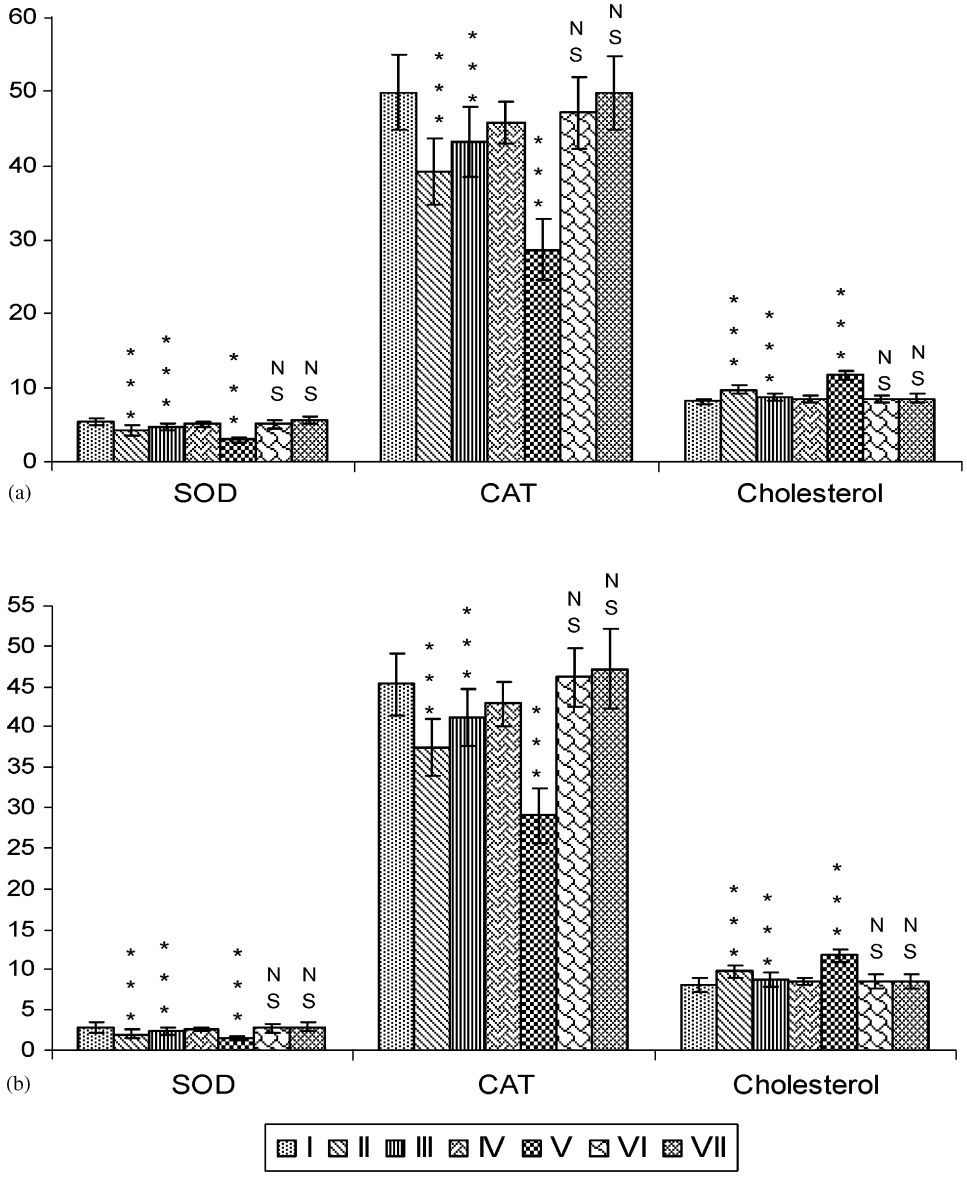

Figure 1. Activities of SOD (U/mg protein) and CAT (min/mg protein) of liver (a) and kidney (b) and the level of cholesterol in experimental groups of rats.

Table 2. Levels of DNA and RNA in liver and kidney (mg/g wet tissue) of experimental groups of rats

Parameters		Experimental groups[a]						
		I[b]	II[c]	III[d]	IV[e]	V[f]	VI[g]	VII[h]
DNA	Liver	7.3 ± 0.17	7.68 ± 0.19^{k}	7.75 ± 0.23^{k}	7.53 ± 0.17^{k}	8.74 ± 0.25^{k}	7.09 ± 0.14^{NS}	7.12 ± 0.15^{NS}
	Kidney	6.7 ± 0.14	6.79 ± 0.15^{k}	7.06 ± 0.16^{k}	6.59 ± 0.12^{k}	7.87 ± 0.2^{k}	6.61 ± 0.13^{NS}	6.8 ± 0.14^{NS}
RNA	Liver	8.9+0.39	9.23+0.38[j]	9.16 ± 0.4^{i}	9.01 ± 0.35^{k}	9.86 ± 0.45^{k}	9.18 ± 0.36^{NS}	9.12 ± 0.37^{NS}
	Kidney	8.15+0.33	8.37+0.31[k]	8.7 ± 0.35^{i}	7.95 ± 0.5^{k}	9.25 ± 0.4^{k}	7.73 ± 0.2^{NS}	7.82 ± 0.3^{NS}

[a]Results are mean $\pm$ SD, $n = 6$. Comparisons are made with Group I vs. Group V, Group I vs. Group VII, Group I vs. Group VI, Group V vs. Group II, Group IV vs. Group V and Group III vs. Group IV.
[b]Control.
[c]Fibrosarcoma+*Dunaliella salina* 500 mg.
[d]Fibrosarcoma+*Dunaliella salina* 1.0 g.
[e]Fibrosarcoma+vit E+Cisplatin.
[f]Fibrosarcoma induced.
[g]*Dunaliella salina* 500 mg.
[h]*Dunaliella salina* 1.0 g. Student's '*t*' test.
[i]$P < 0.05$.
[j]$P < 0.01$.
[k]$P < 0.001$; [NS] – non-significant.

Histopathological changes in tumor tissue

The Group V animals showed the spindle cells arranged in bundles with hyperchromatic changes suggesting increased mitotic activity. Groups II and III rats showed regenerative and regressive changes (Fig. 2b).

Figure 3 shows visible absorption spectra of 80% acetone extract confirming the presence of 9-*cis*-β-carotene and all-*trans*-β-carotene.

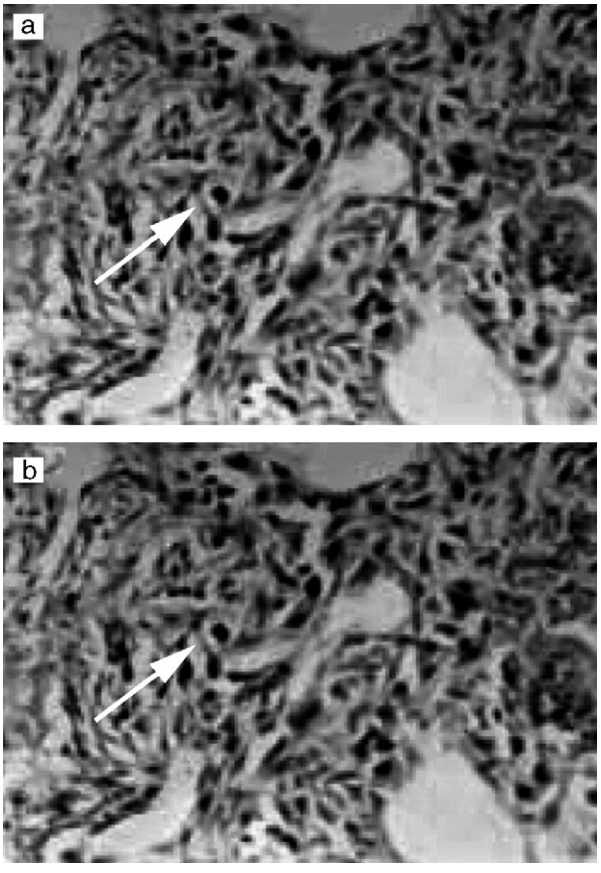

Figure 2. Results are mean $\pm$ SD, $n = 6$. Comparisons are made with Group I vs. Group V, Group I vs. Group VII, Group II vs. Group V, Group III vs. Group IV. NS – non-significant. Student's '*t*' test. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Discussion

Plant derivatives play a major role in curing many types of cancer. It has been implied that the antiperoxidative effect of 9-*cis*-β-carotene compared with that of all-*trans*-isomer is important for its ability to prevent malignant and cardiovascular diseases (Levin et al., 1997; Raja, 2003). Anticarcinogens should have a profound effect on the expression of tumor in experimental animals and ideally have a similar effect on human cancer (Devita et al., 1993). In the mammalian system, blood constituents are maintained within normal biological range. Alterations in the body homeostasis are reflected in the blood parameters. Hence, the study of blood constituents becomes indispensable to understand the normal functioning of the system. Serum pathophysiological enzymes, especially serum AST and serum ALT, are often considered as sensitive indicators of adverse drug

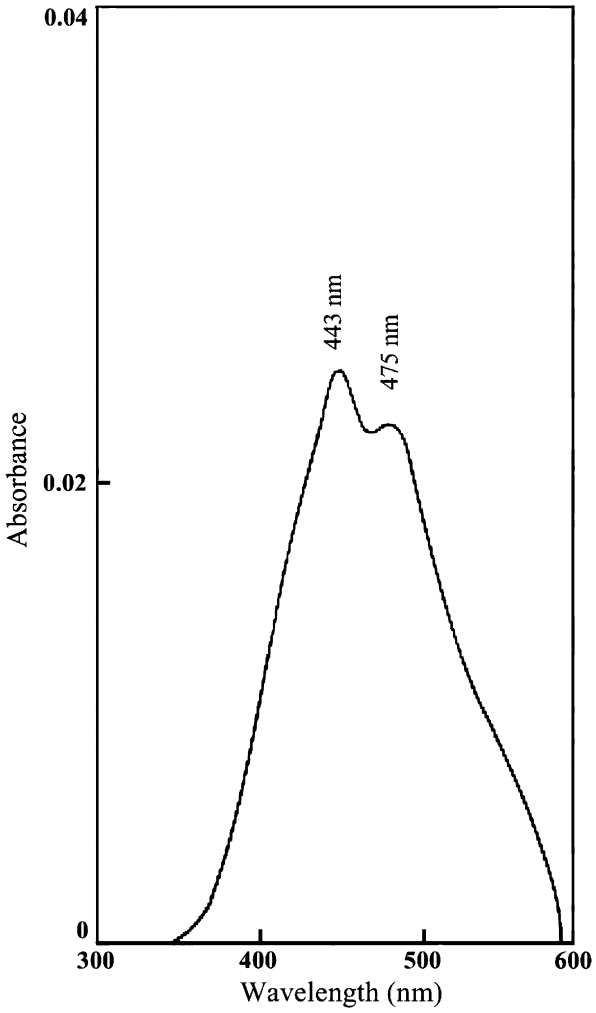

Figure 3. Visible absorption spectrum of β-carotene globules of *Dunaliella salina*.

effect and disease status (Drotman and Lawhorn, 1978). Elevated levels of serum AST and serum ALT are reported in animals treated with toxic chemicals and certain toxic plant derivatives (Malini and Vanithakumari, 1990). In support of our present investigation, the animals (normal) treated with *D. salina* did not show any significant change in the blood constituents and it could be stated that the drug is non-toxic to the mammalian system.

D. bardawil promotes the growth of normal mammary gland cells, but inhibits that of neoplastic cells and that the inhibitory effect of *D. bardawil* on mammary tumor progression is due to its normalizing both the organ-specific and the general metabolism (Fujii et al., 1993). LDH regulates the interconversion of pyruvate and lactate is useful in the recognition of neoplastic disease. Malignant tumors are known to have high rates of glycolytic activity leading to high production of lactic acid. LDH isoenzyme shifts are frequently observed in cancers (Starkweather and Schoch, 1962). They reported a pronounced increase in LDH activity due to a predominance of anaerobic glycolysis. There was an increased level of LDH activity in the malignant cells spreading through the organs of tumor induced rats. The activity of LDH in mouse fibrosarcoma cell strains was found to be five times as high as that of normal muscle tissue (Talageri et al., 1971).

Significant increase in LDH activity was observed in the serum of sarcoma rats. The increase in LDH activity was reversed to near normal by *D. salina* treatment. Significant increase in the activities of the serum AST and serum ALT enzymes was observed in Group V rats. *D. salina* alone treated control rats did not show any change in these marker enzymes, thereby indicating the non-toxic nature of the alga *D. salina*. Groups II and III showed a significant reduction ($P<0.001$) in the activities of marker enzymes as similar to Group I rats, thereby suggesting the protective effect provided by *D. salina*.

Several biological defense mechanisms exist for the prevention of membrane damage due to lipid peroxidation such as CAT and SOD. Accumulation of hydrogen peroxide is highly toxic to cells and catalase plays an important role in the elimination of hydrogen peroxide. It has been suggested that the exposure of erythrocyte to H_2O_2 enhanced the susceptibility of lipid structure of erythrocytes to auto-oxidation and might lead to hemolysis (Halliwell and Gutteridge, 1989). In the present study, CAT and SOD activities decreased significantly in sarcoma rats. The activities of CAT and SOD in liver and kidney of fibrosarcoma rats increased significantly after *D. salina* treatment, indicating its anticancer property. Further, the algal administration has been found to reduce the level of cholesterol in tumor induced rats.

Nucleic acid plays an important role during the neoplastic transformation. It has been reported that the concentrations of DNA and RNA increase in liver, lung and spleen cancers. An alteration in nucleic acid patterns in the adrenals and liver of mice bearing sarcoma-180 has been reported (Hilf et al., 1962). The fluphenazine hydrochloride caused a decrease in the growth of a mammary adrenocarcinoma of the Fischer rat and simultaneous decline in the DNA levels (Hilf et al., 1971). Hydroxyurea inhibits 98% of DNA synthesis in virally transformed fibroblast cells (De Hann and Parker, 1988). In the present study, the levels of DNA and RNA of liver and kidney increased significantly in fibrosarcoma rats. The increase in DNA was more than RNA in sarcoma rats. The present observation is in accordance with the earlier reports on the

levels of nucleic acids in tumor conditions. *D. salina* treatment showed a decrease in the levels of nucleic acids in Groups II and III rats when compared to Group V rats, which indicates the curative nature of the alga on sarcoma.

Histopathological examinations revealed that there was an alteration in the architecture of tumor tissue. This alteration was corrected to near normal by *D. salina* administration showing the curative nature of *D. salina* on fibrosarcoma condition. Groups II and III animals showed regenerative and regressive change due to the administration of *D. salina*, especially in Group III. Thus, the algal therapy efficiently changes the growth pattern of the tumor.

Our earlier studies revealed that *D. salina* grown in De Walne's medium was able to concentrate 6.2 mg/L of β-carotene and the alga grown in the salt refinery effluent also had a capacity to concentrate a minimum of 0.4185 mg/L β-carotene (De Hann and Parker, 1988; Raja et al., 2004a, b). In support of our investigation, visible absorption spectra (Fig. 3) at 443 and 475 nm clearly indicate the presence of 9-*cis*- and all-*trans*-β-carotene isomers (Ben-Amotz et al., 1982). The 9-*cis*-β-carotene has the ability to prevent malignant and cardiovascular diseases due to its antiperoxidative effect (Levin et al., 1997; Raja, 2003). So, in our present study, 9-*cis*-β-carotene might play a possible role in the reduction of cancer.

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References

Albanes, D., Heinonen, O.P., Taylor, P.R., Virtamo, J., Edwards, B.K., Rautalahti, M., Hartman, A.M., Palmgren, J., Freedman, L.S., Haapakoshi, J., Barett, M.J., Teppo, L., 1996. Alpha tocopherol and β-carotene supplements and lung cancer incidence in the alpha-tocopherol, β-carotene cancer prevention study: effects of base-line characteristics and study compliance. J. Natl. Cancer Inst. 88, 1560–1570.

Ben-Amotz, A., Katz, A., Avron, M., 1982. Accumulation of β-carotene in halotolerant algae: purification and characterization of β-carotene rich globules from *Dunaliella bardawil* (Chlorphyceae). J. Phycol. 18, 529–537.

Bertram, J.S., Pung, A., Churley, M., Kappok, T.J., Wilkens, L.R., Cooney, R.V., 1991. Diverse carotenoids protect against chemically induced neoplastic transformation. Carcinogenesis 12, 671–678.

Bitterman, N., Melamed, Y., Ben-Amotz, A., 1994. β-carotene and CNS oxygen toxicity in rats. J. Appl. Physiol. 76, 1073–1076.

Borowitzka, M.A., Borowitzka, L.J., 1988. Dunaliella. In: Borowitzka, M.A., Borowitzka, L.J. (Eds.), Microalgal Biotechnology. Cambridge University Press, New York, pp. 27–58.

Burton, G.W., Ingold, K.U., 1984. β-carotene: an unusual type of lipid antioxidant. Science 224, 569–573.

Burton, K., 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimeteric estimation of deoxyribonucleic acid. Biochem. J. 62, 315.

Chabner, B.A., Shoemaker, D., 1989. Drug development for cancer-implications for chemical modifiers International. J. Rad. Oncol. Biol. Phy. 16, 907–909.

Chandrasekaran, B., Nagarajan, B., 1983. Growth inhibitory effect of echitamine and plumbagin on fibrosarcoma in rats *Arokya*. J. Health Sci. 9, 60–63.

De Hann, J.B., Parker, M.I., 1988. Differential effects of DNA synthesis inhibitors on DNA methylation in normal and transformed cells. Anticancer Res. 8, 617–620.

Devita Jr., V.J., Hellman, S., Rosenburg, S.A., 1993. Principles and Practice of Oncology. JB. Lippincott co., Philadelphia, p. 1436.

Driscoll, J.S., 1984. The preclinical new drug research program of the National Cancer Institute. Cancer Treat. Rep. 68, 63–76.

Drotman, R.B., Lawhorn, G.T., 1978. Serum enzymes as indicators of chemical induced liver damage. Chem. Toxicol. 1, 163–171.

El-Tinay, A.H., Chichester, C.O., 1970. Oxidation of β-carotene. Site of initial attack. J. Org. Chem. 35, 2290–2293.

Fisher, R.A., 1990. Statistical Methods. Experimental Design and Scientific Interence. Oxford University Press, United Kingdom, Europe.

Fujii, Y., Ben-Amotz, A., Sakamoto, S., Nagasawa, H., 1993. Effects of β-carotene rich algae *Dunaliella bardawil* on the dynamic changes of normal and neoplastic mammary cells and general metabolism in mice. Anticancer Res. 13, 389–393.

Halliwell, B., Gutteridge, J.M.C., 1989. Free Radicals in Biology and Medicine. Clarendon Press, Oxford, pp. 188–276.

Hemaiswarya, S., Doble, M., 2006. Potential synergism of natural products in the treatment of cancer. Phyto. Res. 20 (4), 239–249.

Hilf, R., Cowett, V.W., Johnson, M.L., Bormen, A., 1962. Effects of sarcoma-180 and other stressing agents upon adrenal nucleic acids. Cancer Res. 22, 449.

Hilf, R., Bell, C., Goldenberg, H., Michel, I., 1971. Effect of fluphenazine HCl on R-3230 AC mammary carcinoma and mammary glands of the rat. Cancer Res. 31, 1111–1117.

King, J., 1965. Practical Clinical Enzymology. Philadelphia, Duan Nostrand Co., London.

Krinsky, N.I., 1988. The evidence for the role of carotenes in preventive health. Clin. Nutr. 7, 107–114.

Levin, G., Yeshurun, M., Mokady, S., 1997. In vivo per oxidative effect of 9-cis-β-carotene compared with that of the all-trans isomer. Nutr. Cancer 27, 293–297.

Litterest, C.L., LeRoy, A.F., Guarino, A.M., 1979. Disposition and distribution of platinum following parental administration of Cis dichlorodiamine platinum-II to animals. Cancer Treat. Rep. 63, 1485–1492.

Malini, T., Vanithakumari, G., 1990. Rat toxicity studies with β-sitosterol. J. Ethanopharmacol. 28, 221–314.

Misra, H.P., Fridovich, I., 1972. The role superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170–3175.

Mohur, A.F., Coore, I.J.Y., 1975. Simple method of measuring serum level of glutamate oxaloacetic acid and glutamate pyruvic transaminase in routine laboratories. J. Clin. Pathol. 10, 394–399.

O'keefe, E., Cuatrecases, P., 1974. Insulin receptors in murin mammary cells: comparison in pregnant and non-pregnant animals. Biochim. Biophys. Acta 343, 64–77.

Orset, S., Young, A.J., 1999. Exposure to low irradiances favours the synthesis of 9 cis-β-carotene in *Dunaliella salina* (Teod). J. Phycol. 35, 527–549.

Parekh, A.C., Jung, D.H., 1970. Cholesterol determination with ferrictic acetate-Uranium acetate and sulfuric acid-ferrous sulphate reagents. Anal. Chem. 42, 1423–1427.

Raja, R., 2003. Studies on *Dunaliella salina* (Dunal) Teod. with special reference to its anticancer properties. Ph.D. Thesis, University of Madras, Chennai, India.

Raja, R., Anbazhagan, C., Senthil, S., Lakshmi, D., Rengasamy, R., 2004a. Studies on *Dunaliella salina* (Volvocales, Chlorophyta) under laboratory conditions. Sea. Res. Utili. 26, 1–20.

Raja, R., Anbazhagan, C., Ganesan, V., Rengasamy, R., 2004b. Efficacy of *Dunaliella salina* (Volvocales, Chlorophyta) in salt refinery effluent treatment. Asian J. Chem. 16, 1081–1089.

Raja, R., Hemaiswarya, S., Balasubramanyam, D., Rengasamy, R., 2006. PCR-Identification of *Dunaliella salina* (Volvocales, Chlorophyta) and its growth characteristics. M. Res. 162, in press.

Rawal, U.M., Patel, U.S., Rao, G.N., Desai, R.R., 1977. Clinical and Biochemical studies on cataractous human lenses. Arogya. J. Heal. Sci. 3, 69–72.

Shirwaikar, A., Khan, S., Malini, M., 2003. Antiosteoporotic effect of ethanol extract of *Cissus quadrangularis* Linn. On ovariectomized rat. J. Ethanopharmacol. 89, 245–250.

Simpson, K.L., Chichester, C.O., 1982. Metabolism and nutritional significance of carotenoids. Ann. Rev. Nutr. 1, 351–374.

Starkweather, W.H., Schoch, H.R., 1962. Some observations on the lactate dehydrogenase of human neoplastic tissue. Biochem. Biophys. Acta 62, 440–442.

Takahara, S., Hamilton, B.T., Nell, J.U., Kobra, T.Y., Ogura, Y., Nishimura, E.T., 1960. Hypocatalase a new genetic carrier state. J. Clin. Invest. 29, 610–619.

Talageri, V.R., Revankar, S.N., Mashellar, B.N., Ranadive, K.J., 1971. Biochemical characterization of mouse and human malignant cells in vitro. Indian J. Biochem. Biophy. 8, 179–182.

Wald, N.J., Boreham, J., Hayward, J.L., Bulbrook, R.D., 1984. Plasma retinol, β-carotene and vitamin E levels in relation to the future risk of breast cancer. Br. J. Cancer 49, 321–324.

Ward, J., Fauviek, 1976. The nephrotoxic effects of Cis dichlorodiamine platinum-II (NSC-119875) in male F344 rats. Toxicol. Appl. Pharmacol. 38, 535–547.

Williams, A.W., Boileau, T.W-M., Zhou, J.R., Clinton, S.K., Erdman Jr., J.W., 2000. β-carotene modulates human prostrate cancer cell growth and may undergo intracellular metabolism to retinol. J. Nutr. 130, 728–732.