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MODULATORY INFLUENCE OF *SPIRULINA FUSIFORMIS* ON 7,12-DIMETHYLBENZ(A)ANTHRACENE INDUCED PAPILLOMAGENESIS IN THE SKIN OF MICE

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ABSTRACT

The chemopreventive properties of a Spirulina fusiformis *(blue green algae) suspension in olive oil on 7,12-dimethylbenz(a)anthracene (DMBA) induced skin papillomagenesis in female Swiss albino mice are being reported. A significant reduction in the values of tumor incidence, tumor burden and cumulative number of papillomas was observed in mice treated orally with* Spirulina fusiformis *suspension continuously at pre-, peri- and post-initiation stages of papillomagenesis as compared to the control group. Treatment of* Spirulina fusiformis *suspension by oral gavage for 15 days resulted in a significant decrease in the cytochrome P-450 content of the liver* ($p < 0.01$). *Conversely, glutathione S-transferase activity was observed to be significantly elevated as compared with the control group* ($p < 0.1$) *following treatment with* Spirulina fusiformis *suspension.*

INTRODUCTION

Spirulina fusiformis [RECON Ltd., Bangalore], a blue green algae, is a cyanobacterium belonging to the Oscillatoriaceae. It is known to have a high content of protein (Kapoor, 1994) and natural bio-chelated vitamins, especially β-carotene. (Prescott, 1978; Seshadri, 1991). It is also rich in essential fatty acids and natural pigments. It has no proven toxicity and hence is being used as a popular health food supplement in the form of algal cakes, tablets, and capsules being marketed by pharmaceutical companies (Carmichael, 1994).

The β-carotene (provitamin A) content in *Spirulina* is several times higher than that of carrots (Henrikson, 1989), and is known to be a potent antioxidant, inhibiting several types of cancers (Bendich, 1989). *Spirulina* is also known to contain the enzyme superoxide dismutase (SOD), which is found to quench free radicals, products formed during metabolic activation of certain chemical carcinogens.

Based on these properties, we tested the modulatory effect of *Spirulina* suspension on mouse hepatic detoxication enzymes of cytochrome P-450, glutathione *S*-transferase (GST) and the endogenous sulfhydryl content. Also, its presumptive role in chemoprevention of DMBA-induced skin papillomagenesis in Swiss albino mice was studied.

MATERIALS AND METHODS

Animals

Random bred, female Swiss albino mice (7–8 weeks old) were obtained from the Animal Facility (JNU, New Delhi). The animals were given standard mice feed (Hindustan Lever, India) and tap water *ad libitum*. The dorsal skin on the back area of the animals was shaved 3 days before the commencement of the experiment and only those animals in the resting phase of the hair cycle were chosen for the study.

Chemicals

DMBA, croton oil, 1-chloro-2,4-dinitrobenzene (CDNB), 5-dithiobis-2-nitrobenzoic acid (DTNB),

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reduced glutathione, and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO). DMBA was dissolved in acetone at a concentration of 100 µg/50 µl. Croton oil was diluted in acetone to give a 1% dilution.

Preparation of *Spirulina*

Spirulina fusiformis powder was obtained from RECON Ltd.. Bangalore, India. For the dose level of 800 mg/kg body weight, *Spirulina* was suspended in the vehicle, olive oil. *Spirulina* suspension (0.05 ml) was given to each mouse by oral gavage daily.

Preparation of Homogenate and Supernatant Fraction

Animals were killed by cervical dislocation and the entire liver was then perfused immediately with cold 0.9% NaCl and thereafter carefully removed, trimmed free of extraneous tissue and rinsed in chilled 0.15 M Tris HCl buffer (pH 7.4). The liver was then blotted dry, weighed quickly and homogenised in ice cold 0.15 M Tris HCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. This was then centrifuged at 10,000 × g for 20 min. The resultant supernatant was transferred into pre-cooled ultra-centrifugation tubes and centrifuged at 105,000 × g for 60 min in a Beckman ultra-centrifuge (model L 870 M). The supernatant (cytosol fraction), after discarding any floating lipid layer and appropriate dilution, was used for the assay of total cytosolic glutathione *S*-transferase enzyme whereas the pellet representing microsomes was suspended in homogenizing buffer and used for assaying cytochrome P-450 content.

Assay Methods

Determination of cytochrome P-450 content. Determined in microsomal suspension by the method of Omuro and Sato (1964) by recording the difference in absorbance between 450 nm and 490 nm and using an absorption coefficient of 91 $mmol^{-1}\,cm^{-1}$.

Determination of glutathione S-transferase (GST) activity. The cytosolic glutathione *S*-transferase activity was determined spectrophotometrically at 37°C according to the procedure of Habig et al. (1974). The reaction mixture (3 ml) contained 1.7 ml of 100 mM phosphate buffer (pH 6.5), 0.1 ml of 30 mM glutathione and 0.1 ml of 30 mM CDNB. After preincubating the reaction mixture at 37°C for 5 min, the reaction was started by the addition of 0.1 ml diluted cytosol and the absorbance was followed for 5 min at 340 nm. The reaction mixture without the enzyme was used as the blank. The specific activity of glutathione S-transferase is expressed as nmoles of GSH–CDNB conjugate formed per minute per mg protein using an extinction coefficient of 9.6 $mM^{-1}\,cm^{-1}$.

The protein content was measured according to Bradford's (1976) method using BSA as a standard.

Determination of Glutathione (GSH) Content

Hepatic levels of glutathione were determined by the method as described by Moron et al. (1979). Homogenates were immediately precipitated with 0.1 ml of 25% trichloroacetic acid and the precipitate was removed after centrifugation. Free -SH groups were assayed in a total 3 ml volume by the addition of 2 ml of 0.6 mM DTNB prepared in a 0.2 M phosphate buffer (pH 8.0) to 0.1 ml of the supernatant and the absorbance was read at 412 nm using a Shimadzu UV-160 spectrophotometer. Glutathione was used as a standard to calculate µmoles GSH/g tissue.

Statistical Analysis

Statistical significance of difference between control and experimental groups was determined by Student's *t*-test.

EXPERIMENTAL DESIGN

(a) Tumor Induction

Effect of *Spirulina* suspension on DMBA-induced skin papillomagenesis.

Group A: (control) n = 10. All the animals of this group were treated with DMBA (100 µg/50 µl acetone/animal) on day 0 and two weeks later, 0.1 ml croton oil (1% in 100 µl acetone) was applied on the same area. This treatment was continued three-times weekly until the termination of the experiment.

Group B: (treatment) n = 10. All the animals of this group were treated with *Spirulina* suspension (800 mg/kg body wt) for 15 days before the treatment with DMBA on day 0 and also throughout the entire experimental protocol. The croton oil treatment was given three-times weekly until the termination of 16 weeks of the experiment.

During the 16 weeks of experiment, mice of both the groups were weighed weekly and also at the time of autopsy. They were carefully examined once a week for the presence of skin papillomas and the number of papillomas on each affected mouse was recorded. Skin

papillomas were defined as lesions with a diameter greater than 1 mm that were present for at least two consecutive observations.

(b) Cytochrome P-450 Content, GST Activity and GSH Content

The effect of *Spirulina* suspension on cytochrome P-450 content, GST activity and GSH content in the mouse liver was investigated in the following two groups.

Group 1 (n = 10). Mice were placed on a normal diet and were given 50 µl olive oil (vehicle) by gastric intubation for 15 days, daily.

Group 2 (n = 10). Animals were placed on a normal diet and were orally fed 50 µl of *Spirulina* suspension for 15 days, daily.

The body weights of mice were recorded initially and at the end of the experiment.

RESULTS

In the control group, in which a single topical application of DMBA was followed 2 weeks later by repeated applications of croton oil, skin papillomas appeared in all (100%) animals and the cumulative number of papillomas induced during the observation period was 41. The mean number of tumors per effective mouse was observed to be 7.2. Mice of the treatment group, given a continuous treatment of *Spirulina* suspension orally at pre-, peri- and post-initiational phases, showed a significant reduction in the incidence of tumors (70%) as well as the cumulative number of papillomas (32) and the mean number of tumors per effective mouse (4.61) (Figs. 1–3). However, even though the tumors appeared later (7th week) in the treatment group than in the control group (4th week), the average latent period of the treatment group (11.2 weeks) did not differ significantly from the control group (10.6 weeks).

Gastric intubation with *Spirulina* suspension for 15 days resulted in a decrease in the hepatic cytochrome P-450 content ($p < 0.01$). Conversely, GST activity was observed to be significantly elevated in the treatment group than in the control group ($p < 0.1$). Reduced glutathione content in the liver showed no significant difference in the groups (Table 1).

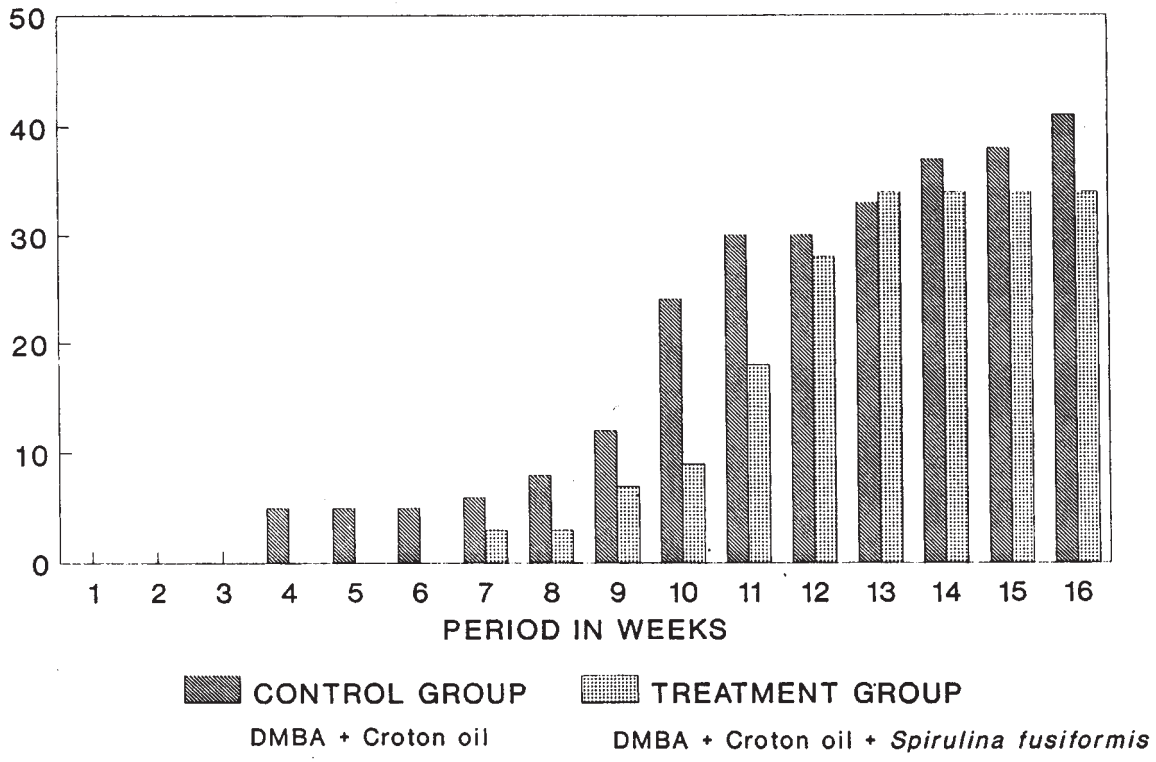

Fig. 1. Cumulative number of papillomas.

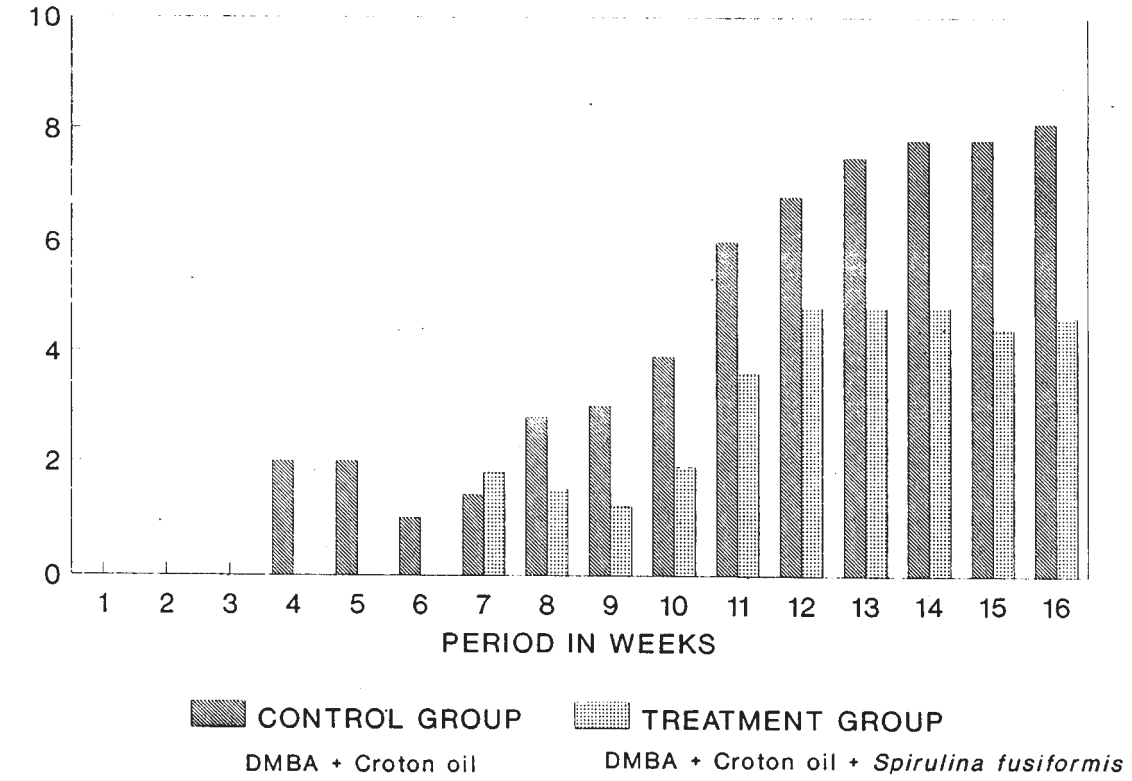

Fig. 2. Tumor burden.

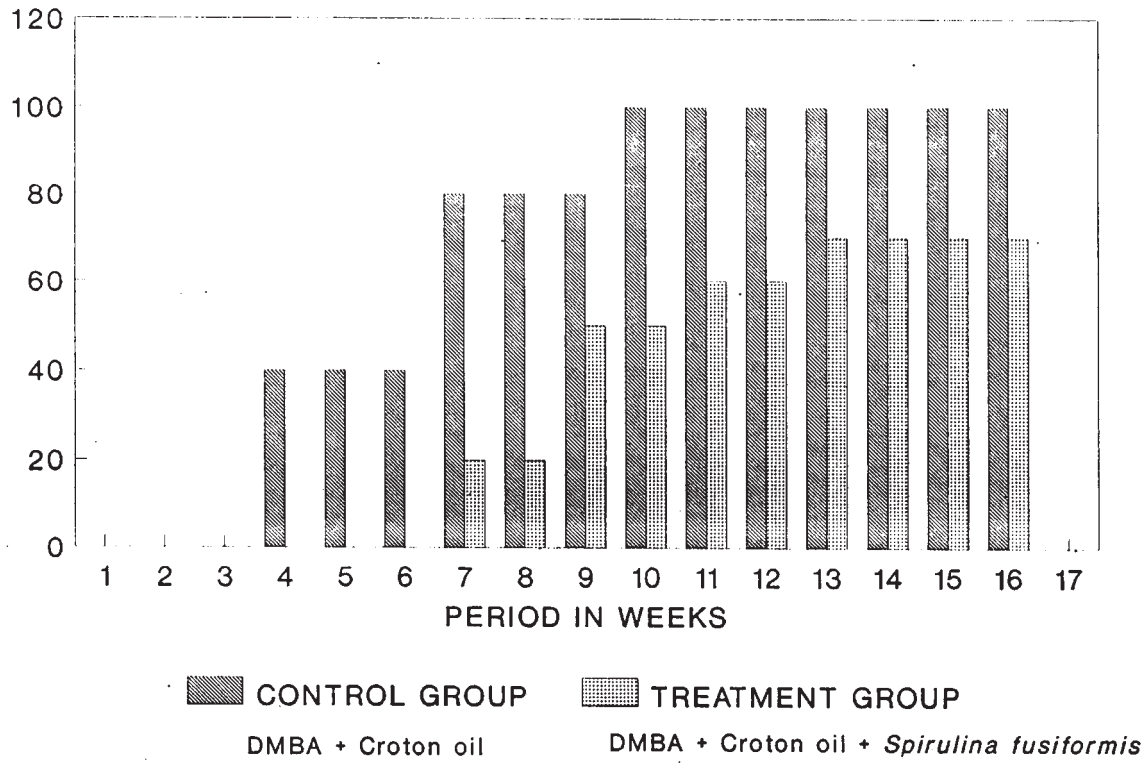

Fig. 3. Percentage of mice with papillomas.

TABLE 1. Modulatory effect of *Spirulina fusiformis* on the hepatic carcinogen metabolising enzymes.

	Treatment	[a]Cyt P-450* content	[b]GST$^+$ activity	[c]GSH conc.
Group 1	Olive oil	0.155 ± 0.008	2358.83 ± 297.9	6.81 ± 0.36
Group 2	*Spirulina* suspension in olive oil	0.117 ± 0.006	2865.01 ± 331.6	6.68 ± 0.43

Results indicate mean ± standard error mean.
[a]Cytochrome P-450 content given in nanomoles /mg protein.
[b]GST activity measured in n mole of GSH-CDNB conjugate formed/min/mg protein.
[c]GSH level expressed as μ moles/gram liver.
*Values differ significantly at $p < 0.01$.
$^+$Values differ significantly at $p < 0.1$.

DISCUSSION

Our data indicate a chemopreventive effect of the *Spirulina fusiformis* suspension on DMBA induced skin papillomagenesis in female Swiss albino mice. The animals treated orally with *Spirulina fusiformis* during the single DMBA treatment topically (100 μg/50 μl acetone) followed by repeated applications of croton oil (1% in acetone, three times per week) showed significant reduction in the cumulative number of papillomas and the values of the tumor burden as compared to the animals treated with DMBA and croton oil alone.

Many phytochemicals are known to modulate the hepatic detoxication enzyme system which activate and detoxify xenobiotics in the body including chemical carcinogens (Hodnan, 1989).

The chemopreventive role of *Spirulina fusiformis* may be attributed to the presence of β-carotene and the enzyme superoxide dismutase (Ben Amotz, 1987; Henrikson, 1989).

Carotenoids have been known to show chemopreventive properties in several types of cancer (Bendich et al., 1989) by acting as blocking agents (Wattenberg, 1992; Ziegler, 1992). Being an antioxidant, it scavenges free radicals derived from endogenous metabolic processes or exogenous sources. Thus, they act as anti-initiators and block the formation of reactive carcinogens (Greenwald, 1993) thereby detoxifying them in the process. It also induces the detoxication enzymes. It also contains the mitochondrial enzyme, superoxide dismutase which is found to quench free radicals and prevents tissue damage.

In the present study, decrease in the cytochrome P-450 content and an increase in the GST activity was observed in the liver of mice with 15 days oral treatment with *Spirulina fusiformis*. Decrease in P-450 content implies depression of the oxidative biotransformation of administered xenobiotic compounds (Sipes et al., 1982) in turn leading to lesser production of reactive electrophilic metabolites from carcinogens, hence offering a protective role to the mouse.

Further, increased level of GST activity detoxifies hydrophobic electrophiles either by binding them covalently or by establishing their conjugation with GSH (Chasseaud, 1979) since electrophiles represent the ultimate reactive carcinogenic forms of carcinogens. Increased level of GST activity detoxicates the chemical carcinogens.

The endogenous sulfhydryl content in the liver showed no significant difference.

Thus, *Spirulina fusiformis*, with no proven toxicity, can be used as a safe and effective chemopreventive agent for skin papillomas. Further investigation on different tumors is in progress.

CONCLUSIONS

In conclusion, our results provide evidence for the first time that the *Spirulina fusiformis* (blue green algae) suspension has a modulatory influence on the cytochrome P-450 content and GST activity in the liver and exhibits a chemopreventive action on DMBA induced skin papillomagenesis.

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