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Evaluation of Radioprotective Effects of *Spirulina* in Swiss Albino Mice

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ABSTRACT : The present study reports the effect of *Spirulina* on radiation induced hematological and biochemical alterations in Swiss albino mice. Animals of Group-I (Control, radiation alone) were exposed to gamma radiation (8 Gy), while, animals of Group-II (Experimental, *Spirulina* + Radiation), received *Spirulina* (800 mg/kg body weight) for seven consecutive days and were exposed to gamma radiation (as in Group-I). Hematological parameters were assessed at different intervals of post-irradiation from day 1 to 14. The average hemoglobin, total erythrocyte count and total leucocyte count in experimental group were significantly elevated as compared to the control group of animals. Treatment with *Spirulina* also caused a significant decrease in malondialdehyde (MDA) formation in the liver, suggesting its role in protection against radiation induced membrane and cellular damage. Results suggest that *Spirulina* modulate the radiation induced hematological and biochemical alterations in Swiss albino mice.

Keywords : Radioprotection, Spirulina, Swiss albino mice, Hematological parameters.

Introduction :

Ionizing radiations are widely used for the treatment of cancer. However, one of the limitations for using radiation is its toxic effect on normal tissues. Radiation induced damage to normal tissues can be partially reduced by the use of radioprotectors that scavenge free radicals produced during irradiation, sparing cancer tissues (Huang *et al.*, 1988). The potential application of radioprotective chemicals in the event of planned exposures or radiation accidents/ incidents has been investigated (Weiss and Simic, 1988, Bump and Malaker, 1998). It has been considered possible that radiation therapy for cancer patients could be improved by the use of radioprotectors to protect normal tissues.

Some antioxidant nutrients and phytochemicals have the advantage of low toxicity and are protective when administered at pharmacological doses. Naturally occurring antioxidants provide protection against low-dose and low-dose-rate irradiation, including therapeutic potential when administered after irradiation. A number of phytochemicals, including caffeine, genistein and melatoxin, have multiple physiological effects as well as antioxidant activity that result in radioprotection *in vivo* (Weiss and Landauer, 2003).

Recently interest has increased in the development of potential drugs of plant origin for the modification of radiation effects. Plant extract such as garlic (Gupta, 1988), ginseng (Pande *et al.*, 1998a), Aloe vera (Pande *et al.*, 1998b), Podophyllum (Goel *et al.*, 1999), Ocimum (Uma Devi *et al.*, 2000), Amaranthus and Spinacea (Bhatia & Jain, 2003a,b) and Mentha (Samarth and Kumar, 2003) have been found to have an advantage over the synthetic compounds in terms of low/no toxicity at the effective dose with minimum side effects.

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Spirulina is a fresh water filamentous blue green algae belonging to family Oscillitoriaceae, kingdom Monera. It is a rich source of beta-carotene, vitamin E, vitamin B complex, cyanocobalamine, SOD, proteins, iron, phosphorus, zinc, calcium, copper, magnesium, manganese, chromium, potassium, selenium, carbohydrates and essential fatty acids. Based on the nutritional aspects of blue green algae, *Spirulina*, the present study has been undertaken to evaluate the effect of *Spirulina* on radiation induced hematological alterations in Swiss albino mice.

Materials and Methods :

Animals : Adult female Swiss albino mice (8-weeks old) were obtained from the animal facility (JNU, New Delhi). The animals were provided with standard mice feed (Ashirwad feeds, Chandigarh, India) and tap water *ad libitum*. The colony was maintained at room temperature of 25 ± 2 ^{0}C and the light: dark exposure of 12 hr: 12 hr.

Source of Irradiation : The animals were whole body exposed to gamma radiation with a dose of 8 Gy by a Co^{60} source (dose rate = 1.69 Gy/min), at a distance of 77.5 cm from the source at the Department of Radiotherapy, SMS Medical College and Hospital, Jaipur, India.

***Spirulina* :** The *Spirulina* extract in powder form was obtained gratis from M/s Recon Pharmaceuticals, Bangalore, India. It was dissolved in double distilled water for being given to the animals at proper dose levels.

Experimental Design : The mice were divided into two groups consisting of six animals in each, Group-I animals were fed orally (using 22-gauge oral feeding needle; Popper and Sons Inc., New York, U.S.A.) with 0.1 ml of double distilled water once a day for 7 days before radiation and served as the control group, while Group-II received 800 mg/kg body weight of *Spirulina* in 0.1 ml double distilled water in a similar fashion. One hour after administration on day 7, the animals of both groups were exposed to 8 Gy of gamma radiation.

Hematological Study : The blood samples were collected from each group of various post-exposure intervals between 1 to 14 days from the orbital venous plexus by puncturing with the tip of capillary tube. The blood was collected in a vial containing 2% ethylenediamine tetra acetic acid (EDTA) as anticoagulant. Parameters such as total leucocyte count (TLC), total erythrocyte count (TEC) and hemoglobin (HB) level were determined at 1, 3, 5, 9 and 14 days after radiation exposure by adopting standard procedures.

Biochemical Study :

Reduced glutathione (GSH) : The GSH level was determined in liver by method as described by (Moron *et al.*, 1979). Homogenates were immediately precipitated with 0.1 ml of 25% TCA 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 and 0.9 ml 0.2 Mm sodium phosphate buffer (pH 8.0) to 0.1 ml of the supernatant and the absorbance was read at 412 nm using a UV-VIS Systronics spectrophotometer. Glutathione was used as a standard to calculate mmole GSH/gm tissue.

Lipid peroxidation (LPO) : The LPO in microsomes prepared from liver, was estimated spectrophotometrically by Thiobarbituric acid reactive substances (TBARS) by method of (Ohkawa *et al.*, 1979) and is expressed in terms of malondialdehyde (MDA) formed per mg protein. In brief, 0.4 ml of microsomal sample was mixed with 1.6 ml of 0.15 M

Tris KCl buffer to which 0.5 ml of 30% TCA was added. Then 0.5 ml of 52 mM TBA was added and placed in a water bath for 25 min 80^0 C, cooled in ice and centrifuged at room temperature for 10 min at 3,000 rpm. The absorbance of the clear supernatant was measured against reference blank of distilled water at 531.8 nm.

Statistical Analysis : The results obtained were expressed as mean ± SE. Student's 't' test was used to make a statistical comparison between the groups. A statistical comparison was completed with the irradiation alone group Vs the normal and irradiation alone group Vs the *Spirulina* and radiation combined group. The significance levels were set at $P<0.005$.

Results :

Hematology : *Spirulina* treatment for seven consecutive days prior to radiation exposure (8 Gy) showed significant increase in hematological parameters such as Hb, TEC and TLC as compared to control (radiation alone) group of animals.

The HB levels of control and *Spirulina* treated groups were considerably decreased after radiation exposure (8 Gy), however, the maximum decrease was observed at day 5 post-irradiation (Table 1). The hemoglobin levels and the percentage decrease in control groups was 21.5% whereas, it was 12.7% in *Spirulina* treated group.

The TEC decreased continuously till day 5 in both control and *Spirulina* treated group. It decreased to 31.2% in control group of animals, while it was 24.5% in animals of *Spirulina* treated group following day 5 post-irradiation (Table 1). Thereafter, the TEC was slightly increased; the percentage decrease was 16.6% in control and 7.1% in the *Spirulina* treated group on day 14 post-irradiation.

The average TLC of mice are shown in Table 1. The decrease in number of leucocytes was observed till day 5. A decrease in the TLC of 61.9% was noticed in the control animals, whereas, decrease of 58.3% was observed in the *Spirulina* treated group on day 5. It lies increased after day 5, but remained below normal in both control and experimental groups. However, the TLC in the *Spirulina* treated group was significantly higher at all the studied intervals (Table 1).

Liver Biochemistry : There was significant decrease in the GSH of mice exposed to gamma radiation, whereas *Spirulina* treated group showed significant increase in the hepatic GSH level. The LPO level (MDA formation) in liver was found to be significantly decreased after exposure to gamma radiation. In *Spirulina* treated group the MDA level was observed significantly higher than the control group (Table 2).

Discussion :

The results from the present study indicate that the pretreatment of *Spirulina* protects from radiation induced hematological and biochemical alterations in Swiss albino mice. The radioprotective effect of *Spirulina* was demonstrated by evaluating the hematological parameters such as Hb, TEC and TLC on various post-irradiation time intervals i.e., from day 1 to 14. Also biochemical parameters such as GSH and LPO were assessed. A significant radioprotection was achieved when *Spirulina* was given orally (800 mg/kg body weight/day) for seven consecutive days before radiation exposure (8 Gy gamma radiation).

In the present study, a significant decrease in the hematological constituents of peripheral blood in animals of the irradiation alone group was observed. The decline in hematological constituents may be attributed to a direct damage by radiation. Although,

Table 1 : Effect of *Spirulina* on hematological parameters of Swiss albino mice at various post-irradiation time intervals

Hematological parameter	**Group**	**Post-irradiation time intervals (in days)**				
		1	3	5	9	14
Hb (14.10±0.036g/100ml)	Group-I	11.20±0.03 (20.5)	10.10±0.03 (28.3)	8.81±0.01 (37.5)	10.85±0.05 (23.0)	11.06±0.05 (21.5)
	Group-II	12.48±0.04 (11.5)	11.27±0.04 (20.0)	10.30±0.03 (26.9)	11.80±0.03 (16.3)	12.30±0.09 (12.7)
TEC (11.10±0.365/mm^3)	Group-I	9.50±0.41 (14.4)	8.46±0.34 (23.7)	7.63±0.44 (31.2)	8.51±0.35 (23.3)	9.25±0.38 (16.6)
	Group-II	10.40±0.40 (6.3)	9.33±0.27 (15.9)	8.38±0.41 (24.5)	9.43±0.28 (15.0)	10.31±0.39 (7.1)
TLC (5233±55.77/mm^3)	Group-I (56.5)	2275±21.41 (61.0)	2040±23.94 (61.9)	1991±19.00 (48.0)	2716±23.76 (24.5)	3950±48.30
	Group-II	2350±3.65 (55.0)	2150±9.67 (58.9)	2180±7.30 (58.3)	2975±9.12 (43.1)	4233±8.63 (19.1)

Group I = Radiation alone (8 Gy)
Group II = *Spirulina* + Radiation

Table 2 : Radiomodulatory influence of *Spirulina* on GSH and LPO levels in liver of Swiss albino mice

Groups	Biochemical Parameters	
	GSH***	LPO****
	(38.6±1.60)	(0.521±0.037)
Group I	28.6±1.80	0.731±0.087
Group II	34.4±2.10	0.415±0.035

***GSH level was measured as mmole of GSH/g tissue.
****LPO level was measured as nmole MDA formed/mg protein.
Group I = Radiation alone (8 Gy)
Group II = *Spirulina* + Radiation

3 Gy total body dose is required to produce detectable depletion in total erythrocyte cells, the whole body irradiation of the moderate dose range (5-10 Gy) leads to a decreased concentration of all the cellular elements in the blood. This may be due to a direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage, or leakage through capillary walls and loss of production of cells (Casarett, 1968). Mitotically active precursor cells are sterilized by radiation, and the subsequent supply of RBCs, WBCs and platelets is thereby diminished. The time at which the number of circulating cells in the blood reaches minimum value since, mature circulating cells begins to die off and the supply of new cells from the depleted precursor population is inadequate to replace them so that the full effect of radiation becomes apparent (Hall, 2000).

In the present investigation, *Spirulina* pretreatment showed a gradual recovery of hematological constituents in the peripheral blood of Swiss albino mice. Also it was observed that *Spirulina* treatment significantly elevated GSH level and decreased MDA formation in the liver of Swiss albino mice. The GSH is present in all mammalian cells in substantial concentrations. It represents an important defense against oxygen derived free radicals and cellular lethality from exposure to anticancer drugs or ionizing radiation (Orrhtinius and Moldeus, 1984, Biaglow *et al.*, 1989). Thus, radiomodulatory effect observed in the present study may be due to the significant elevation in GSH level.

Several pathways of radioprotection have been suggested for the mechanism of protective action in mammalian cells against the damaging effects of ionizing radiation. The mechanisms implicated in the protection of cells by radioprotectors include free radical scavenging that protects against reactive oxygen species (ROS) generated by ionizing radiation or chemotherapeutic agents, and hydrogen atom donation to facilitate direct chemical repair at sites of DNA damage. The ROS generated by ionizing radiation are scavenged by radioprotectors before they can interact with biochemical molecules, thus reducing the harmful effects of radiation.

Thus, results of the present study suggests *Spirulina* modulates the radiation induced hematological and biochemical alterations in Swiss albino mice. The

radiomodyfying property of *Spirulina* is mainly attributed to the high contents of beta-carotene, which is singlet oxygen quencher. *Spirulina* also contains vitamin E, which is an effective lipid soluble antioxidant and free radical scavenger, protecting cell membrane from peroxidative damage. The antioxidant function of vitamin E seems to play a major role in preventing radiation damage.

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