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Role of Spinacia oleracea as Antioxidant : A Biochemical Study on Mice Brain after Exposure of Gamma Radiation

Rajesh Kumar Verma, R. Sisodia and A. L. Bhatia
Neurobiology Laboratory
Department of Zoology,
University of Rajasthan, Jaipur-302004 (India)

Present study is an attempt to investigate the radioprotective efficacy of spinach against radiation induced oxidative stress, since its leaves are rich in antioxidants like carotenoids (lutein, β-carotene, zeaxanthin), p-Coumaric acid, ascorbic acid, proteins, vitamins etc. Healthy Swiss albino male mice of 6-week-old age groups were selected from an inbred colony; maintained on standard mice feed and water *ad libitum*. For the experiments, mice were divided in four groups. Group I (normal) it did not received any treatment. Group II (drug treated) was orally supplemented spinach extract once daily at the dose of 1100 mg/kg.b.wt. /day for 15 consecutive days dissolved in double distilled water. Group III (experimental) was also administered orally spinach extract at the dose of 1100 mg/kg.b.wt./day for 15 consecutive days thereafter exposed to single dose of 5 Gy of gamma radiation at the dose rate of 1.07 Gy/min. Group IV (control) received distilled water orally equivalent to spinach extract for 15 days thereafter it was exposed to 5 Gy of gamma radiation. After the exposure mice were sacrificed at different autopsy intervals viz. 1,3,7,15 and 30 days. Brain was removed and processed to estimate lipid peroxidation (LPO).

Radiation induced significant elevation in the LPO values, which were lowered by supplementation of spinach prior to irradiation at all the intervals studied. At day 30th LPO values attained normalcy in the experimental group, but in the control group LPO values was still higher by approximately 12%. The levels of LPO products in brain of SE supplemented mice activates antioxidant enzymes in brain suggesting that spinach leaf extract reduces LPO values by quenching free radicals. The protection rendered with SE in LPO value of brain in the present study indicates the possible role *Spinacia* as radioprotector to some extent if taken continuously which might be due to synergistic effect of antioxidant constituents present in the spinach.

Key Words : *Spinacia oleracea*, Lipid peroxidation, Radioprotection, Mice.

Introduction :

Synthetic protectors have toxicity, which limit their value in the clinical field. In this context, now search is on for some natural compounds, which can quench the reactive energy of free radicals and eliminate oxygen, one of major participants in lipid peroxidation. A large number of compounds from various plant sources have been shown to possess antioxidant properties (Bhattacharya *et al.*, 1996, Yen *et al.*, 1996). Antioxidants of plant origin are

vitamin E, C, selenium, phenolic compounds, carotenoids, flavanoids etc. (Chandha, 1997). It has been assumed that nutritional intervention by increased intake of phyto-antioxidants may reduce the threat of free radicals.

Carotenoids are substances synthesized only in plants, which serve to protect the plants from the free radicals generated during photosynthesis. β-carotene, one of the carotenoids, has been thought to be of value to human and other species not only as precursor to vitamin A but also for having excellent antioxidant properties (Krinsky, 1989). *Spinacia oleracea* L. (English name: Spinach, Tamil-Pasalai Keerai, Telugu- Mathubucchali, Gujarati-Palak, Kashmiri- Palakh, Hindi-Palak) is a rich source of carotenoids (lutein, β-carotene, zeaxanthin), p-Coumaric acid, ascorbic acid, proteins, vitamins and other substances. The chemical composition of *Spinacia oleracea* is calcium-73mg/100gm, magnesium-84mg/100gm, iron-10.9%, phosphours-1mg/100gm, pottasium-206mg/100gm and vitamins C, A, thiamin, riboflavin, lutein and zeaxanthin (Gopalan *et al.*, 1966).

Radiation induced LPO as reflected by the thiobarbituric acid reactive substance (TBARS) equivalents at various *post- irradiation* intervals were studied to elucidate the amount of damage and protection provide by the spinach extract (SE). Recently, the interest has been developed to search for potential drugs, especially of plant origin, that are capable of modifying immune and radiation responses with comparatively low side effects. Many phytochemicals from plant are known to possess antioxidant properties. Indeed, human beings are consuming a variety of antioxidants in their diet and are thus protected from damage. Present study is to investigate protective effect of SE against radiation induced LPO in brain of Swiss albino mice, which is a rich source of carotene and other substances.

Material and Methods :

***Animals* :** Healthy Swiss albino male mice of 6 week old with an average weight 22±3 gms. were selected from an inbred colony. The animals were fed with standard mice feed obtained Hindustan Liver Ltd., New Delhi and water was provided *ad libitum.* Mice were maintened in a ventilated laboratory at constant temperature (18-22^0 C).

***Radiation Exposure*:** Theratern model, ^{60}Co beam therapy unit supplied by AER, Canada, was used for the irradiation by the animal at dose of 5 Gy at Radiotherapy unit, SMS Medical College and Hospital, Jaipur (India).

Plant Extract Preparation: The extract was prepared by fresh leaves of *Spinacia oleracea*; family-Chenopodiaceae. Spinach leaves were washed, air dried, powdered and extracted with alcoholic and water by refluxing for 48 hr. (16×3 hrs.) at 40^0 C. Thus obtained crude extract was vacuum evaporated so as to make it in powder from which was dissolved in double distilled water (DDW), just before oral administration.

***Drug Tolerance Study* :** Optimum dose was determined for the animals by dividing them in to four groups of 10 each and SE given orally 400, 600,800,1100 mg/kg.b.wt./day. Thirty minutes after last administration, mice were exposed to whole body 9.0 Gy gamma radiation. All such mice were observed till 30 days for symptoms of radiation sickness, mortality, and toxicity if any. The optimum dose (1100 mg/kg.b.wt/day) thus obtained, was used for experiment in detail.

***Experimental Design* :** Mice were divided in to four groups. Group I (normal) it did not received any treatment. Group II (drug treated) was orally supplemented spinach extract (50% methanolic extract) once daily at the dose of 1100 mg/kg.b.wt./day for 15 consecutive days dissolved in double distilled water. Group III (experimental) was also administered orally spinach extract (50% methanolic extract) at the dose of 1100 mg/kg.b.wt./day for 15 consecutive days thereafter exposed to single dose of 5 Gy of gamma radiation at the dose rate of 1.07 Gy/min. Group IV (control) received distilled water orally equivalent to spinach extract for 15 days thereafter it was exposed to single dose of 5 Gy of gamma radiation at the dose rate of 1.07 Gy/min with a source to surface distance of 77.5 cm. The mice were autopsied at 1, 3, 7, 15 and 30 days post-irradiation. Brain was removed and a homogenate was prepared for estimation of Lipid peroxidation by the method of Okhawa *et al.*, (1979), using tetramethoxy propane (Malondialdehyde) at the standard.

Statistical analysis :

The results obtained were expressed, as Mean+SEM. Student 't' test was used to make a statistical comparison between the groups. Significance levels were set at $P< 0.001$.

Result and Discussion :

Graph-1 shows lipid peroxidation (LPO) increases after irradiation up to day 7^{th} in both the groups (experimental and control group). Thereafter decrease in LPO values was observed from day 15^{th} onwards in both the groups. LPO values were significantly lower in experimental group from

their respective control group at all the intervals, which compensated by day 30th. In experimental group, the values were higher by 31.80% compared to the normal. Percentage protection observed in LPO level in experimental group was 14.38%, 17.72%, 19.98%, 15.24% and 12.21% at 1,3,7,15,30 days *post exposure* respectively.

Van het Hof (2000) reported the β-carotene supplementation meal increased plasma concentration of β-carotene effectively. All vegetable meals increased the plasma concentration of lutein and vitamin C significantly. A significant increase in plasma folate concentration was found only after consumption of the spinach-supplemented meal, which provided the highest level of folate. Disruption of the spinach matrix increased the plasma responses to both lutein and folate, whereas it did not affect the response to β-carotene. Guil *et al.* (1997) studied the nutritional (ascorbic acid, dehydroascorbic acid and carotenes), anti-nutritional and toxic components (oxalic acid, nitrate and uric acid) determined in sixteen popular species of wild edible plants, which were collected for human consumption in South East Spain. Bickford *et al.,* (2000) reported that diets supplemented with spinach are one of the nutritional sources of antioxidants, reverse age-induced decline in β-adrenergic function in cerebellar Purkinje neurons measured using electrophysiological techniques. The spinach diet improved learning on a runway motor task, previously shown to be modulated by cerebellar norepinephrine. Joseph (1999) that the supplements of strawberry, spinach, or blueberry to 19 month-old fischer 344 rats were effective in reversing age-related deficits in several neuronal and cognitive function suggesting that phytochemicals present in antioxidant-rich foods may be beneficial in reversing the course of neuronal and behavioral aging.

The damage occurs due to radiation is a free radical process. Free radical are potentially dangerous for cell. The reaction of free radicals, e.g. hydroxyl and peroxyl radicals on bio molecules are important in physical and pathology (Halliwell *et al*., 1989). Radiation induced Lipid peroxidation has been reported to be caused by superoxide radicals (Petkau and Chelack, 1976). However, later studies indicated that the hydroxyl radicals is the most active species involved in radiation induced Lipid peroxidation (Raleigh *et al*., 1977). The discovery that β-carotene acts as a powerful quencher of singlet oxygen and a scavenger of free radicals (Foote *et al*., 1970), especially at low partial oxygen pressure (Burton and Ingold, 1984), provided a

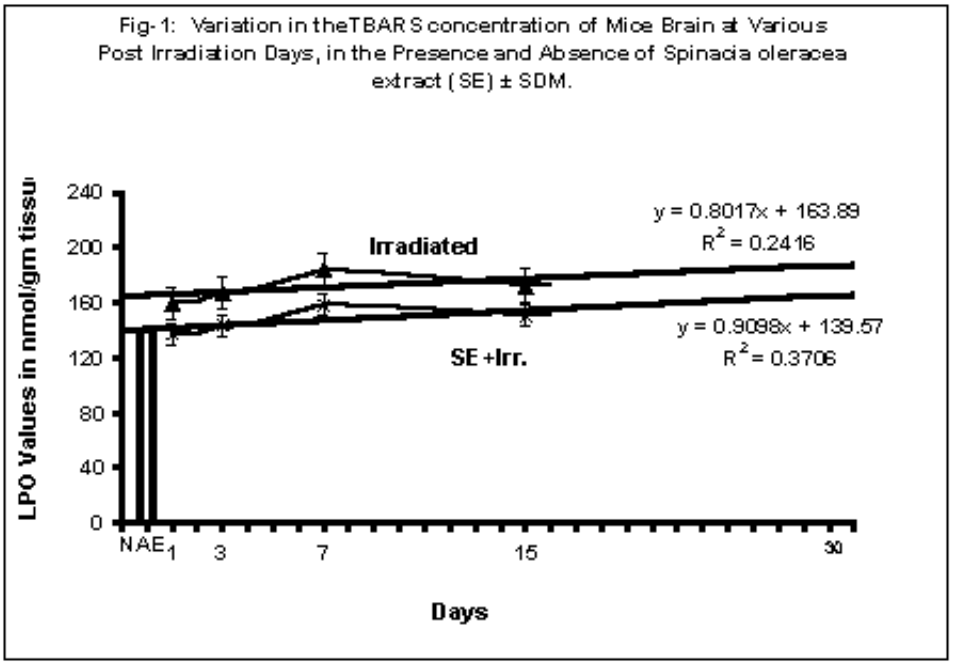
theoretical background for the present work on its possible effect against radiation. Lipid peroxidation brings about several changes in biological membranes (Leyko and Bartosz, 1986). It is a highly destructive process and cellular organelles and whole organism, lose biochemical function and structural architecture (Kale and Sitasawad, 1990), which may lead to damage or death of cell.

The targets of cell radicals attack include proteins, lipids, carbohydrates and most important, DNA. Singlet oxygen can damage DNA by causing single strand breaks, which potentially include mutation while the hydroxyl radical can stimulate lipid peroxidation leading to the destruction of lipid cell membranes and other lipid structures. The oxidative stress may lead to DNA damage and mutagenesis, protein and carbohydrate and metabolic disorders (Sies, 1986). The antioxidative mechanism of β-carotene has been suggested to be singlet oxygen quenching, free radicals scavenging and chain breaking during lipid peroxidation (Gerster, 1993).

Conclusion :

The result shows that the SE renders protection against radiation-induced oxidative stress. The reduction in the amount of TBARS equivalents in the SE administered irradiated animals suggests that SE may scavenge the free radicals formed during radiation exposure. The antioxidative mechanism of β-carotene has been suggested to be singlet oxygen quenching. We used plant leaf extract, which aside from having high concentration of β-carotene content other constituents common to green plants the possible synergistic effect and possible beneficial potency of the plant nutrient obscure and warrants further research.

Present study finding that SE reduces the amount of TBARS equivalents in mice brain when given before radiation exposure make it a potential preventive agent against lipid peroxidation that can be induced by exposure to radiation. Some preliminary experiments of plant extract indicates certain clues that the increased herbal supplementation of spinach may exert an antiradiation influence in the body organs with cell renewal system like liver and non- cell renewal, brain. But more experiments are needed to prove their efficacy. The experiments are still underway in our laboratory with regards to the explorations of the anti-radiation and anti-dimensions of this herb.



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