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Estimation of Lipid Peroxidation of Spinach Leaf Extract's after Radiation Exposure to Mice Brain

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Abstract

This study seeks to investigate the radioprotective efficacy of spinach in mitigating oxidative stress induced by radiation, given its leaves' richness in antioxidants, including proteins, vitamins, ascorbic acid, p-coumaric acid, and carotenoids (lutein, beta-carotene, and zeaxanthin). Healthy male 6-week-old Swiss albino mice were chosen from an inbred colony and kept on regular mouse food and water without restriction. Four groups of mice were used for the tests. There was no treatment given to Group 1 (normal). Group II (drug treated) received a daily oral supplement of spinach extract dissolved in double-distilled water at a level of 1100 mg/kg.b.wt./day for 15 days in a row. For 15 days in a row after being exposed, Group III (experimental) also received oral spinach extract at a rate of 1100 mg/kg.b.wt/day. Supplementing with spinach before irradiation lowers the LPO values, which were significantly elevated by radiation at all examined intervals. Day 30° saw the experimental group's LPO values return to normal, while the control group's LPO values were remained increased by about 12%. It is possible that spinach leaf extract lowers LPO values by squelching free radicals because the amounts of LPO products in the brains of mice given SE supplement activate antioxidant enzymes in the brain. The present study's protective effect of SE on the brain's LPO value suggests that spinach may have some radioprotective properties if consumed regularly. This could be because of the antioxidants' synergistic effects.
Keywords*:* Lipid peroxidation, Radioprotection, Gamma exposure, Mice, Spinach

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Introduction

Antioxidants possess the capacity to engage with free radicals safely and halt the chain reaction prior to inflicting harm on vital molecules. Vitamin E, beta-carotene, and vitamin C are the primary micronutrient antioxidants, despite the presence of additional enzymatic systems in the body that neutralize free radicals. Cosmic rays and other ionizing radiation are absorbed by living things, producing free radicals as an intermediary in regular metabolic processes. The toxicity of synthetic protectors restricts their use in therapeutic settings. Currently, efforts are underway to find natural substances that can reduce the reactive energy of free radicals and get rid of oxygen, which is a key player in lipid peroxidation. Numerous substances from different plant sources have been demonstrated to exhibit antioxidant qualities (Bhattacharya *et al*., 1996, Yen *et al*., 1996). Plant-based antioxidants include flavanoids, carotenoids, phenolic compounds, vitamin E, vitamin C, and selenium (Chandha, 1997). The damage posed by free radicals has been thought to be mitigated by nutritional intervention through greater consumption of phyto-antioxidants. Carotenoids are compounds that are solely produced by plants and help shield them from the free radicals that are produced during photosynthesis. It has been believed that ß -carotene, one of the carotenoids, is beneficial to humans and other species since it is a precursor to vitamin A and has good antioxidant qualities (Krinsky, 1989). Spinach, also known as Tamil-Pasalai Keerai, Telugu-Mathubucchali, Gujarati-Palak, Kashmiri Palakh, and Hindi-Palak, is a plant that is rich in proteins, vitamins, ascorbic acid, lutein, ß-carotene, and zeaxanthin, among other compounds. Calcium (73 mg/100 g), magnesium (84 mg/100 g), iron (10.9%), phosphorus (1 mg/100 g), potassium (206 mg/100 g), and vitamins C, A, thiamin, riboflavin, lutein, and zeaxanthin compose the chemical composition of Spinacia oleracea (Gopalan *et al*., 1966). The extent of damage and the protective effect of spinach extract (SE) were assessed by analyzing radiation-induced lipid peroxidation (LPO) as measured by thiobarbituric acid reactive substances (TBARS) equivalents at various post-irradiation time points. The pursuit of possible medications, particularly those derived from plants that can alter radiation and immunological responses while having very few adverse effects has gained attention recently. Numerous plant phytochemicals are known to have antioxidant qualities. It is true that people are shielded from harm since they eat a range of antioxidants. Swiss albino mice, whose brains are rich in carotene and other compounds, are being used in this study to examine the preventive impact of SE against radiation-induced LPO.

Material and Methods

Animals: Six-week-old Swiss albino male mice in good health, weighing an average of 22±3 grams, were chosen from an inbred colony. Standard mouse feed from Hindustan Liver Ltd. in New Delhi was given to the animals, and they were given unlimited access to water. Mice were kept at a consistent temperature (18–22° C) in a ventilated lab.

Radiation Exposure: - The animal received 5 Gy of radiation at the radiotherapy unit of SMS Medical College and Hospital in Jaipur, India, utilizing a Theratron type Co beam treatment system supplied by AER, Canada.

Plant Extract Preparation:-Fresh *Spinacia oleracea* leaves from the Chenopodiaceae family were used to make the extract. Spinach leaves were cleaned, allowed to air dry, ground into a powder, and then refluxed with water and alcohol for 48 hours (16 x 3 hours) at 40° C. Before being taken orally, the resulting crude extract was vacuum-evaporated to create a powder, which was then mixed in double-distilled water (DDW).

Drug Tolerance Study:-The animals were divided into four groups of ten each, and the SE was administered orally at 400, 600, 800, and 1100 mg/kg.b.wt./day in order to find the optimal dose. Mice were exposed to 9.0 Gy of gamma radiation throughout their bodies 30 minutes after the last injection. All of these mice were monitored for signs of radiation illness, death, and any toxicity for 30 days. A detailed experiment was conducted using the optimal dose of 1100 mg/kg.b.wt/day that was thus obtained.

Experimental Design:-There were four groups of mice. There was no therapy given to Group I (normal). For 15 days in a row, Group II (drug treated) received a daily oral supplement of spinach extract (50% methanolic extract) dissolved in double-distilled water at a dose of 1100 mg/kg.b.wt./day. Furthermore, Group III (experimental) was administered a single dose of 5 Gy of gamma radiation at a rate of 1.07 Gy/min following 15 consecutive days of oral administration of spinach extract (50% methanolic extract) at a dosage of 1100 mg/kg body weight per day. Group IV (control) received a single dose of 5 Gy of gamma radiation at a dosage rate of 1.07 Gy/min, following a 15-day regimen of oral administration of distilled water comparable to spinach extract.

Statistical analysis:-The acquired data were presented as Mean±SEM. A statistical comparison between the groups was conducted using the student 't' test. The significance threshold was set at $P<0.001$.

Result and Discussion

Table 1 demonstrates that both the experimental and control groups' levels of lipid peroxidation (LPO) increased following irradiation until day seven. LPO readings then started to decline in both groups on day 15 and continued to do so. By day 30, the experimental group's LPO readings had significantly decreased compared to the control group at every interval. Compared to the normal, the experimental group's values were 31.80% higher. The experimental group's percentage protection in LPO levels at 1, 3, 7, 15, and

30 days after exposure was 14.38%, 17.72%, 19.98%, 15.24%, and 12.21%, respectively.

Table 1:- Variation in the Lipid peroxidation (n mol/gm) of mice at various post irradiation days, in the presence and absence of plant extract (SEM)

N- 141.65±1.52 (100%)

SE- 135.77 ±1.80 (95.84%) NS

GROUPS	DAYS POST TREATMENT				
	1	3	7	15	30
IR	159.38±2.06 (112.51%)	168.3±1.64 (118.81%)	186.7±1.90 (131.80%)	172.2±1.21 (121.56%)	161.6±1.13 (114.08%)
SE + IR	139.01±1.61 (98.13%)	143.2±1.17 (101.09%)	158.4±1.45 (111.82%)	150.61±1.31 (106.32%)	144.31± 1.06 (101.87%)

N-Normal SE- Spinach extract IR- γ-irradiated P<0.001, NS-non significant

Normal and SE only treated animals shows the mean values of all autopsy intervals as there was insignificant variation in different autopsy intervals.

According to Van Het Hof (2000), a ß -carotene supplementation meal successfully raised the plasma concentration of ß -carotene. Every veggie meal markedly raised the plasma levels of vitamin C and lutein. Only after eating the spinach-supplemented lunch, which had the highest level of folate, was there a noticeable rise in plasma folate concentration. While the response to ß -carotene was unaffected, the disruption of the spinach matrix increased the plasma responses to both lutein and folate. The nutritious (ascorbic acid, dehydroascorbic acid, and carotenes), anti-nutritional, and poisonous (oxalic acid, nitrate, and uric acid) components of sixteen common species of wild edible plants that were gathered for human use in South East Spain were examined by Guil *et al*. (1997). According to Bickford *et al*. (2000), diets enhanced with Radiation causes damage through a mechanism known as free radicals. Free radicals have the ability to harm cells. Free radicals, such as hydroxyl and peroxyl radicals, have a significant impact on biomolecules in both disease and physics (Halliwell *et al*., 1989). Superoxide radicals have been implicated in radiation-induced lipid peroxidation (Petkau and Chelack, 1976). Later research, however, showed that the most active species in radiation-induced lipid peroxidation are hydroxyl radicals (Raleigh *et al*., 1977). The current study on its potential impact was theoretically supported by the finding that ß -carotene is a potent singlet oxygen quencher and free radical scavenger (Foote *et al*., 1970), particularly at low partial oxygen pressure (Burton and Ingold, 1984).

Proteins, lipids, carbohydrates, and—above all—DNA are the focus of cell radical attacks. While the hydroxyl radical can promote lipid peroxidation, which results in the breakdown of lipid cell membranes and other lipid structures, singlet oxygen can harm DNA by producing single strand breaks, which may include mutation. Oxidative stress can cause metabolic abnormalities, protein and carbohydrate problems, mutagenesis, and damage to DNA (Sies, 1986). It has been proposed that ß -carotene's antioxidative mechanism involves chain breaking during lipid peroxidation, free radical scavenging, and singlet oxygen quenching (Gerster, 1993).

Conclusion

The outcome demonstrates that the SE provides defense against oxidative damage brought on by radiation. SE may scavenge the free radicals produced following radiation exposure, as evidenced by the decrease in TBARS equivalents in the animals given SE. Singlet oxygen quenching has been proposed as ß-carotene's antioxidative mechanism. In addition to having a high concentration of ß -carotene and other components common to green plants, the plant leaf extract we utilized has an unknown synergistic effect and potential positive potency that merits more investigation.

The current study's discovery that SE lowers the quantity of TBARS equivalents in mice's brains when administered before to radiation exposure suggests that it may be used as a preventive measure against lipid peroxidation, which radiation exposure can cause. According to some early plant extract research; there are hints that increasing herbal supplementation of spinach may have an antiradiation effect on the body's organs that have cell renewal systems, such as the liver, and non-cell renewal organs, such as the brain. However, additional research is required to demonstrate their effectiveness. Our laboratory is still doing tests to investigate this herb's anti-radiation and anti-dimension properties.

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